Ion S5 and Ion S5 XL Systems

Targeted sequencing has never been simpler

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Introducing the Ion S5 and Ion S5 XL systems

Now, adopting next-generation sequencing in your lab is simpler than ever.

The Ion S5™ and Ion S5™ XL systems provide the simplest DNA-to-data workflow for targeted sequencing with industry-leading speed and affordability. So you can spend less time doing repetitive lab work and more time answering the critical questions in your research.

Want to sequence small gene panels or bacterial genomes on Monday and exomes or transcriptomes on Wednesday? The new Ion S5 and Ion S5 XL systems let you leverage a single benchtop instrument that scales to your application and throughput needs.

Ion Torrent™ technology has been referenced in over 1,500 publications in the first four years since its introduction. Now you can drive your research forward using this highly-cited technology with the latest innovation in leading-edge benchtop next-generation sequencing: the Ion S5 and Ion S5 XL systems.

Ion S5 systems

- Ion S5 System—Simple workflow, fast sequencing, lower weekly throughput
- Ion S5 XL System—Simple workflow, faster data analysis, higher weekly throughput


Ion S5
ionorrent

“That’s it? That’s all I have to do? This is really easy to set up”

Susana Häggqvist,
Research Engineer,
Uppsala Genome Center, Sweden

The S is for

Simplicity

Ready. Set. Sequence.

The Ion S5 and Ion S5 XL systems come with load-and-go reagents and a straightforward user interface, so you can set up the sequencer with less than 15 minutes of hands-on time.

You can also track your consumables with ease and accuracy with the automated RFID tracking feature of the systems.

By adding an Ion Chef™ System to your lab, you can benefit from automated library and template preparation, so you can go from DNA to data with less than 45 minutes of hands-on time.

Cartridge-based reagents

Less than 15 minutes of sequencing setup time

Ion Chef System

30 minutes of hands-on time for library and template prep

Automated consumable tracking

Easy and accurate RFID tracking

The S is for

Scalability

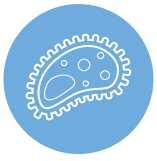
Single sequencer.
Multiple applications.

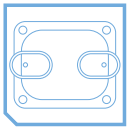
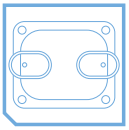
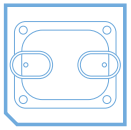
Focus your efforts on applications that have the potential to be impactful.

Each Ion S5 and Ion S5 XL system currently supports three different chip types, so you can run multiple applications on a single sequencer. This flexibility also means no more batching of samples to achieve the optimum cost efficiency. Just select the chip type that matches your specific throughput or application needs.

"I need to be able to vary the number and type of samples I put onto a run in any given week, but not compromise on cost per sample. If we have a lower number of samples we can run them on the smaller throughput chips. The Ion S5 XL offers this flexibility."

Dr. Jean François Laes
Chief Technology Officer,
OncoDNA, Belgium

Research area
 Cancer research
 Inherited disease research
 Screening for aneuploidy detection
 Infectious disease research

Application	Examples of popular panels	Number of samples per run		
		 Ion 520 Chip	 Ion 530 Chip	 Ion 540 Chip
Identify tumor specific genomic alterations from 10 ng FFPE DNA	Ion AmpliSeq Cancer Hotspot Panel v2 (50 genes)*	16	48	96
Detect SNVs, indels, CNVs, and gene fusions in a single run	Oncomine Comprehensive Assay (143 genes)	1	4	8
Research global gene expression levels from 10 ng of FFPE RNA	Ion AmpliSeq Transcriptome Panel	-	2	8
Investigate known variants associated with drug metabolism	Ion AmpliSeq Pharmacogenomics Research Panel (40 genes)†	96	384	More than 384‡
Examine causal variants for research of specific disease pathways	Ion AmpliSeq Cardiovascular Research Panel (424 genes)§	2	8	32
Discover SNPs, indels, and CNVs for research of rare or unknown disorders by whole-exome sequencing	Ion AmpliSeq Exome RDY Panel	-	-	2
High-throughput copy number and aneuploidy assessment	NA	24	96	384
Identify and type microbes through genome sequencing**	NA	12	48	96
Assess known variants associated with antibiotic resistance	Ion AmpliSeq TB Research Panel	36	144	384
Identify bacterial species in mixed samples	Ion 16S Metagenomics Kit	48	192	384

* Assuming 1,000x coverage. † Assuming 400x coverage. ‡ Assuming availability of >384 barcodes. § Assuming 175x coverage.
** Assuming 5Mb bacterial genome at 30x coverage. Assuming—400 bp for Ion 520/530 chips and 200 bp for Ion 540 chips.

The S is for

Speed

Because every hour counts.

A few hours could make all the difference in your quest for the right answer.

Unlike other light-based sequencers, there is no cumbersome optics or labeled nucleotides with semiconductor sequencing technology. The Ion S5 systems are the only benchtop NGS platforms with run times as little as 2.5 hours, and rapid DNA-to-data workflows that can be completed in as little as 24 hours.

Sequencing run times of 2.5–4 hours

- DNA to data in as little as 24 hours

"Speed is important for our service. But so is processing of 8–12 exomes a day without batching. Ion S5 XL offers us both."

Dr. Sara Alvarez
Medical Director,
NIMGenetics, Spain

The S is for

Small sample input

Because every sample matters.

Explore complex pathways from your low-input or degraded samples with Ion AmpliSeq™ technology, which enables you to prepare libraries from just a few nanograms of low-quality DNA or RNA.

Cited by hundreds of publications in just three years—you can count on this highly-referenced technology for consistent and robust library preparation performance.

10 ng of DNA or RNA

For low-quality, low-input samples

The S is for

Simple data analysis and storage

Go from data to variants in a few clicks.

Whether you are new to next-generation sequencing or an experienced user, Torrent Suite™ Software and Ion Reporter™ Software make it easy for you to get started with next-generation sequencing.

Primary analysis:

- Plan, monitor, track, and analyze your runs in Torrent Suite™ Software

Secondary analysis:

- Integrate, annotate, and interpret variants in Ion Reporter Software.
- Cloud or onsite options available based on your needs

Data storage:

- Securely store/retrieve sequencing runs and data results Torrent Suite™ Storage Systems or DataSafe™ Solutions.

The S is for

Ion S5 systems

		Ion S5 System			Ion S5 XL System		
		 Simple workflow for panels, microbes, exomes, and transcriptomes			 Simple, rapid workflow for panels, microbes, exomes, and transcriptomes		
		Ion 520 Chip	**Ion 530 Chip**	**Ion 540 Chip**	**Ion 520 Chip**	**Ion 530 Chip**	**Ion 540 Chip**
Reads		3–5 million	15–20 million	60–80 million	3–5 million	15–20 million	60–80 million
Output*	**200 bp**	0.6–1 Gb	3–4 Gb	10–15 Gb	0.6–1 Gb	3–4 Gb	10–15 Gb
	400 bp	1.2–2 Gb	6–8 Gb	—	1.2–2 Gb	6–8 Gb	—
Run times	**200 bp**	2.5 hr	2.5 hr	2.5 hr	2.5 hr	2.5 hr	2.5 hr
	400 bp	4 hr	4 hr	—	4 hr	4 hr	—
Analysis time†	**200 bp**	5 hr	8 hr	16.5 hr	1 hr	2.5 hr	5 hr
	400 bp	8 hr	17.5 hr	—	2 hr	4 hr	—

* Expected output with >99% aligned/measured accuracy. Output dependent on read length and application.
† Analysis time to aligned BAM files.

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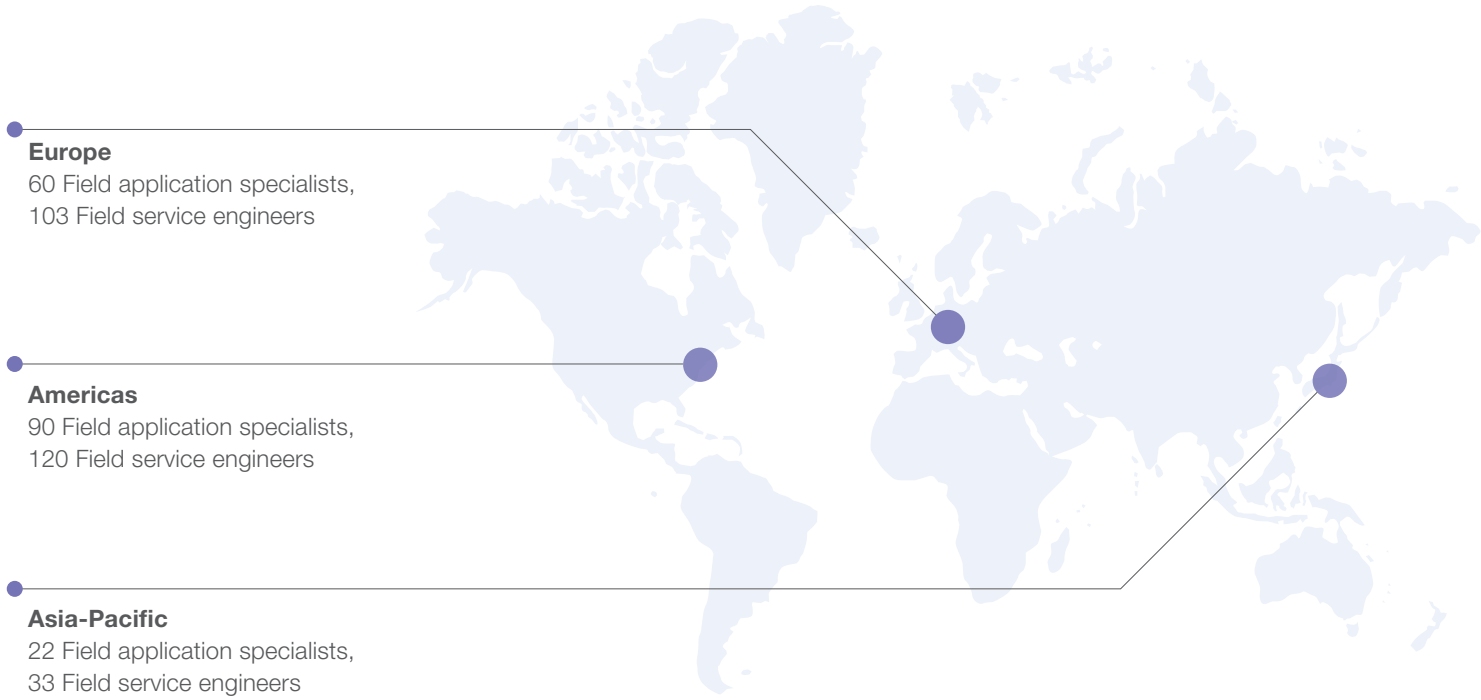
Service and support

We are here to help.

With more than 3,700+ global sales, service, and technical support specialists available to assist you in person, by phone, or online you can be confident that we will be there when and where you need us most.

In addition to flexible service plans to meet your unique needs, and online or hands-on training programs to get your site up and running quickly, you can connect with peers in the Ion Torrent™ next-generation sequencing community through regular user group meetings held at over 30 cities around the world. With over 30,000 members, the Ion Community is a web-based forum that allows Ion Torrent™ researchers to openly share methods and data, to both evaluate the technology and build on it.

We invite you to start sequencing with the Ion S5 and Ion S5 XL systems, based on technology adopted by your peers and supported by our scientists.



Europe
60 Field application specialists,
103 Field service engineers

Americas
90 Field application specialists,
120 Field service engineers

Asia-Pacific
22 Field application specialists,
33 Field service engineers

Start your sequencing journey at **thermofisher.com/ions5**

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Ion GeneStudio S5 series

Speed and throughput flexibility across a broad range of NGS applications

Chip type	Number of reads	Read length (output*)	Ion GeneStudio™ S5 System	Ion GeneStudio™ S5 Plus System	Ion GeneStudio™ S5 Prime System
			Turnaround time (sequencing run** plus analysis time)		
Ion 510™ Chip	2–3 million	200 bp (0.3–0.5 Gb)	4.5 hr	3 hr	3 hr
		400 bp (0.6–1 Gb)	10.5 hr	5 hr	5 hr
Ion 520™ Chip	4–6 million	200 bp (0.6–1 Gb)	7.5 hr	3.5 hr	3 hr
		400 bp (1.2–2 Gb)	12 hr	5.5 hr	5.5 hr
	3–4 million	600 bp (0.5–1.5 Gb)	12 hr	5.5 hr	5.5 hr
Ion 530™ Chip	15–20 million	200 bp (3–4 Gb)	10.5 hr	5 hr	4 hr
		400 bp (6–8 Gb)	21.5 hr	8 hr	6.5 hr
	9–12 million	600 bp (1.5–4.5 Gb)	21 hr	8 hr	7 hr
Ion 540™ Chip	60–80 million	200 bp (10–15 Gb)	19 hr	10 hr	6.5 hr
		200 bp (20–30 Gb) 2 runs in 1 day	NA	20 hr	10 hr†
Ion 550™ Chip	100–130 million	200 bp (20–25 Gb)	NA	11.5 hr	8.5 hr
		200 bp (40–50 Gb) 2 runs in 1 day	NA	NA	12 hr†

* Expected output with >99% aligned or measured accuracy. Output dependent on read length and application.
** Sequencing run times are between 2.5 and 4 hr.
† Analysis of first run occurs concurrently with the second sequencing run.

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	Ion GeneStudio S5 System	Ion GeneStudio S5 Plus System	Ion GeneStudio S5 Prime System
Compatible chips	Ion 510, 520, 530, and 540 Chips	Ion 510, 520, 530, 540, and 550 Chips	
Dimensions (W x D x H)	54.2 x 80.6 x 50.9 cm		
Weight	63.5 kg		
Power	100–240 VAC, 50/60 Hz, 6.5–14.5 A	100–240 VAC, 50/60 Hz, 6.5–14.5 A	100–240 VAC, 50/60 Hz, 6.5–14.5 A
Instrument clearance	Top = 30.5 cm (12.0 in.) Front = 30.5 cm (12.0 in.) Left = 10.0 cm (4.0 in.) Right = 30.5 cm (12.0 in.) Back = 30.5 cm (12.0 in.)		
Working environment	• Temperature: 20–30°C (68–86°F) • Humidity: 40–60%, noncondensing • Altitude: Up to 2,000 m (6,500 ft) above sea level • Thermal output at typical power draw of 1,200 W: 4,094 BTU/hr	• Temperature: 20–30°C (68–86°F) • Humidity: 40–60%, noncondensing • Altitude: Up to 2,000 m (6,500 ft) above sea level • Thermal output at typical power draw of 1,200 W: 4,094 BTU/hr	• Temperature: 20–30°C (68–86°F) • Humidity: 40–80%, noncondensing • Altitude: Up to 2,000 m (6,500 ft) above sea level • Thermal output at typical power draw of 1,200 W: 4,094 BTU/hr (instrument) and 1100 W: 3,752 BTU/hr
Other connections	1 GigE Ethernet; 2 x USB 2.0; RJ45-type connector		
Server storage	~12 TB	~24 TB	~25 TB
Server dimensions (W x D x H) and weight	NA	NA	30.5 x 70.9 x 44.4 cm, 41.8 kg
Software	Alignment and variant calling with Torrent Suite Software; compatibility with laboratory information management systems as well as native integration with Ion Reporter™ Software (cloud and local server)		

Ordering information

Product	Cat. No.
Ion GeneStudio S5 series	
Ion GeneStudio S5 System	A38194
Ion GeneStudio S5 Plus System	A38195
Ion GeneStudio S5 Prime System	A38196
Instruments for sample prep automation	
Ion Chef System	4484177
Ion OneTouch 2 System	4474779

Find out more at **thermofisher.com/genestudio**

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Ion GeneStudio S5 Series Systems

Focused, scalable next-generation sequencing has never been faster

ThermoFisher SCIENTIFIC

Choose faster, more scalable next-generation sequencing

Multi-instrument portfolio in one comprehensive NGS system

Since its inception, next-generation sequencing (NGS) has revolutionized genomic research, enabling accelerated biological discoveries and clinical advancements. Its power and application potential as a research tool continues to evolve, driving demand for faster, more accurate sequencers.

Built on Ion Torrent™ technology, the Ion GeneStudio™ S5 Systems provide the simplest sample-to-data workflow for targeted sequencing with industry-leading speed and scalability, allowing you to spend more time answering critical questions in your research.

- **Ion 550™ Chip**
  100–130M reads
- **Ion 540™ Chip**
  60–80M reads
- **Ion 530™ Chip**
  15–20M reads
- **Ion 520™ Chip**
  4–6M reads
- **Ion 510™ Chip**
  2–3M reads

Five Ion S5™ chip options enable a sequencing throughput range of 2M to 130M reads

Sequencing and analysis in as little as 3 hours with the Ion GeneStudio S5 Prime System

Answer your research questions with less total workflow time and less hands-on time

Sequence fast

Ion GeneStudio S5 System

Turnaround time: 19 hr*

and faster

Ion GeneStudio S5 Plus System

Turnaround time: 10 hr*

and faster

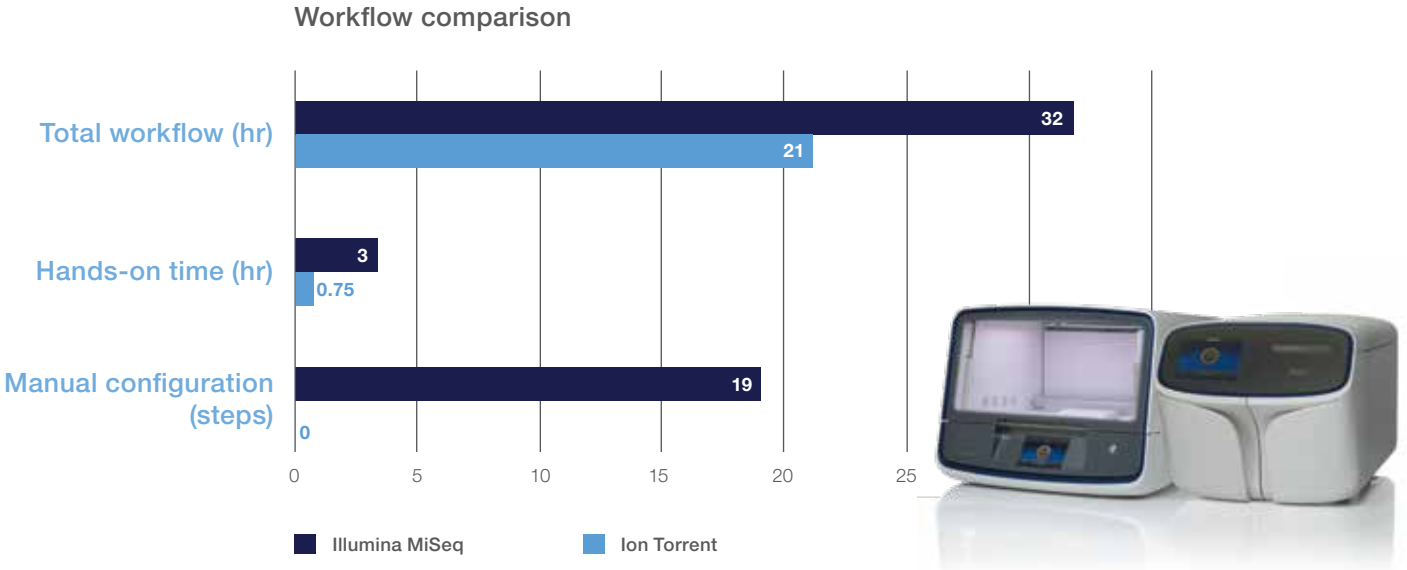
Ion GeneStudio S5 Prime System

Turnaround time: 6.5 hr*

Throughput scalability and application flexibility—all on the same platform. Choose the system configuration that best fits your budget and turnaround time needs.

*Sequencing and analysis times based on Ion 540 Chip.

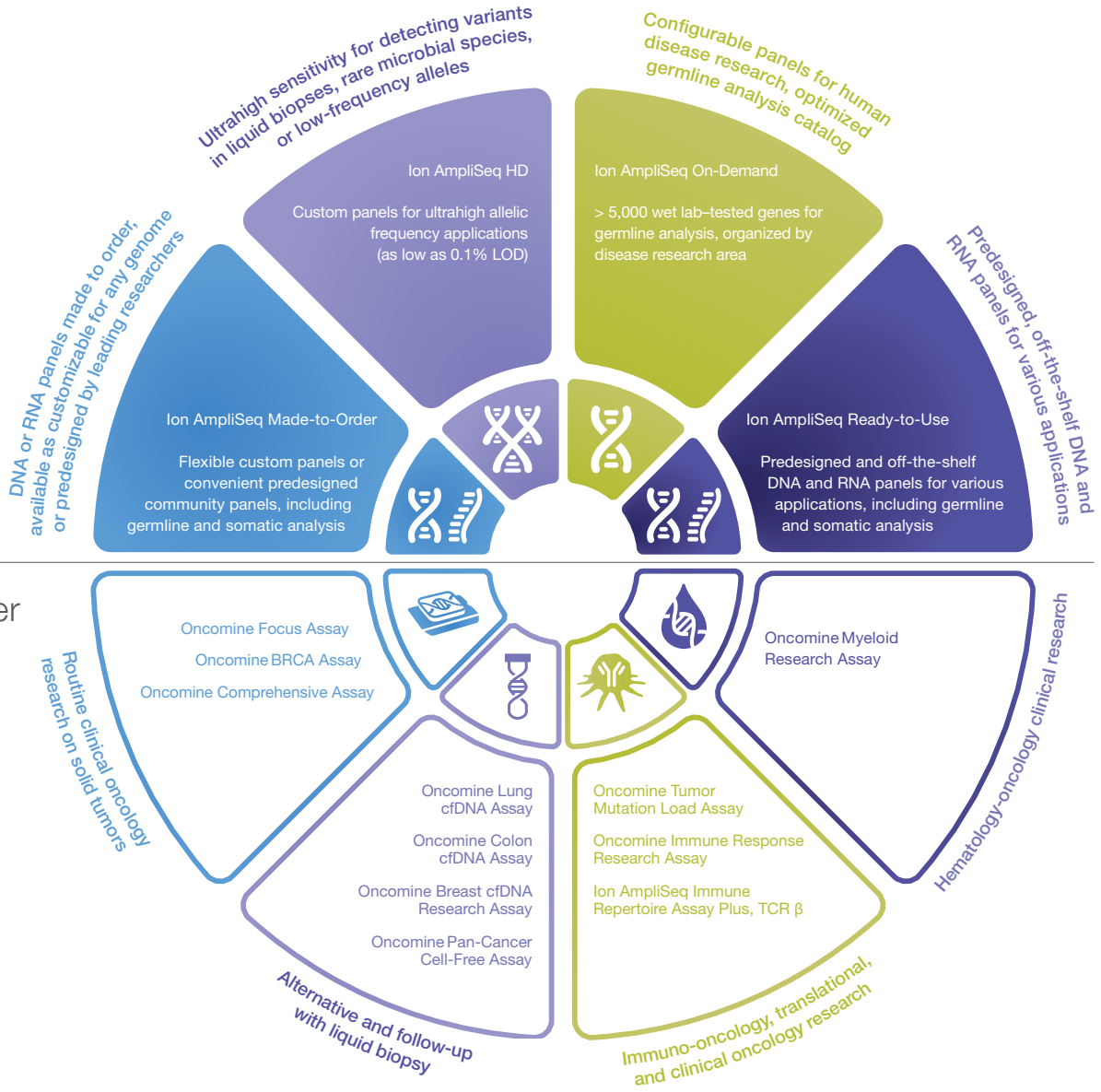
By combining the Ion Chef™ System with the Ion GeneStudio S5 System, collect data up to 30% faster, with 75% less hands-on time than with the Illumina™ MiSeq System.



Choose targeted sequencing

The research content you want

Optimized, off-the-shelf panels and custom designs



The automation you need

Fast workflow, from library to data

1

Library and template prep

30 min of hands-on time for library prep, template prep, and chip loading with the Ion Chef System

2

Sequencing

2.5 hr sequencing runs on Ion GeneStudio S5 instruments with <15 min of setup time

3

Data analysis

Transform data to insight with analysis, QC reporting, and variant calling on Thermo Fisher™ Cloud or an in-house Ion Reporter™ Server

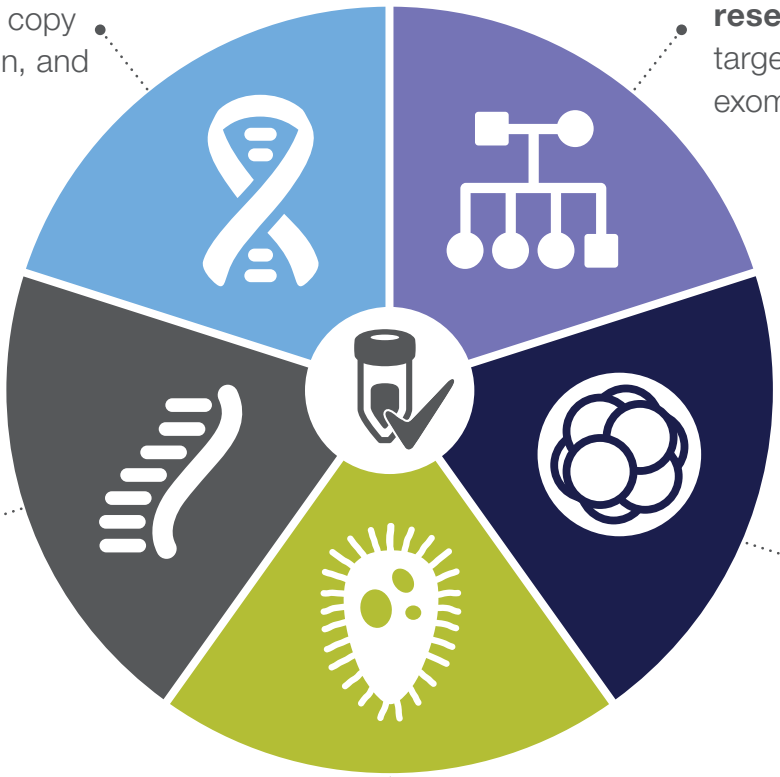
One sequencer—multiple applications

The Ion GeneStudio S5 Series system enables small and large next-generation sequencing (NGS) projects

Designed to optimize throughput and cost, the Ion GeneStudio S5 system uses semiconductor sequencing on chips to enable flexibility in analysis—from gene panels to exomes, gene expression profiling to transcriptomes, or microbial genomes to microbiomes.

Cancer research: gene panels for SNPs, indels, copy number, gene expression, and gene fusion analysis

Inherited disease research: panels for targeted gene or whole exome analysis



Gene expression analysis: whole transcriptome RNA-Seq, targeted RNA sequencing, and small RNA sequencing

Reproductive health research: aneuploidy detection

Microbiology/infectious disease research: microbial whole genomes, microbial genotyping, and metagenomics

> Exome trios are straightforward in a single Ion 550 chip run. We've also tried mouse transcriptome, DNA, and RNA panels. Data quality is excellent, comparable with our Ion 540 chip data. The 550 workflow can support double the samples per chip.

Adam Ameur, bioinformatician
Department of Immunology, Genetics and Pathology
National Genomics Infrastructure (NGI), SciLifeLab Uppsala

System performance

	Ion GeneStudio S5 System	Ion GeneStudio S5 Plus System	Ion GeneStudio S5 Prime System
Maximum throughput/day (chip type)	15 Gb (one Ion 540 chip)	30 Gb (two Ion 540 chips)	50 Gb (two Ion 550 chips)
Total sequencing and analysis time at maximum throughput	19 hr (Ion 540 chip)	10 hr (Ion 540 chip) 11.5 hr (Ion 550 chip)	8.5 hr (Ion 540 chip) 8.5 hr (Ion 550 chip)
Compatible chips	Ion 510, 520, 530, and 540 chips	Ion 510, 520, 530, 540, and 550 chips	

> In my institute, many customers outsource for exome and whole genome studies. They come to me for speed, and flexibility for different gene panels, tailored to disease areas of interest. This is the most elegant workflow on the market today, and I can now offer a range of applications and deliver the data in the fastest turnaround time in the market, at costs that are competitive.

Morten Dunoe, lab manager
Molecular Genetic Laboratory, Department of Clinical Genetics
Rigshospitalet, Copenhagen University Hospital, Denmark

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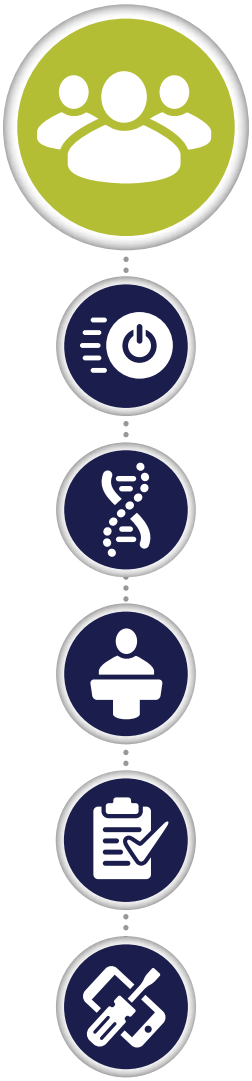

More than 1,300 service and support specialists worldwide partner with you

- **SmartStart orientation**—hands-on training in your laboratory to get you up and running quickly
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- **Training services**—training programs available at our training centers located throughout the world, in your lab, or through web-based instruction
- **Compliance services**—audit-ready documentation managed by a compliance specialist to ensure your instrument is installed and performing to manufacturer's specifications
- **Service plans**—scheduled planned maintenance and guaranteed response times

Ordering information

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Ion GeneStudio S5 Plus System	A38195
Ion GeneStudio S5 Prime System	A38196
Ion Chef Instrument	4484177

Start your sequencing journey at
thermofisher.com/genestudio

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Rapid sequencing of microorganisms with the versatile Ion S5 next-generation sequencing systems

Ion S5™ Systems and infectious disease research

- Targeted sequencing of viruses, bacteria, or fungi from biological materials without culturing
- Robust sequencing of whole bacterial genomes from isolates or mixed cultures
- Low cost of ownership and low cost of sequencing per sample
- Rapid workflows that allow sequencing results from microbial samples in less than 24 hours
- Integrated software for simple run setup and data analysis

Figure 1. The Ion S5 System and infectious disease research applications. Efficient semiconductor-based sequencing provides rapid results at low cost with up to 80 million DNA fragments sequenced in 7.5 hours on the Ion S5 XL System (sequencing and primary data analysis).

Next-generation sequencing technologies have transformed our understanding of the microbial universe. Complete bacterial genomes can now be sequenced at low cost and often without the need to culture the organism. Approaches that isolate a single bacterial cell followed by whole genome amplification and sequencing further expand the database of unknown and unculturable organisms. In addition, targeted approaches based on the 16S rRNA gene or other specific genes or regions of viral, bacterial, and fungal genomes have provided a highly effective strategy to identify organisms without the requirement for host nucleic acid subtraction.

Sequencing of microbes directly from biological samples is expected to have a significant impact on detection rates in the future. Currently, 20% of pneumonial samples and 50–70% of diarrheal, meningitis, and encephalitis samples cannot be associated with a pathogen by conventional microbial analyses. Furthermore, blood cultures are associated with a low detection rate, <25%, for bloodstream infections.

Targeted or whole genome sequencing (WGS) approaches can be employed effectively on the Ion S5 and Ion S5 XL Systems for research that may be potentially used to help trace the origin of outbreaks, to monitor biologics manufacturing processes, ensure food and beverage safety, and for clinical microbiology applications (Figure 1).

The tunable sequencing output of the Ion S5 System, with 3 million to 80 million reads, enables a broad set of microbial research applications and sample throughputs to be addressed effectively (Table 1).

Sequencing workflow

The sequencing workflow consists of four main steps (Figure 2).

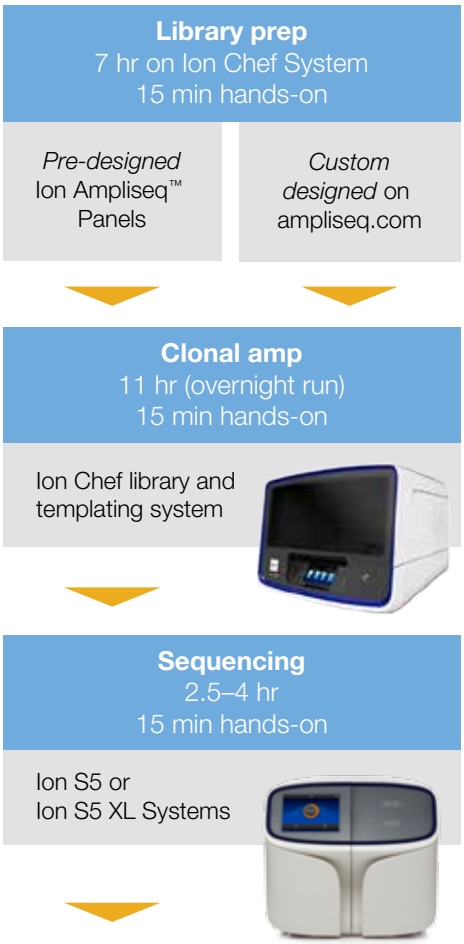


Figure 2. Targeted sequencing workflow. Ion AmpliSeq libraries are prepared manually or with the Ion Chef System. Libraries are then placed in the Ion Chef System for emulsion PCR, enrichment, and loading onto Ion S5 chips. Chips are placed in the Ion S5 System with reagents for sequencing. Primary data analysis is performed with Torrent Suite Software.

Table 1. Number of microbial samples that can be run on Ion S5 chips (barcodes are available for up to 96 samples per run).

		Ion 520™ Chip	Ion 530™ Chip	Ion 540™ Chip
Reads (millions/chip)		3–5	15–20	60–80
Output	200 bp reads	0.6–1 Gb	3–4 Gb	10–15 Gb
	400 bp reads	1.2–2 Gb	6–8 Gb	–
Samples per run				
Ion 16S Metagenomics Kit*		48	192	–
Ion AmpliSeq TB Research Panel†		36	144	384**
Viral whole genome‡		72	288	384**
Bacterial whole genome§		12	48	96††

* Assumes 1,000x coverage of mid-complexity sample.
† Assumes 1,000x coverage.
‡ Assumes 275 kb genome at 100x coverage.
§ Assumes 5 Mb genome at 30x coverage.
** Upon availability of 384 barcodes. The content provided herein may relate to products that have not been officially released and is subject to change without notice.
†† 200 bp reads only.

1. Library preparation

For targeted sequencing:

Genomic DNA (10–100 ng) isolated from the sample is converted to a sequencing library by selective amplification of regions of interest using the Ion AmpliSeq protocol that enables highly multiplexed PCR amplification of up to 20,000 targets in a single tube. Eliminating primer-dimers a fraction of the target-specific Ion AmpliSeq primers are digested away from the amplified product, followed by a ligation step to add adapter sequences to the amplicons. Pre-designed microbial panels are available, such as the Ion AmpliSeq TB Research Panel and the Ion AmpliSeq Ebola Research Panel, or custom panels can be easily developed using the Ion AmpliSeq Designer online tool.

The Ion AmpliSeq library preparation workflow may be performed manually or can be performed automatically for 8 samples per run of 1- or 2-pool panel designs on the Ion Chef™ System. Requiring less than 2 pipetting steps per sample, Ion AmpliSeq library preparation on the Ion Chef System reduces hands-on time, generating pooled libraries ready for downstream template preparation.

For whole genome sequencing:

The Ion Xpress™ Plus Fragment Library Kit is used to produce a whole genome library from genomic DNA. The kit uses an enzyme-based random fragmentation method followed by ligation of adapters and amplification. Alternatively, the Thermo Scientific™ MuSeek™

Library Preparation Kit provides a fast and simple transposon-based method for preparing high-quality genomic DNA libraries. The kit utilizes MuA transposase enzyme for fragmentation and simultaneous tagging of the target DNA, eliminating the need for separate shearing, end repair, and adaptor ligation steps. This enables library construction in as little as 80 minutes with consistent yields from low DNA sample amounts (100 ng).

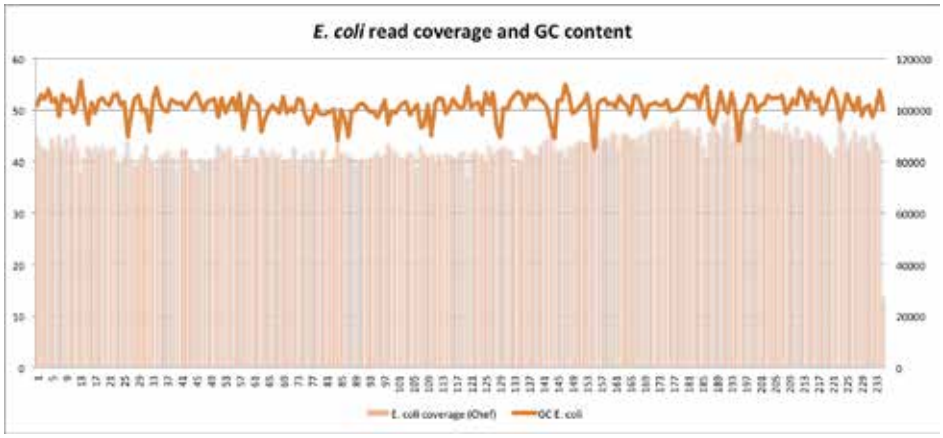
2. Clonal amplification (template preparation)

Libraries that are prepared manually or by automation are then clonally amplified on the Ion Chef System by emulsion PCR of library molecules captured on beads. The Ion Chef System performs all template preparation steps, including creating the emulsion mixture, performing the PCR, carrying out the post-PCR purifications, and finally loading of the purified templated beads onto the Ion S5 chips. The prepared chips are ready for sequencing on the Ion S5 System.

3. Sequencing

A sequencing run on the Ion S5 and Ion S5 XL Systems is initiated by loading a reagent cartridge, buffer, cleaning solution, and waste container. The Ion S5 chip is then loaded and the run is started. The addition of bases by the DNA polymerase results in the production of hydrogen ions, changing the pH which is coverted to sequencing signal through ion-sensitive wells that hold the templated beads. Read lengths of up to 400 bp can be produced with three available semiconductor chips that produce up to 5, 20, or 80 million reads per chip (Table 1).

A



B

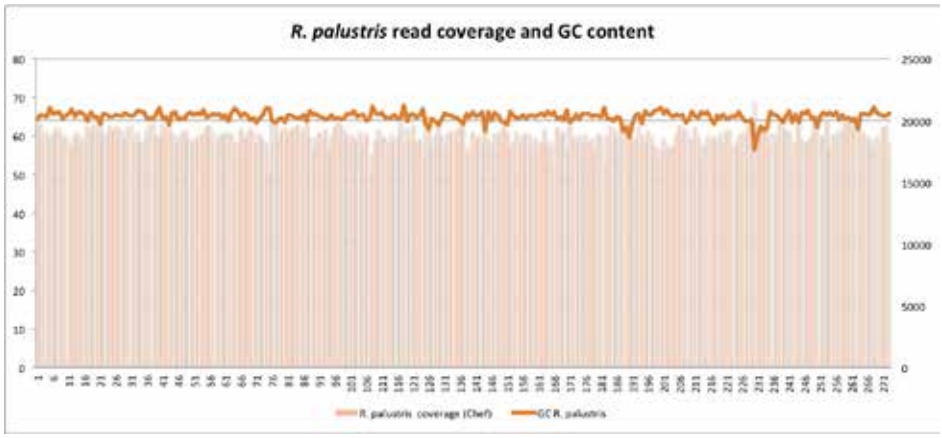


Figure 3. Whole genome sequencing on the Ion S5 System. Fragment libraries were sequenced on the Ion 520 Chip. Coverage plots for libraries prepared with the Ion Chef System (20 Kb window) compared with GC content indicate high levels of coverage uniformity across the genome. **(A)** *E. coli* genome with balanced GC content. **(B)** *R. palustris* genome with high GC content.

4. Data analysis

Torrent Suite Software coordinates all the experiment planning, from run setup to data processing, and primary analysis of microbial sequencing data, with plugins for assembly and microbial-specific analysis. Furthermore, 16S rRNA profiling data can be uploaded to Ion Reporter Software for further analysis with a suite of data analysis and visualization tools.

Results

The flexibility, simple workflows, and cost effectiveness of the Ion S5 Systems enable highly efficient sequencing-based microbial analysis.

Sequencing bacterial whole genomes

For epidemiological research applications such as outbreak investigations, surveillance, and determining disease etiologies, whole genome sequencing provides the highest data resolution.

As an example of rapid WGS, fragment libraries were generated from *Escherichia coli* and *Rhodopseudomonas palustris* with

the Ion Xpress Plus Fragment Kit followed by clonal amplification and chip loading on the Ion Chef System, sequencing on the Ion S5 System, and *de novo* assembly using the SPAdes v3.1 plugin. Data from the rapid whole genome sequencing workflow is shown in Figure 3.

Metagenome sequencing research

16S rRNA profiling is an efficient method for characterizing bacterial populations at low cost and with minimal bioinformatics effort. The Ion 16S™ Metagenomics Kit has been designed to provide comprehensive identification (to >80% of Greengenes sequences) by simultaneously sequencing 7 hypervariable regions in the bacterial 16S rRNA gene. When applied to ground beef samples, the 16S sequencing results showed high-resolution identification of bacterial species. Species diversity was substantially reduced on culture (Figure 4).

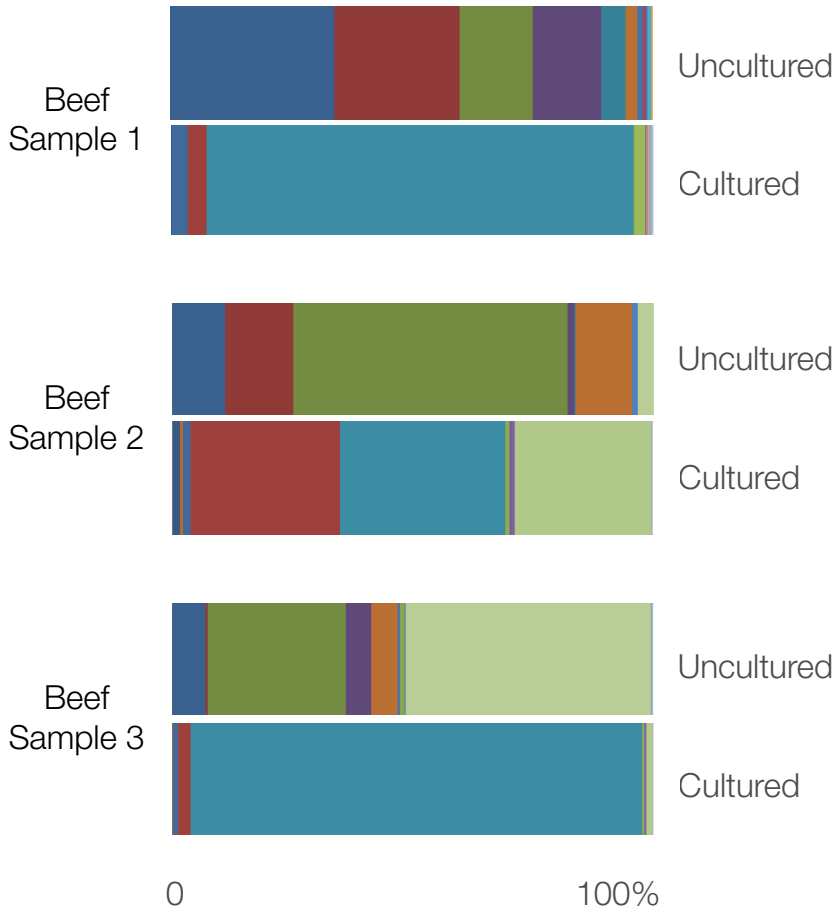


Figure 4. 16S rRNA sequence analysis of ground beef samples. Libraries generated with the Ion 16S Metagenomics Kit were generated from ground beef samples and sequenced on the Ion 530 Chip. Automated analysis, annotation, and taxonomical assignment were generated using Ion Reporter Software. Uncultured ground beef samples show a greater diversity of genera compared to cultured samples, suggesting that culturing introduces bias. Of note, the number and diversity of bacterial genera differ in ground beef from different sources as shown by color..

Sequencing targeted regions of bacteria

Targeted sequencing approaches that query specific genes or regions of interest can be used for species or subtype identification, or for the identification of known variants associated with drug resistance or virulence markers. Sequencing output requirements can be substantially reduced by targeted sequencing, especially for complex samples with a background of large amounts of host nucleic acids (e.g., characterization of microbes directly from blood or sputum samples). Optimized Ion S5 System workflows can also offer rapid data generation capabilities of less than 24 hours for research into culture-free identification. Ion AmpliSeq technology, with its capability to amplify and sequence thousands of amplicons simultaneously, offers a robust approach for microbial characterization such as drug resistance in *Mycobacterium tuberculosis*.

Targeted sequencing of *M. tuberculosis*

The Ion AmpliSeq TB Research Panel was developed to enable rapid genotyping of known variants that may be associated with antibiotic resistance. Eight genes (*embB*, *eis*, *gyrA*, *inhA*, *katG*, *pncA*, *rpoB*, *rpsL*) in *M. tuberculosis* are targeted by 109 amplicons that cover protein coding regions and additional upstream and downstream regions to maximize discovery of known and novel variants. Sequencing of 6 wild type (H37Rv) and 6 rifampicin-resistant (mutation in *rpoB*) strains using the Ion AmpliSeq TB Research Panel on a single Ion 520 Chip detected all mutations associated with resistance (Table 2).

Detection of virions directly from CSF

To demonstrate the utility of Ion S5 sequencing for the detection of virions directly from biological samples, experiments were performed by sequencing bulk nucleic acids from CSF on the Ion 540 Chip. Reads that mapped to the human genome were bioinformatically filtered out and remaining reads were analyzed for viral sequences (Table 3).

Table 2. Variants identified from DNA isolated from archived pure *M. tuberculosis* cultures. WT indicates wild type. A red circle indicates presence of mutation S531L (TCG/TTG) 761155.

	WT1..6	*R1*	*R2*	*R3*	*R4*	*R5*	*R6*
embB							
eis							
gyrA							
inhA							
katG							
pncA							
rpoB		●	●	●	●	●	●
rpsL							

Table 3. Detection of virions in CSF.

Sample	Spiked-in virus	Viral reads	Total reads
1	La Crosse virus	311,259	39,320,183
2	Human adenovirus C	36,649	44,845,792

ion torrent

Ordering information

Product	Description	Quantity	Cat. No.
Microbial targeted sequencing panels			
Ion 16S Metagenomics Kit	Two primer pools to amplify seven hypervariable regions (V2, V3, V4, V6, V7, V8, and V9) of bacterial 16S rRNA	100 reactions	A26216
Ion AmpliSeq TB Research Panel	Targets genes associated with antimicrobial resistance in *M. tuberculosis* (TB). The research panel assesses 109 amplicons (two pools) from 8 genes (*embB*, *eis*, *gyrA*, *inhA*, *katG*, *pncA*, *rpoB*, *rpsL*). The panel is designed to be used on cultured sputum extract.	Custom order	ampliseq.com
Ion AmpliSeq Ebola Research Panel	Targets genes associated with the of Ebola virus. The panel assesses 145 amplicons across the Ebola virus genome.	Custom order	ampliseq.com
PathAmp FluA Reagents	A set of highly specific, universal influenza primers combined with a high-fidelity master mix for the amplification of all eight influenza A genomic segments in a single tube	50 preps	4485019
Ion AmpliSeq library preparation			
Ion AmpliSeq Library Kit 2.0 Manual	Ion AmpliSeq library preparation	8 reactions	4475345
		96 reactions	4480441
		384 reactions	4480442
Ion Xpress Barcode Adapters 1-96 Kit	96 unique barcode adapters	1 kit	4474517
Ion Library Equalizer Kit	Bead-based solution replacing the need for library quantification and library dilutions for library normalization	96 reactions	4482298
Automated library preparation			
Ion AmpliSeq Kit for Chef DL8	Automated Ion AmpliSeq library preparation supplied with IonCode barcodes	4 x 8 reactions	A29024
Microbial whole genome sequencing			
Ion Xpress Plus Fragment Library Kit	Enzymatic fragment library construction method	10 reactions	4471269
Ion TrueMate™ Plus Library Kit	Generation of mate-pair libraries (2–8 kb inserts) from any genomic DNA	10 reactions	A25656
Template preparation			
Ion Chef System	Automates template preparation and chip loading	1 system	4484177
Ion 520/530 Kit-Chef	Template preparation for Ion 520 and Ion 530 Chips on the Ion Chef System	8 reactions	A27757
Ion 540 Kit-Chef	Template preparation for Ion 540 Chips on the Ion Chef System	8 reactions	A27759
Sequencing			
Ion S5 System	Ion S5 next-generation sequencing system	1 system	A27212
Ion S5 XL System	Ion S5 XL next-generation sequencing system	1 system	A27214
Ion 520 Chip Kit	Sequencing reagents including an Ion 520 Chip	8 reactions	A27762
Ion 530 Chip Kit	Sequencing reagents including an Ion 530 Chip	8 reactions	A27764
Ion 540 Chip Kit	Sequencing reagents including an Ion 540 Chip	8 reactions	A27766
Sequencing			
Ion Reporter Software	16S Metagenomics Analysis		thermofisher.com/ionreporter

Find out more at **thermofisher.com/ioninfectiousdisease**

ThermoFisher SCIENTIFIC

ThinkPad L15 Gen 3 (Intel) 21C3007CMH

PSREF
Product Specifications Reference

PERFORMANCE

Processor
Intel® Core™ i7-1255U, 10C (2P + 8E) / 12T, P-core 1.7 / 4.7GHz, E-core 1.2 / 3.5GHz, 12MB

Graphics
Integrated Intel Iris® Xe Graphics Functions as UHD Graphics

Chipset
Intel SoC Platform

Memory
1x 16GB SO-DIMM DDR4-3200

Memory Slots
Two DDR4 SO-DIMM slots, dual-channel capable

Max Memory
Up to 64GB DDR4-3200

Storage
512GB SSD M.2 2242 PCIe® 4.0x4 NVMe® Opal 2.0

Storage Support
One drive, up to 1TB M.2 2242 SSD

Storage Slot
One M.2 2242 PCIe 4.0 x4 slot

Card Reader
MicroSD Card Reader

Audio Chip
High Definition (HD) Audio, Realtek® ALC3287 codec

Speakers
Stereo speakers, 2W x2, Dolby® Audio™

Camera
FHD 1080p + IR Hybrid with Privacy Shutter

Microphone
2x, Array

Battery
Integrated 57Wh

Power Adapter
65W USB-C® (3-pin)

DESIGN

Display
15.6" FHD (1920x1080) IPS 250nits Anti-glare

Keyboard
Backlit, English (EU)

Case Color
Thunder Black

Case Material
PC-ABS (Top), PC-ABS (Bottom)

Dimensions (WxDxH)
360.2 x 237 x 19.93 mm (14.18 x 9.33 x 0.78 inches)

Weight
Starting at 1.76 kg (3.87 lbs)

SOFTWARE

Operating System
Windows® 11 Pro, WE (EN/FR/DE/NL/IT)

CONNECTIVITY

Ethernet
100/1000M (RJ-45)

WLAN + Bluetooth®
Intel Wi-Fi® 6 AX201, 11ax 2x2 + BT5.1

WWAN
WWAN Upgradable to 4G

Standard Ports
- 1x Ethernet (RJ-45)
- 1x USB 3.2 Gen 1
- 1x Thunderbolt™ 4 / USB4® 40Gbps (support data transfer, Power Delivery 3.0 and DisplayPort™ 1.4)
- 1x Headphone / microphone combo jack (3.5mm)
- 1x HDMI®, up to 4K/60Hz
- 1x USB-C 3.2 Gen 1 (support data transfer, Power Delivery 3.0 and DisplayPort 1.4)
- 1x USB 3.2 Gen 1 (Always On)
- 1x microSD card reader

Optional Ports (configured)
- 1x Nano-SIM Card Slot
- 1x Smart Card Reader

Docking
Various docking solutions supported via Thunderbolt or USB-C.
For more compatible docking solutions, please visit Docking for ThinkPad® L15 Gen 3 (Intel)

SECURITY & PRIVACY

Smart Card Reader
Smart Card Reader

Security Chip
Discrete TPM 2.0

Fingerprint Reader
Touch Style, Match-on-Chip, Integrated in Power Button

Physical Locks
Kensington® Nano Security Slot™, 2.5 x 6 mm

Other Security
- IR camera for Windows® Hello
- Glance by Mirametrix®
- Tile ready
- Camera privacy shutter

MANAGEABILITY

System Management
Non-vPro®

SERVICE

Base Warranty
1-year, Courier or Carry-in

Included Upgrade
CO2 Offset 1 ton (5WS0Z74929), 1Y Premier TP Main WHB (CPN) (5WS1B09491)

CERTIFICATIONS

Green Certifications
- EPEAT™ Gold
- TCO Certified
- ENERGY STAR® 8.0
- RoHS compliant
- ErP Lot 3

Mil-Spec Test
MIL-STD-810H military test passed

MODEL

TopSeller	: Yes	Announce Date	: 2022-05-24
EAN / UPC / JAN	: 0196801509320	End of Support	: N/A

· Lenovo reserves the right to change specifications or other product information without notice.
· Product image on this page is a sample, it may not match the real color of this model
· Battery life is an estimated maximum. Actual battery life may vary based on many factors, including screen brightness, active applications, features, power management settings, battery age and conditioning, and other customer preferences

Premier Support - **A direct 24x7x365 line to our most elite technicians.**

- Advanced technical support, 24x7x365, in more than 100 markets
- Technical Account Managers for proactive relationship and escalation management
- Comprehensive hardware & OEM software support
- Priority on service delivery and repair parts
- Single point of contact for simplified end-to-end case management
- Lenovo Service Connect Portal for customized reporting and product support

Accidental Damage Protection - **Protection of your PC from the unexpected.**

Experience shows that a significant portion of devices may be damaged (and not covered under warranty) in the first three years. Lenovo's Accidental Damage Protection service covers accidents beyond the system warranty such as drops, spills, bumps and even electrical surges. Make sure your new device has maximum protection, avoid unplanned costs and get peace of mind.

Base Warranty - **Included with the systems you purchase, default 1- or 3-year coverage window.**

Warranty Extensions & Upgrades - **Service and protection that's right for your circumstances.**

- Pick Up & Return Courier
- Onsite Support
- Technician Installed Customer
- Replaceable Unit Service
- International Warranty Upgrade

Recommended Accessories

ThinkPad USB-C Dock Gen 2

40AS0090US

- Lenovo understands that modern business depends on a wide variety of PC's and even brands to meet their business needs. To make this easier Lenovo USB-C dock is compatible with Lenovo notebooks as well as note...

Recommended Services

BEST	3Y Premier Support Plus upgrade from 1Y Premier Support (5WS1L39505)
BETTER	3Y Lenovo Support (Premier Support + KYD + 3Y SBTY + International Upg) (5PS1J31171)
GOOD	3Y Premier Support upgrade from 1Y Premier Support (5WS1B38518)

For a full list of accessories and services, please visit:
https://smartfind.Lenovo.com

Lenovo **PSREF**
Product Specifications Reference

ion torrent
life technologies

Ion Chef™ System

Publication Number MAN0007956 **Revision** E.0

This guide contains the information needed to prepare your site for installation of the Ion Chef™ System (4484177).

Site preparation workflow

A Life Technologies representative will contact you to schedule the installation. When the installation is scheduled:

1. Receive and inspect the shipment (see "Receive and inspect the shipment" on page 14).
2. Move the crated instrument to the installation site (see "Move the crated instrument to the installation site" on page 14).
3. Complete the site preparation checklist (see "Site preparation checklist" on page 2).
4. Ensure:
   - The site preparation checklist is complete.
   - The purchase order for the installation and training materials is complete.

Installation timeline and training

After the Ion Chef™ System is uncrated, installation and testing of the Ion Chef™ System takes 4-6 hrs.

During and/or after installation, the service representative reviews data and provides some basic operator training. For additional training and reference information, see the user documents provided with the Ion Chef™ System.

life technologies

Site preparation checklist

IMPORTANT! Complete all activities below before the scheduled installation date. If the activities are not complete when the service representative arrives, the scheduled installation may be postponed.

- Review the customer responsibilities and assign personnel (see "Customer responsibilities" on page 3).
- Identify an installation site that meets all requirements in this guide:
  - Space and clearance (see "Instrument clearances" on page 5).
  - Environmental (see "Electrical requirements for the Ion Chef™ System" on page 10).
  - Ventilation and waste collection (see "Ventilation and waste collection requirements" on page 9).
  - Electrical (see "Electrical requirements" on page 10).
  - Network (see "Network configuration" on page 11).
  - Safety (see "Safety requirements" on page 12).
- Confirm that all materials needed for installation and operation are available (see "Materials for installation and training" on page 13).
- Confirm that the product was received and inspected (see"Receive and inspect the shipment" on page 14):
  - All items on the shipping list are the same items ordered at the time of purchase.
  - Any damage to shipping containers was reported to field service.
  - Any damage or mishandling was recorded on the shipping documents.
  - The reagents box was unpacked and stored as specified.
- Confirm the installation site is cleared and ready for instrument installation ("Move the crated instrument to the installation site" on page 14).
- Confirm the crated instrument and other shipping containers have been moved to the installation site (see "Move the crated instrument to the installation site" on page 14).

Customer responsibilities

Personnel	Responsibilities
Site preparation/ installation coordinator	• Reviews the site preparation guide for safety information and instrument requirements. • Coordinates personnel and tasks. • Chooses the site. • Reviews checklists with applicable personnel, then with the service representative to verify that the site is properly prepared. • Receives and inspects the Ion Chef™ System. • Stores the reagents box according to the specifications indicated in the product inserts. • Schedules the installation and informs personnel of the installation day. • Ensures that the site is clear of unnecessary material on the installation day. • Is available to assist the service representative throughout installation.
Laboratory safety representative	• Reviews the site preparation guide for safety information. • Ensures that the required safety practices and equipment are in place. • Is in the vicinity and available to the service representative at all times while the service representative is at the customer's facility.
Laboratory personnel/ primary users	• Review safety information. • Ensures that all customer-provided materials for installation are present at the site. • Ensures that primary users (responsible for training other users) are available during the installation, so that they can be trained on the instrument.
Facilities personnel	• Ensures that the installation requirements are met for: – Space at the installation site – Building clearances – Temperature and humidity – Waste collection – Electrical supply – Computer – Safety and installation materials • If possible, moves the crated Ion Chef™ System to the site before the installation date. • Is available to assist service representative and laboratory personnel throughout installation. • If applicable, ensures that at least two people are available to help the service representative move and position the instrument.

Personnel	Responsibilities
Network or IT specialist (if the instrument will be connected to a network)	• Ensures that active, tested local area network (LAN) connections are in place before the scheduled installation date. • Ensures that network hardware is compatible with an RJ45-type connector. • If necessary, supplies additional cables. • Is available during installation to connect the Ion Chef™ System to the network. • If applicable, provides and installs a network or dedicated printer. ⚠ **CAUTION!** Do not attempt to connect the Ion Chef™ System components to the network before the service representative arrives.

Site requirements

Dimensions and weights

To prepare for installation, provide space for receipt and configuration of the following components. This section provides dimensions and weights for the crates and packages you will receive, and it describes the dimensions of the Ion Chef™ System after it has been installed and configured.

IMPORTANT! We do not install, service, or repair instruments in areas designated BioSafety Level 3 (BSL-3) or BioSafety Level 4 (BSL-4).

Crate dimensions and weight

Crate	Height	Length (depth)	Width	Weight
Ion Chef™ System	71.1 cm (28 in)	86.4 cm (34 in)	86.4 cm (34 in)	134.0 kg (295.0 lbs)

Components dimensions and weights

Component	Height		Length (depth)	Width	Weight
	Open	Closed			
Ion Chef™ System	83.9 cm (33.0 in)	56.1 cm (22.1 in)	70.0 cm (27.6 in)	71.4 cm (28.1 in)	68.2 kg (150 lbs)

Instrument clearances

During instrument setup and maintenance, it is necessary to access the back and sides of the instrument. If the back of the instrument faces a wall, it will be necessary to have enough space to rotate the instrument on the bench for access.

IMPORTANT! For safety, the power outlet used for powering the instrument must be accessible at all times.

Component	Top	Front	Left	Right	Back
Ion Chef™ System	35.6 cm (14 in)	17.0 cm (6.7 in)	10.0 cm (4.0 in)	10.0 cm (4.0 in)	10.0 cm (4.0 in)

Placement of the instruments and server

If you will connect the Ion Chef™ System directly to the Torrent Server, the installation room must accommodate both the Ion Chef™ System, Ion Sequencer, and Torrent Server. If the components will be placed on a stationary or mobile bench, verify that the bench meets the requirements in "Dimensions and weights" on page 4.

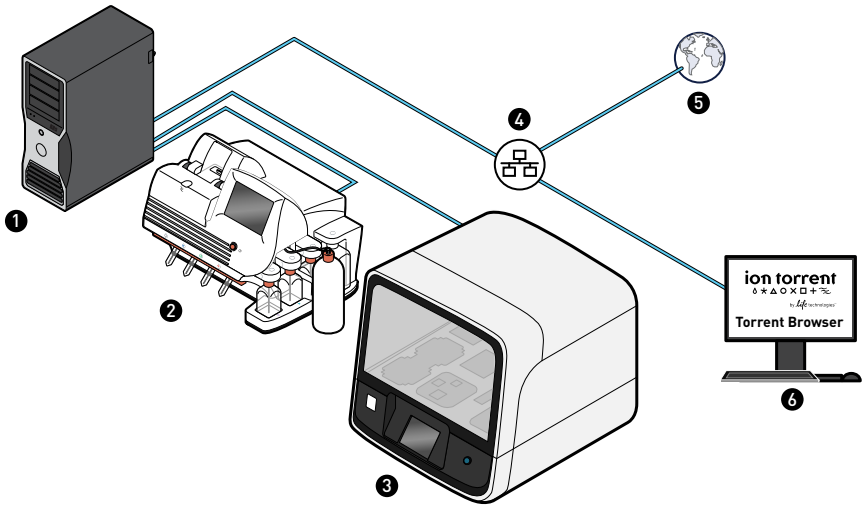
IMPORTANT! The Ion Chef™ System and Ion Sequencer:

- Must be installed on level surfaces.
- Should be located in close proximity for workflow convenience.

Note: Life Technologies is not responsible for any damage caused by using a laboratory bench that does not meet the minimum weight capacities requirements.

System components

The Ion Chef™ System must be connected to the Torrent Server either directly using a category 6 Ethernet cable, or indirectly through a local area network that has been configured to permit HTTP-443, SSH-22, and FTP-20/21 traffic. Unlike the Ion Sequencer, the Ion Chef™ System does not require a robust network connection to the Torrent Server and can accommodate standard network connection speeds.



① Torrent Server
② Ion Sequencer
③ Ion Chef™ System
④ Local area network
⑤ Internet
⑥ Client computer

Planning the installation

In preparation for the Ion Chef™ System installation, you must plan the layout of your laboratory to accommodate library and template preparation activities, in addition to those related to chip preparation and sequencing. This section will familiarize you with the stations involved in the Ion Chef™ System workflow and help you understand the basic laboratory layouts.

Laboratory layout

When designing your PCR laboratory layout, follow good laboratory practices to ensure reliable and contamination-free PCR results. Pay particular attention to the need to separate the areas for pre- and post-PCR activities. Isolating the amplicon source, separating pre-PCR from post-PCR activities, and dedicating laboratory supplies and/or equipment to each space can significantly reduce the potential for contamination.

As shown in the illustrations below, the Ion Sequencer and Ion Chef™ Instrument can be deployed in both one- and two-room laboratory configurations. The two-room layout is highly recommended due to the protection that it affords against contamination; however, the sub-optimal one-room layout will produce acceptable results if proper precautions are observed.

Note: The positions of the stations in the pre-and post- PCR rooms are not important.

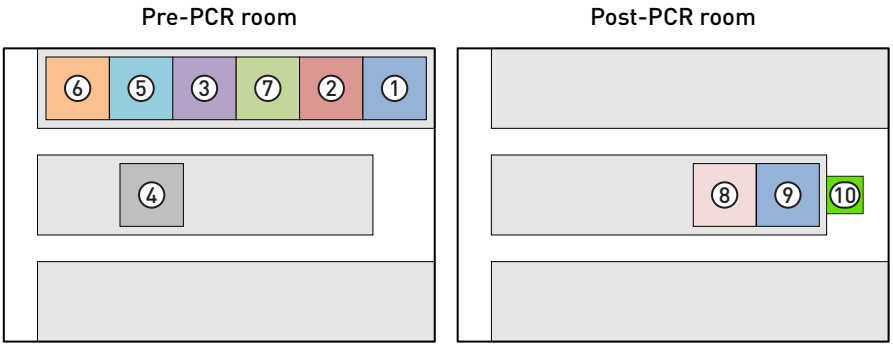


Figure 1 Two-room layout

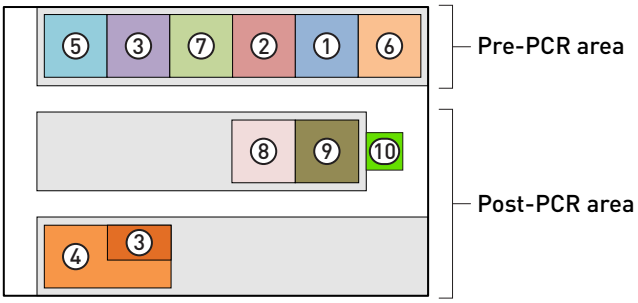


Figure 2 One-room layout

① Agilent® 2100 Bioanalyzer® instrument
② (*Optional*) Bioruptor® system
③ Centrifuge
④ Amplification mixture setup hood
⑤ Library area setup hood
⑥ Pipettes
⑦ (*Optional*) Pippen Prep™ instrument
⑧ Ion Chef™ System
⑨ Ion Sequencer and Torrent Server
⑩ Gas cylinder

Laboratory workstations

The following table describes the workstations associated with the generic laboratory layout for the Ion Sequencer and Ion Chef™ Instrument. The stations are categorized in terms of their involvement pre- and post-PCR activities.

#	Station	Location	Description/requirements
1	Agilent® 2100 Bioanalyzer® Instrument	Pre-PCR area	The Agilent® 2100 Bioanalyzer® Instrument is used to perform library construction quality control, providing yield and size characterization. When planning the instrument placement, consider isolating the Agilent® 2100 Bioanalyzer® Instrument from the sequencer, preferably in a room dedicated for library preparation, to minimize contamination. **Note:** If necessary, a qPCR instrument can be substituted for the Agilent® 2100 Bioanalyzer® Instrument. In general, qPCR does not yield size distribution but does provide more accurate counts.
2	(Optional) Bioruptor® System	Pre-PCR area	If required, the Bioruptor® Instrument performs acoustic shearing/ fragmentation of DNA samples for library preparation. DNA shearing can be accomplished through the use of a Covaris® Focused-Ultrasonicator or Shear™ DNA fragmentation (part of the Ion Xpress™ Library Kit), both of which are acceptable substitutes for the Bioruptor® Instrument. Mechanical shearing is recommended (using Bioruptor® or Covaris® instruments), but enzymatic shearing is acceptable and can be less expensive. When planning Bioruptor® or Covaris® instrument placement: • Consider placing the Bioruptor® or Covaris® instrument in an isolated room to minimize the noise hazard. • Consider isolating the instrument from the sequencer, preferably in a dedicated room for library preparation, to minimize contamination.
3	Centrifuge	Pre-PCR areas	The centrifuges is used during library preparation. When planning centrifuge placement, place it in a convenient location.
4	Amplification mixture setup hood	Pre-PCR area	Whenever possible, amplification mixture preparation should be performed within a dedicated hood. When selecting a location for amplification mixture preparation: • The amplification mixture setup station requires a set of dedicated pipettes. • If only one hood is available within the pre-PCR area, dedicate the hood for amplification mixture preparation and perform library preparation elsewhere. • If a hood is unavailable within the pre-PCR area, select a bench that is sterilized regularly and preferably isolated from the neighboring stations. **IMPORTANT!** Separation of the amplification mixture preparation area from the other stations is critical to preventing contamination. **IMPORTANT!** If possible, physically separate the library and amplification mixture setup areas.

#	Station	Location	Description/requirements
5	Library area setup hood	Pre-PCR area	Whenever possible, library preparation should be performed within a dedicated hood. The use of a dedicated hood allows for UV treatment of the library preparation area and minimizes contamination from post-PCR material containing adaptors. When selecting a location for library preparation: • In a one-room laboratory layout, the use of a dedicated hood for library preparation is highly recommended, but not required. • The library area setup station requires a set of dedicated pipettes. • If a dedicated hood is unavailable, select a bench that is preferably isolated from the neighboring stations.
6	Pipettes	Pre-PCR areas	Both the pre- and post-PCR areas require a complete set of dedicated pipettes. If necessary, the pipettes can be shared between neighboring stations, except for the amplification mixture and library setup stations, both of which require dedicated sets of pipettes.
7	(Optional) Pippin Prep™ Instrument	Pre-PCR area	If required, the Pippin Prep™ Instrument is used to perform automated size selection prior to template preparation. Use of the Pippin Prep™ Instrument is necessary only if size selection is required for your sample type. **Note:** Size selection can also be accomplished through the use of the Invitrogen E-Gel® Agarose Gel Electrophoresis and Documentation System, an acceptable substitute for the Pippin Prep™ Instrument.
8	Ion Chef™ Instrument	Post-PCR area	When planning the placement of the Ion Chef™ Instrument, confirm that the location meets all clearance and environmental requirements described in this document. **Note:** If possible, install the Ion Sequencer and Ion Chef™ Instrument to a dedicated power outlet.
9	Ion Sequencer and Torrent Server	Post-PCR area	When planning the placement of the Ion Sequencer and Torrent Server, confirm that the location meets all clearance and environmental requirements described in the *Site Preparation Guide* for your Ion Sequencer. The sequencer requires a constant supply of nitrogen gas, typically provided by a gas cylinder located within 3m (10ft) of the instrument. **IMPORTANT!** The gas cylinder must be chained to a wall or bench. **IMPORTANT!** The Ion Sequencer is sensitive to both electrical noise and temperature changes. **Note:** Because the Torrent Server requires a direct connection to the Ion Sequencer via a category 6 Ethernet cable, the server is typically installed to the bench directly beneath the instrument.

Environmental requirements

Ensure that the installation room is maintained under correct environmental conditions. Avoid placing the sequencer or server adjacent to heaters, cooling ducts, or in direct sunlight. Place the sequencer at least a meter away from major sources of electronic noise such as refrigerators or microwaves. Fluctuations between day and night temperatures can cause system instability.

Component	Acceptable range
Altitude	Located between sea level and 6500 ft (2000 m) above sea level
Humidity	Relative humidity 40-60%, noncondensing
Operating Temperature	20-25°C (68-77°F)
Vibration	Install the Ion Chef™ System on benches that are free of vibration and have no contact with equipment that causes vibration (freezers, pumps, and similar equipment). Significant vibration during sequencing can add noise and reduce the quality of the sequencing measurements.
Pollution	The Ion Chef™ System has a Pollution Degree rating of II (2). It can be installed in an environment that has nonconductive pollutants such as dust particles or wood chips. Typical environments with a Pollution Degree II (2) rating are laboratories and sales and commercial areas.
Overvoltage category	The Ion Chef™ System has an installation (overvoltage) category of II (2), and is classified as portable equipment.
Other conditions	For indoor use only. The Ion Chef™ System is away from any vents that could expel particulate material on the system components.

Ventilation and waste collection requirements

WARNING! The instrumentation must be installed and operated in a well-ventilated environment as defined as having a minimum airflow of 6–10 air changes per hour. Contact your environmental health and safety coordinator to confirm that all instruments are installed and operated in an environment with sufficient ventilation.

Ventilation requirements

Allow at least 10 cm (4 in.) of clearance around the Ion Chef™ Instrument for ventilation.

Cleaning or decontamination

Refer to the user documentation for your Ion Chef™ System for information on how to clean or decontaminate the instrument.

Wear appropriate protection, including gloves, laboratory goggles, and coat whenever you work with the fluids used on this instrument, or parts that may come into contact with these fluids.

Use only the cleaning agents as described in the user documentation for your Ion Chef™ System. Use of cleaning agents not described in this manual can impair the instrument. Contact Technical Support if you have questions.

Wipe off any liquid on or around the instrument using a lint-free tissue.

Disposing of waste

WARNING! CHEMICAL HAZARD. Refer to Safety Data Sheets (SDSs) and local regulations for handling and disposing of plastic consumables. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of plastic consumables.

WARNING! CHEMICAL HAZARD. Before handling chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. Waste produced by instruments can be hazardous and can cause injury, illness, or death.

Electrical requirements

CAUTION! Do not unpack or plug in any components until the Field Service Engineers (FSEs) have configured the system for the proper operating voltage.

WARNING! For safety, the power outlet used for powering the instrument must be accessible at all times. See "Instrument clearances" on page 5 for information about the space needed between the wall and the instrument. In case of emergency, you must be able to immediately disconnect the main power supply to all the equipment. Allow adequate space between the wall and the equipment so that the power cords can be disconnected in case of emergency.

- Electric receptacle required: 2-prong with ground pin
- Mains AC line voltage tolerances must not be greater than ± 10% percent of nominal voltage

Device	Rated voltage[1]	Rated frequency	Rated current[2]
Ion Chef™ System	100-240 VAC	50/60 Hz	12A

[1] If the supplied power fluctuates beyond the rated voltage, a power line regulator may be required. High or low voltages can adversely affect the electronic components of the instrument.

[2] Based on rated current at minimum input voltage.

Electrical requirements for the Ion Chef™ System

- Facility to supply via building code compliant 15A circuit.
- Use an approved UL Listed detachable power supply cord, as supplied, to connect the system to the wall.

Network requirements

The instrument is factory-configured for IPv4 TCP/IP communication and includes a fast Ethernet adapter (10/100 Mbps) with a RJ45-type connector for integrating the device into a local area network (LAN). If the instrument will be connected to a LAN, an active, tested network jack must be in place before the scheduled installation date. Also, a representative from your information technologies department must be available during the installation to help connect the instrument to your network.

Network configuration

If you plan to connect the Ion Chef™ Instrument to your network, then the following requirements must be met before the installation can take place. Discuss any discrepancies with your concierge representative prior to the visit.

- If necessary, an information technologies resource must be available to assist with the network connection on the date of the Ion Chef™ Instrument installation.
- The room in which the Ion Chef™ Instrument will be installed must contain at least one active network jack.
- If the Ion Chef™ Instrument will be located more than 10 feet from the network jack, you must provide a standard Category 6 Ethernet cable of sufficient length.
- If dynamic network configuration (DCHP) is not available or will not be used, a static IP address must be reserved for the Ion Chef™ Instrument.
- The onsite domain name system (DNS) server must be configured to resolve the URL of the Torrent Server to be accessed by the Ion Chef™ Instrument.
- The Ion Chef™ Instrument can see only one Torrent Server. Planned Run sharing will need to be enabled to see multiple Torrent Servers connected to a LAN. Refer to the *Ion Chef™ and Torrent Server Network Setup User Guide* (Pub. no. MAN0013444) for information on enabling Planned Run sharing.
- If your network employs a firewall that restricts traffic between devices, it must be configured to permit HTTPS-443, SSH-22, and FTP-20/21 communication between the Ion Chef™ Instrument and the Torrent Server.
- The Ion Chef™ Instrument requires outbound internet access through HTTPS-443 and SSH-22 to allow remote support via the Axeda Remote System Monitoring (RSM) Agent. The instrument includes the Axeda Agent to assist you in maintaining your Ion Chef™ Instrument and to provide timely technical support (for more information, see **http://www.axeda.com/community/customers/applied-biosystems**).

  At minimum, whitelist the following outbound addresses for the instrument:
  - `drm.appliedbiosystems.com` on HTTPS-443
  - `rssh.iontorrent.net` on SSH-22

  IMPORTANT! Without access through HTTPS-443 and SSH-22, we cannot support your Ion Chef™ Instrument in a timely fashion and we may forego remote support for your site altogether at our discretion.

  Note: The Axeda Agent is already in use on many of our products to perform instrument diagnostics, preventive maintenance, failure prediction, and proactive notification. The agent does not collect any sequencing reports, results, or data. For more information on the Axeda Agent, see: **http://lifetech-it.hosted.jivesoftware.com/message/1546#1546**
- If the Ion Chef™ Instrument will use Network Time Protocol (NTP) for date/time synchronization, the instrument must have outbound Internet access through UDP-123 to the server pool at `pool.ntp.org`.

Safety requirements

Safety practices

A safety representative from your facility must ensure that:

- Personnel establish and follow all applicable safety practices and policies to protect laboratory personnel from potential hazards.
- All applicable safety devices and equipment are available at all times.

Required safety equipment

Your laboratory has specific safety practices and policies designed to protect laboratory personnel from potential hazards that are present. Follow all applicable safety-related procedures at all times.

The following safety equipment and protection from hazards must be available at the installation site:

- Protection from any sources of hazardous chemicals, radiation (for example, lasers, radioisotopes, radioactive wastes, and contaminated equipment), and potentially infectious biological material that may be present in the area where the service representative will work.
- Appropriate fire extinguisher:
  - You are responsible for providing an appropriate fire extinguisher for use on or near the equipment.
  - The types and sizes of fire extinguishers shall be suitable for use on electrical and chemical fires as specified in current codes, regulations, and/or standards, and with approval of the Fire Marshall or other authority having jurisdiction.
  - The installation of appropriate fire extinguishers shall be in addition to other fire-protection systems and not as a substitute or alternative to them.
- Eyewash
- Safety shower
- Eye and hand protection
- Adequate ventilation, including vent line/fume hood, if applicable
- Biohazard waste container, if applicable
- First-aid equipment
- Spill cleanup equipment
- Applicable Safety Data Sheets (SDSs)

Materials for installation and training

IMPORTANT! The materials for template and library preparation are not required for the Ion Chef™ System installation and training.

Template and library preparation

For a complete list of materials and equipment required for template and library preparation, see the associated documentation in the following table.

For information on...	Refer to the...
Ion Fragment Library preparation	*Ion Xpress™ Plus gDNA Library Preparation User Guide* (Pub no. 4471989)
Library quantitation	*Ion Library Quantitation Kit User Guide* (Pub no. 4468986)

Note: The documents listed in the table above and similar resources are available for download from the Ion Community website (**http://ioncommunity.lifetechnologies.com/community/protocols-home**).

Materials required for installation

Description [1]	Supplier [2]	Catalog no.	Quantity
Ion Chef™ Install Supplies [3]	Life Technologies	4488374	1
Uninterruptible Power Supply[4]	MLS	—	1
Isopropyl Alcohol (IPA) Wipes	MLS	—	Varies

[1] Life Technologies has validated this protocol using this specific material. Substitution may adversely affect system performance.

[2] Where noted, materials are available from Major Laboratory Suppliers (MLS).

[3] The Ion Chef™ Install Supplies are provided with the instrument. No separate order is required.

[4] We recommend using an uninterruptible power supply (UPS) for laboratories that experience frequent power outages or line voltage fluctuations. The UPS must be compatible with 1500 W output or higher. The 1500 VA unit from APCC provides approximately 11 minutes of backup power for an instrument.

Materials required for training

Please refer to the *Ion Chef™ System Pre-Installation Checklist* (Pub. no. MAN0009817) for materials required for Ion Chef™ System training.

IMPORTANT! An Ion Sequencer must be available for use by the trainer during Ion Chef™ System training. Please refer to the *Ion Personal Genome Machine® (PGM™) System Pre-Installation Checklist* or the *Ion Proton™ System Pre-Installation Checklist* for the materials required to run the instrument that will be used during training.

Receive and inspect the shipment

1. Verify that the items shown on the shipping list are the same items that you ordered at the time of purchase.
2. Carefully inspect the shipping containers and report any damage to the service representative. Record any damage or mishandling on the shipping documents.

IMPORTANT! Do not unpack Ion Chef™ System shipping containers, to protect yourself from liability if any damage occurred during shipping.

Move the crated instrument to the installation site

1. Clear the installation site of all unnecessary materials.
2. If possible, move the crated instrument and other shipping containers to the installation site. Do not uncrate.

CAUTION! PHYSICAL INJURY HAZARD. Do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more people.

CAUTION! Do not tip the crated instrument on end. Tipping may damage the instrument hardware and electronics.

Note: After installation, retain the crate and instrument packaging in case you need to relocate the instrument.

Related documents and support

Customer and technical support

Visit **www.lifetechnologies.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

The information in this guide is subject to change without notice.

For support visit **lifetechnologies.com/support** or email **techsupport@lifetech.com**

lifetechnologies.com

11 February 2015

life technologies

A Thermo Fisher Scientific Brand

IonCode™ Barcode Adapters 1–384 Kit

Catalog Number A29751

Pub. No. MAN0014640 **Rev.** A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Description

The IonCode™ Barcode Adapters 1-384 Kit provides a set of 384 unique barcode adapters specifically designed for optimal performance with the Ion PGM™, Ion Proton™, Ion S5™, and Ion S5™ XL sequencers. When used in combination with the Ion AmpliSeq™ Library Kit 2.0, this kit enables pooling of up to 384 amplicon libraries for multiplex sequence analysis. This simplifies the Ion sequencing workflow for a wide range of applications, including targeted enrichment.

Kit contents and storage

Contents	Quantity	Storage
IonCode™ Barcode Adapters 1–384 (3840 reactions): • IonCode™ 0101–0196 in 96-well PCR Plate (red) • IonCode™ 0201–0296 in 96-well PCR Plate (yellow) • IonCode™ 0301–0396 in 96-well PCR Plate (green) • IonCode™ 0401–0496 in 96-well PCR Plate (blue)	1 set of 4 plates (10 reactions per barcode)	–30°C to –5°C

Handling and storage guidelines

To ensure the proper function of the IonCode™ Barcode Adapters, handle and store the associated consumables and reagents according to the following guidelines:

- The kit is shipped on dry ice, but store under the recommended conditions (–30°C to –5°C) and in an upright position.
- Inspect plates for damage upon arrival and again before use.
- Centrifuge plate at 1000 × *g* after thawing to collect contents at the bottom of the wells.

Barcode layout

The Barcode layout in each plate follows the numbering scheme shown here.

Set up a typical amplicon library barcode adapter ligation reaction

IonCode™ Barcode Adapters are premixed with P1 Adapter, so the setup of ligation reactions is simplified.

1. Starting with a 22 µL FuPa Reagent digestion reaction in a 96-well PCR plate, assemble the following ligation reaction:

Component	Volume
Switch Solution	4 µL
IonCode™ Barcode Adapter [1]	2 µL
Total volume (includes 22 µL of digestion reaction)	28 µL

[1] No mixing with P1 Adapter or diluting is necessary.

2. Add 2 µL of DNA Ligase to each well (30 µL total volume).
3. Vortex thoroughly, or mix by pipetting up and down.
4. Seal the reaction plate with MicroAmp™ Clear Adhesive Film (Cat. no. 4306311) or equivalent, and incubate reactions according to the appropriate library preparation guide.
5. Reseal the IonCode™ Barcode Adapter plate with adhesive film and store at -30°C to -5°C.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Corporate entity: Life Technologies | Carlsbad, CA 92008 USA | Toll Free in USA 1.800.955.6288

iontorrent

Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef

USER GUIDE

Instructions for automated template preparation, chip loading, and sequencing

for use with:
Ion Chef™ System
Ion S5™ System
Ion S5™ XL System
Ion GeneStudio™ S5 System
Ion GeneStudio™ S5 Plus System
Ion GeneStudio™ S5 Prime System

Catalog Numbers A34461, A34019
Publication Number MAN0016854
Revision G.0

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

Life Technologies Corporation | 5781 Van Allen Way | Carlsbad, CA 92008

Revision history: Pub. No. MAN0016854

Revision	Date	Description
G.0	7 November 2022	Updated flow information in "Create a Planned Run" on page 19.
F.0	29 October 2020	• Included chip compatibility information in "Ion S5™ Calibration Standard" on page 10. • Added new troubleshooting items: "Chip Check fails" on page 65 and "Reagent Check fails" on page 65. • Updated requirement for Ion AmpliSeq™ panels with amplicons greater than 325 bp. • Added recommendation for chips used for initialization. • Updated run time information.

The information in this guide is subject to change without notice.

Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef (Cat. Nos. A34461, A34019) is used for automated template preparation and sequencing of libraries up to 400 bp using the Ion Chef™ System and the Ion S5™ System, Ion S5™ XL System, or Ion GeneStudio™ S5 System. The kit contains prepackaged single-use template and sequencing reagent cartridges with integrated sample tracking.

Key features of the kit include:

- Automated workflow for sequencing of 8 loaded chips with complete run traceability.
- Maximum flexibility with compatibility for the Ion 510™ Chip, Ion 520™ Chip, and Ion 530™ Chip.

Library compatibility

The Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef can be used with up to 400-base-read libraries of any type prepared using any available Ion Torrent™-branded library kit.

Mixing chip types

You can perform templating runs with two different types of chips, using any combination of the three chips. However, you cannot mix template sizes (200-base and 400-base template run lengths) in a single run.

IMPORTANT! To perform templating runs with mixed chip types, you must update the Torrent Suite™ Software to version 5.6 or later (see "Software compatibility").

Ion GeneStudio™ S5 Series instrument reference

In this document, Ion GeneStudio™ S5 Series Sequencer or Ion GeneStudio™ S5 Series System refers generically to the following systems, unless otherwise specified.

- Ion GeneStudio™ S5 System (Cat. No. A38194)
- Ion GeneStudio™ S5 Plus System (Cat. No. A38195)
- Ion GeneStudio™ S5 Prime System (Cat. No. A38196)

Software compatibility

The Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef is compatible with Torrent Suite™ Software 5.4 and later. Be sure to update your Torrent Suite™ Software to the latest available version before using this kit. For more information, see the *Torrent Suite™ Software User Guide* for your version of the software.

Kit contents and storage

IMPORTANT!

- Do not substitute components from any other sequencing kits. We have verified this protocol using these specific materials. Substitution can adversely affect system performance.
- Store all consumables and cartridges under the recommended conditions and in an upright position. Do NOT store the Ion S5™ Sequencing Reagents (Part No. A27768) on dry ice or in a closed environment where dry ice is present.

Each Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef contains all the supplies that are required to prepare and sequence 8 Ion 510™ Chips, Ion 520™ Chips, or Ion 530™ Chips.

- Catalog No. A34461 supports 4 Ion Chef™ runs (2 chips/Ion Chef™ run) and 4 sequencer initializations (2 sequencing run/sequencer initialization). Select A34461 for templating and sequencing of 200-base–read libraries only.
- Catalog No. A34019 supports 4 Ion Chef™ runs (2 chips/Ion Chef™ run) and 8 sequencer initializations (1 sequencing run/sequencer initialization). Select A34019 for templating and sequencing of 400-base-read libraries.

On arrival, inspect all consumables and contact Technical Support if any of the products have been damaged during shipping.

Kit summary

Component	Part No.	Quantity per kit	
		A34461	A34019
Ion S5™ Chef Supplies	A27755	4 boxes	4 boxes
Ion S5™ Chef Solutions	A27754	1 box	1 box
Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents	A34018	1 box	1 box
Ion S5™ Sequencing Solutions	A27767	1 box	2 boxes
Ion S5™ Sequencing Reagents	A27768	1 box	2 boxes

Ion Chef™ reagents and materials

Contents	Amount / box	Storage
Ion S5™ Chef Supplies (Part No. A27755)		
Chip Adapter	2	15°C to 30°C
Enrichment Cartridge v2	1	
Tip Cartridge v2	1	
PCR Plate	1	
Frame Seal v2	1	
Recovery Station Disposable Lid v2	2	
Recovery Tube v2	12	
Ion S5™ Chef Solutions (Part No. A27754)		
Ion S5™ Chef Solutions	4 cartridges	15°C to 30°C
Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents (Part No. A34018)		
Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents	4 cartridges	–30°C to –10°C

Ion S5™ sequencing reagents and materials

Contents	Amount / box	Storage
Ion S5™ Sequencing Solutions (Part No. A27767)		
Ion S5™ Wash Solution	4 × 1.5 L	15°C to 30°C
Ion S5™ Cleaning Solution	250 mL	

Contents	Amount / box	Storage
Ion S5™ Sequencing Reagents (Part No. A27768)		
Ion S5™ Sequencing Reagents	4 cartridges	–30°C to –10°C[1]

[1] Cartridges ship at 2°C to 8°C. Store as indicated, do not store on dry ice.

IMPORTANT! Do not store the Ion S5™ Sequencing Reagents (Part No. A27768) on dry ice or in a closed environment containing dry ice.

Related products

Compatible Ion Chip™ kits

Description	Catalog No.	Quantity	Storage
Ion 510™ Chip Kit (2 × 4-pack)	A34292	8 chips	15°C to 30°C
Ion 520™ Chip Kit (2 × 4-pack)	A27762	8 chips	
Ion 530™ Chip Kit (2 × 4-pack)	A27764	8 chips	

Ion S5™ Calibration Standard

For *de novo* sequencing applications, the Ion S5™ Calibration Standard can be added to diluted libraries to increase base-calling accuracy. The Ion S5™ Calibration Standard (Cat. No. A27988) is ordered separately.

Use the Ion S5™ Calibration Standard with Ion 520™ Chip or Ion 530™ Chip workflows only. The standard has not been validated for use with the Ion 510™ Chip workflow.

Contents	Amount	Storage
Ion S5™ Calibration Standard	80 µL	–30°C to –10°C

Ion S5™ Controls Kit Plus

The Ion S5™ Controls Kit Plus (Cat. No. A30729) is ordered separately. See the *Ion S5™ Controls Kit Plus Product Information Sheet* (Pub. No. MAN0016206) for details.

Contents	Amount	Storage
Human CEPH Genomic DNA Control (red cap)	30 µL	–30°C to –10°C
Human CEPH Control 200 Library (yellow cap)	12 µL	
E. coli DH10B Control 400 Library (orange cap)	16 µL	

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Ion Chef™ System	4484177
Microcentrifuge[1]	MLS
2-, 20-, 200-, and 1,000-µL pipettes and appropriate filtered tips	MLS
Microcentrifuge tubes, 1.5-mL or 1.7-mL	MLS
Vortex mixer with a rubber platform	MLS
Gloves, powder-free nitrile	MLS
Ice buckets and ice	—
Nuclease-free water, molecular biology grade	MLS
Isopropyl alcohol, 70% solution	MLS
Wipes, disposable lint-free	MLS
(Optional) Uninterruptible Power Supply (UPS) [2]	MLS

[1] Must fit standard 0.2- and 1.5-mL microcentrifuge tubes and generate 21,000 × *g*. To convert the RPMs of your centrifuge to RCF in units of gravity, see **tools.thermofisher.com/content/sfs/brochures/TR0040-Centrifuge-speed.pdf**.

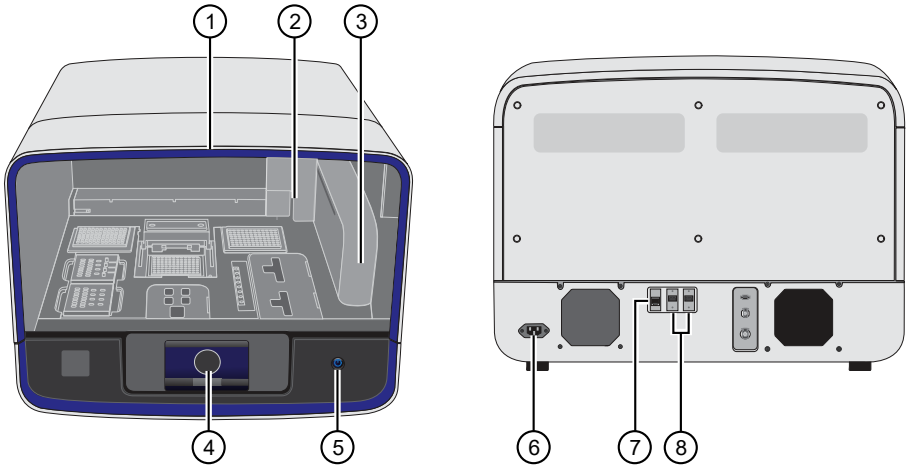
[2] For laboratories that experience frequent power outages or line voltage fluctuations, we recommend that you use an uninterruptible power supply that is compatible with 2500 W output or higher.

About the Ion Chef™ System

The Ion Chef™ System (Cat. No. 4484177) provides automated, high-throughput template preparation and chip loading for use with an Ion S5™ Sequencer, Ion S5™ XL Sequencer, or Ion GeneStudio™ S5 Series Sequencer. The system includes a complete set of cartridge-based consumables and reagents that enable a user to load two chips in approximately 13 hours with less than 15 minutes of hands-on time. The Ion Chef™ System features network integration with the Torrent Suite™ Software to enable sample and reagent traceability throughout the chip preparation workflow.

Ion Chef™ Instrument components

The following figure illustrates the major external and internal features of the Ion Chef™ Instrument.



① **Door** – Provides access to the interior of the instrument. The door is locked in the closed position during operation.

② **Micropipettor** – A mechanical positive-displacement pipettor that performs all fluid transfers during sample and chip preparation.

③ **Robotic arm** – Enables fluid transfer by the Micropipettor. The arm also contains an optical sensor that reads the barcodes of instrument reagents and consumables.

④ **Touchscreen** – Provides access to all instrument functions for operation, maintenance, and troubleshooting.

⑤ **Power button** – Power switch for the Ion Chef™ Instrument, where the states are on (illuminated) and off.

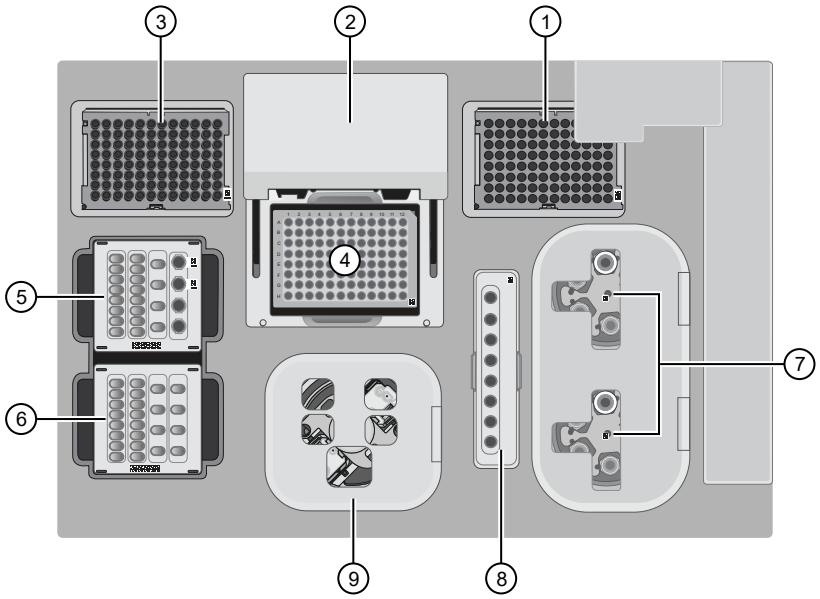
⑥ **Power port** – A 100–240 VAC port that provides power to the Ion Chef™ Instrument.

⑦ **Ethernet port** – An RJ45 port that provides Ethernet (100 Mbit) communication with the Ion Chef™ Instrument.

⑧ **USB port** – Provides USB communication with the Ion Chef™ Instrument. Used to update the instrument firmware and to transfer data during service or maintenance.

Interior hardware and consumables

The following figure illustrates the interior of the Ion Chef™ Instrument and describes the stations involved in the preparation of chips for sequencing on an Ion S5™ Sequencer, Ion S5™ XL Sequencer, or Ion GeneStudio™ S5 Series Sequencer.



① **Waste pipette tip rack** – The position of the rack containing waste (used) pipette tips.

② **Automated heated cover** – Transfers the plate cover to the PCR reaction plate within the sample block. During thermal cycling, the heated cover applies compression to seal the reaction plate and heats the cover to prevent condensation.

③ **New pipette tips** – The position of the rack containing unused pipette tips.

④ **Thermal cycler sample block** – Performs thermal cycling of the sequencing reactions on a 96-well PCR reaction plate.

⑤ **Reagents station** – The position on the instrument deck of the diluted libraries, NaOH, and the Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge.

⑥ **Solutions station** – The position on the instrument deck of the Ion S5™ Chef Solutions cartridge, which is maintained at room temperature.

⑦ **Recovery centrifuges** – Twin stations that perform centrifugation of the ISPs during the recovery phase of template preparation.

⑧ **Enrichment station** – The position of the rack containing consumables for enrichment of the template-positive ISPs.

⑨ **Chip-loading centrifuge** – Performs centrifugation of sequencing chips that have been mounted to chip-loading adapters and loaded with template-positive ISPs.

About the Ion Chef™ System touchscreen interface

The Ion Chef™ System features a simple interface for loading chips, cleaning the instrument, and performing system maintenance and configuration tasks.

① **Set up run** button – Set up the Ion Chef™ template preparation and chip-loading routine. Choose **Step by Step** to have the instrument lead you stepwise through installation of reagents and consumables, or choose **Quick Start** to proceed if you have already installed the consumables.

② **Open Door** button.

③ **Notifications** button – View notifications about instrument status during and between runs.

④ **Quick Start** button – Proceed directly to the Quick Start instrument setup mode. User verifies the loading of a new pipette tip cartridge and an empty pipette tip rack to hold waste tips generated during the run, before proceeding to Deck Scan.

⑤ **Settings** button – Advance to a screen providing the following options:

- **Notifications**: view notifications about instrument status during and between runs
- **Instrument Settings**: view current settings and network configuration, set instrument name, adjust time zone
- **Service tools**: access screens for service-related maintenance and instrument diagnostics
- **Torrent Server**: add and manage Ion Torrent™ Server connections
- **Clean Ion Chef**: proceed directly to the instrument cleaning routine
- **Check for updates**: check availability of system software updates

Workflow

The following workflow shows how to prepare and load samples using the Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef and Ion Chef™ System for sequencing on an Ion S5™ Sequencer, Ion S5™ XL Sequencer, or Ion GeneStudio™ S5 Series Sequencer.

Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef Workflow

Create a Planned Run (page 19)

Create a planned run in Torrent Suite™ Software by entering run parameters for templating and sequencing your samples.

Dilute the library samples (page 25)

If needed, dilute sample and control libraries. Thaw the Reagents cartridge at room temperature for 45 minutes before use.

Load the Ion Chef™ Instrument (page 28)

Load the Ion Chef™ Instrument with sequencing chips, reagents, and sample libraries.

Start the Ion Chef™ run (page 39)

Start the Ion Chef™ run by following the step-by-step instructions on the Ion Chef™ Instrument home touchscreen.

Unload the Ion Chef™ Instrument (page 44)

After the Ion Chef™ Run completes, unload the prepared sequencing chips from the Ion Chef™ Instrument.

Load the prepared sequencing chip into the sequencer and begin the sequencing run (page 50)

Load the prepared sequencing chips on a sequencer and follow the instructions on the touchscreen to start the sequencing run.

2 Before you begin

Precautions

Avoid nucleic acid contamination

IMPORTANT! A primary source of contamination is spurious DNA fragments from previous sample processing steps. Do not introduce amplified DNA into the library preparation laboratory or work area.

Avoid chip damage

IMPORTANT! To avoid possible damage to the chip due to electrostatic discharge, ground yourself before picking up a chip or placing a chip on a surface such as a lab bench. For example, touch the metal trim on the chip compartment before inserting or removing a chip from the chip clamp.

Protection by equipment

WARNING! The protection that is provided by the equipment can be impaired if the instrument is operated outside the environment and use specifications, the user provides inadequate maintenance, or the equipment is used in a manner that is not specified by the manufacturer (Thermo Fisher Scientific).

Guidelines for using Ion Chef™ reagents and consumables

- Store all consumables and cartridges under the recommended conditions and in an upright position.
- Inspect all consumables and cartridges for damage on arrival and again before use.
- Hold sequencing chips by gently gripping them by their edges.
- When the instrument is not in use, remove all consumables and reagents from the deck and close the instrument door.

- Except for the *new* Tip Cartridge v2, do not reuse any of the consumables or reagents. After each run, the empty Tip Cartridge v2 is transferred to the waste tip station.

  IMPORTANT! All components are single-use only.

- Use only Ion Torrent™ kits and supplies with the Ion Chef™ Instrument. The use of third-party reagents and supplies can adversely affect the performance of the instrument and chips.
- Remove and sequence chips within 1 hour after the instrument finishes loading them. If you cannot sequence a loaded chip immediately, store it in a chip storage container at 4°C until you are ready to run it (up to 6–8 hours maximum).

  IMPORTANT! If you choose to store a loaded chip, remove the chip from 4°C storage (but keep it in the chip storage container) at least 20 minutes before running it, allowing the chip to warm to room temperature.

Prepare the Ion Chef™ Instrument for use

- Ensure that the Ion Chef™ Instrument has been cleaned following the previous run. If not, clean the instrument *before* loading it with consumables.

  Note: For more information on the cleaning procedure, see Chapter 7, "Clean the Ion Chef™ System".

- Inspect the empty compartments of the Reagents and Solutions stations for condensation. Condensate can collect in these compartments, depending on temperature and humidity conditions. Before loading consumables into the instrument, wipe the compartments dry with a laboratory wipe or absorbent cloth, if needed.

- Thaw the Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge at room temperature for 45 minutes before use.

- Ensure that the Ion Chef™ Instrument has a connection to the Ion Torrent™ Server. On the instrument home touchscreen, tap **Settings** ▸ **Ion Torrent™ Server** to view the connection status.

  Note: If the instrument is not connected, see the *Ion Chef™ Instrument Network Connection User Guide* (Pub. No. MAN0013444) for instructions on how to configure a direct or indirect network connection.

3 Create a Planned Run

About Planned Runs

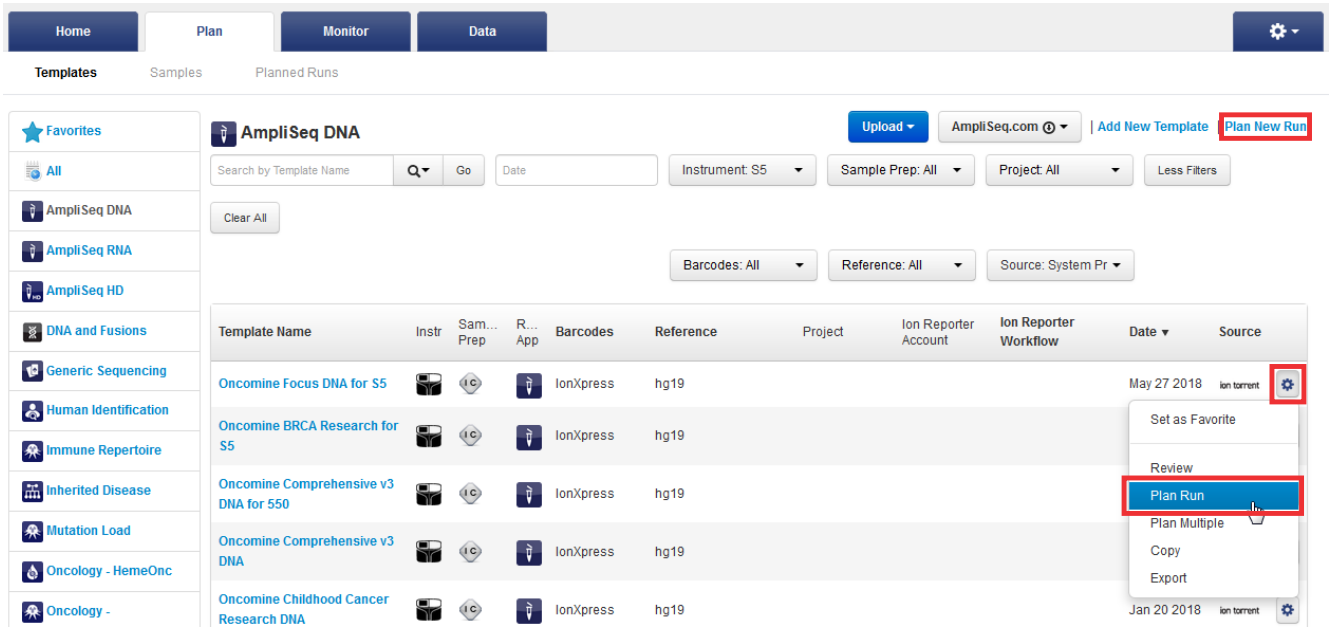
Planned Runs are digital instructions that are created in Torrent Suite™ Software for controlling the template preparation and sequencing instruments. Planned Runs contain settings such as number of flows, kit types, barcodes, sample information, and reference files (if any). Planned Runs are also used to track samples, chips, and reagents throughout the workflow, from template preparation on the Ion Chef™ Instrument through sequencing on an Ion S5™ Sequencer, Ion S5™ XL Sequencer, or Ion GeneStudio™ S5 Series Sequencer and subsequent data analysis. Each chip that is prepared in an Ion Chef™ run requires its own Planned Run.

IMPORTANT! For more information on creating a Planned Run in Torrent Suite™ Software, including a complete description of each field in the **Create Plan** workflow bar, see the *Torrent Suite™ Software Help*, available by clicking the **Help** button in the software.

Create a Planned Run

IMPORTANT! If you are using the Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef with libraries that were prepared using Oncomine™ panels, see the corresponding Oncomine™ Assay User Guide for assay-specific instructions on creating a Planned Run in Torrent Suite™ Software.

1. Sign in to the Torrent Suite™ Software.
2. In the **Plan** tab, in the **Templates** screen, select the application that you want to run (such as AmpliSeq DNA) from the left navigation menu, then click one of the following options:
   - To plan a new run using the **Generic Sequencing** template for the selected application, click **Plan New Run**.
   - To plan a new run using a specific Planned Run template, in the row of the template, click **(Actions)** ▸ **Plan Run**.



3. In the **Create Plan** workflow bar, review the **Ion Reporter** and **Research Application** steps, then make selections appropriate to your run. Click **Next**.
4. In the **Kits** step, make the following selections:
   a. Select **Ion GeneStudio™ S5 System** from the **Instrument** dropdown list.
   b. Select the appropriate chip type from the **Chip Type** dropdown list.
   c. Select the library kit used to prepare your libraries from the **Library Kit Type** dropdown list.
   d. *(Optional)* For barcoded libraries, select the barcode set used during library preparation from the **Barcode Set** dropdown list.

e. Select **IonChef** for **Template Kit**, then select **Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef** from the **Template Kit** dropdown list.

f. Select **Ion S5 Sequencing Kit** from the **Sequencing Kit** dropdown list.

g. Enter the appropriate number of flows in the **Flows** field.

Sequencing type	Number of flows
200-base-read	500
400-base-read	850

Note:

- If you are using Ion AmpliSeq™ On-Demand Panels, set the flows to 550. You can perform two sequencing runs per initialization, with 550 flows each.
- If you are using Ion AmpliSeq™ panels with amplicons longer than 325 bp, increase the number of flows to 650. A file with information about amplicon length can be downloaded from **AmpliSeq.com**. See the *Ion AmpliSeq™ Designer Help* (Pub. No. MAN0018937).

IMPORTANT! Do not exceed 1,100 total flows for one Ion S5™ Sequencing Reagents cartridge. For 200-base-read sequencing, you can run two chips per sequencer initialization. For 400-base-read sequencing, you can run only one chip per sequencer initialization.

h. In the **Advanced Settings** pane, select **Customize**, then select the templating protocol from the **Templating Protocol** dropdown list.

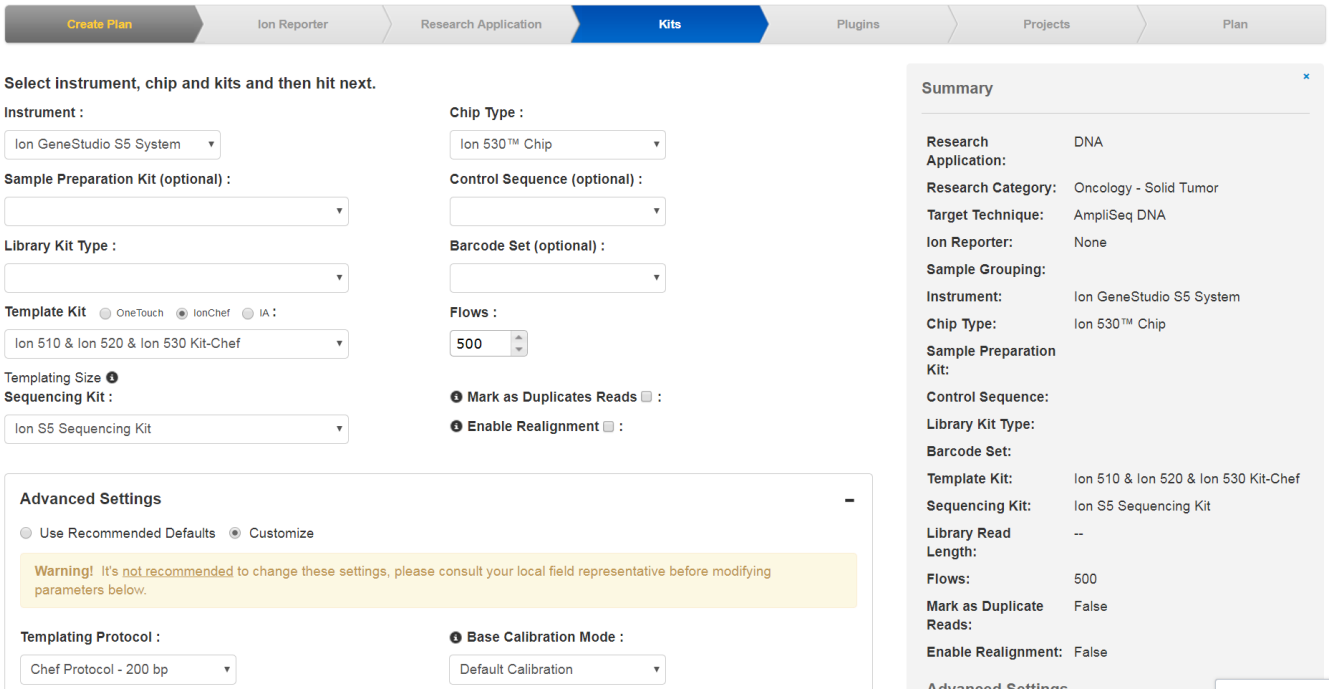
Note:

- Select **Chef Protocol - 200** for libraries with <300-base average read length.
- Select **Chef Protocol - 400** for libraries with ≥300-base average read length.
- For Ion AmpliSeq™ On-Demand Panels, ensure that **Chef Protocol - 200** is selected.

i. Select or edit the remaining optional information fields appropriately for your run.

IMPORTANT! For *de novo* sequencing applications that do not include a reference BAM file, select **Enable Calibration Standard** from the **Base Calibration Mode** dropdown list in the **Advanced Settings** pane. Select this option only if you are adding Ion S5™ Calibration Standard to the Ion Chef™ Library Sample Tubes. Selecting this option and adding the Ion S5™ Calibration Standard to your library allows greater accuracy of base-calling in libraries for which a reference BAM file does not exist.

j. Click **Next**.



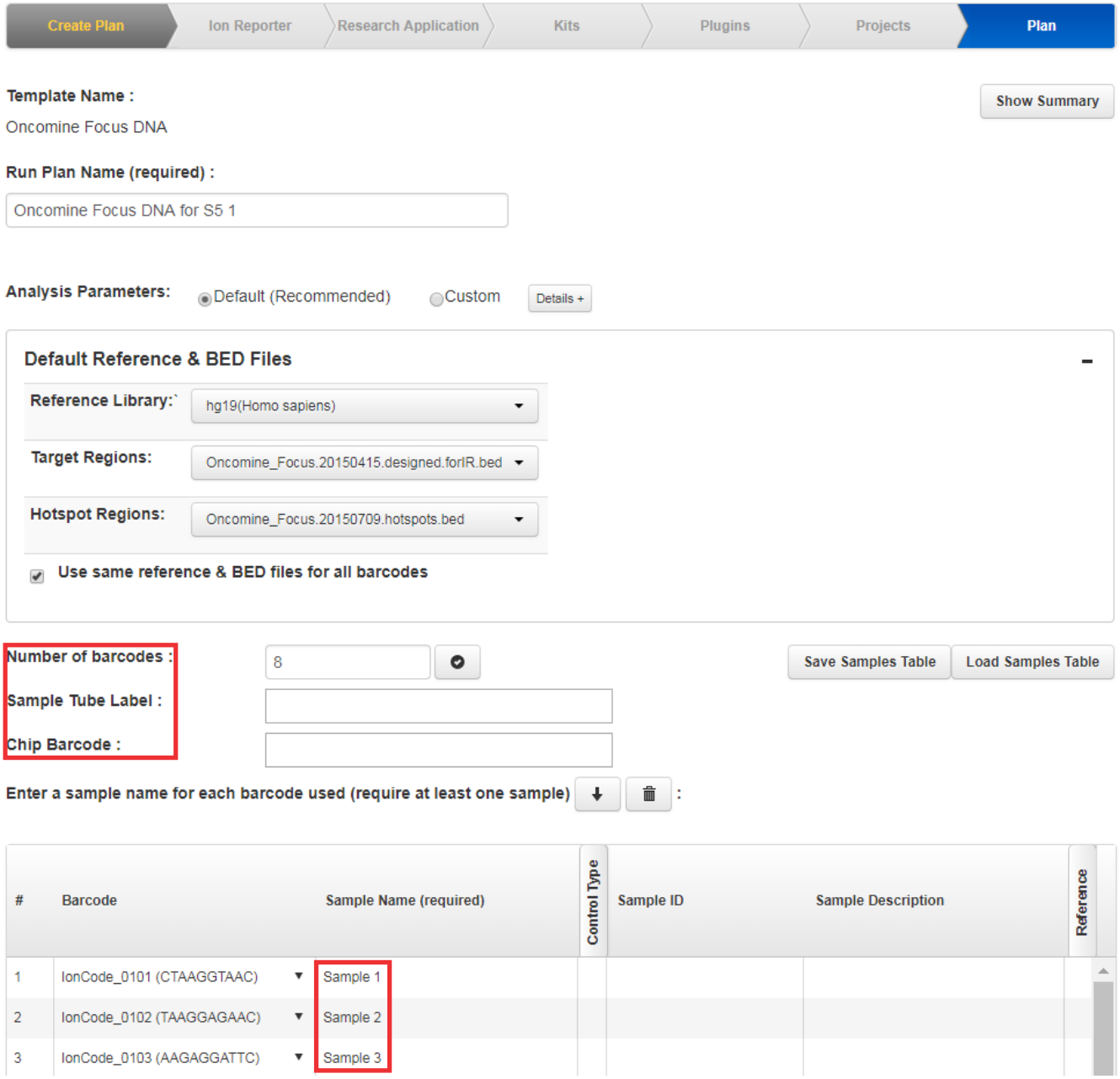
5. Review the **Plugins** and **Projects** steps, then make selections appropriate to your run.

6. In the **Plan** step, enter or make the following selections:
   a. Enter a Run Plan Name, then select Reference and BED files appropriate to your run.
   b. Enter the number of barcodes you are using in your combined library in the **Number of barcodes** field, then click ✔ to the right of this field. Edit the auto-populated list of barcodes that appears, if needed.

      Note: If you did not use barcode adapters in library preparation and did not select a **Barcode Set** in the **Kits** step, fields appear in the **Plan** step where you enter the number of chips that are used, then enter the sample name and chip barcode for each sample.

   c. Scan or enter the barcode of the Ion Chef™ Library Sample Tube into the **Sample Tube Label** field.
   d. Scan or enter the chip barcode into the **Chip Barcode** field.

e. Enter a sample name for each barcode in the appropriate **Sample Name (required)** fields.



7. After you have completed your selections, click **Plan Run** at the bottom right of the **Plan** step screen to save the run.

The run is listed in the **Planned Runs** screen under the name that you specified and is automatically used by the Ion Chef™ System when the associated sample is loaded.

If you are performing a dual-chip run, repeat step 2–step 7 to create a Planned Run for the second chip. You can copy the first Planned Run in the Planned Runs screen, then edit as needed.

Planned Run workflow key fields

Step or field name	Description
Ion Reporter	If the Ion Reporter™ Software is installed and enabled, and you want to analyze the run data using the software, select the **Ion Reporter Account**, then select the Ion Reporter™ workflow from the **Existing Workflow** dropdown list.
Research Application	Select the **Research Application** and **Target Technique** that represent your sequencing experiment.
Instrument	Select **Ion GeneStudio™ S5 System**.
Chip Type	Select the **Ion 510™ Chip, Ion 520™ Chip, or Ion 530™ Chip**.
Library Kit Type	Select the kit used to prepare the library.
Template Kit	Select **Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef**.
Sequencing Kit	Select **Ion S5 Sequencing Kit**.
Barcode Set (optional)	If you are using barcodes with: • **DNA libraries** – Select the appropriate barcode set used. • **RNA libraries prepared using the Ion Total RNA-Seq Kit v2** – Select the **IonXpressRNA** barcode set, which contains all 16 barcodes in the Ion Xpress™ RNA-Seq Barcode 1–16 Kit. If you are *not* using barcodes with: • **DNA libraries** – Leave the Barcode Set field blank. • **RNA libraries prepared using the Ion Total RNA-Seq Kit v2** – Select **RNA_Barcode_None** from the dropdown list. This will ensure that the proper trimming is performed on the resulting sequence when the RNA library does not have a barcode.
Flows	Enter the appropriate number of flows for the read length (for example, 500 flows for 200-base-read sequencing and 850 flows for 400-base-read sequencing).
Plugins	Select the appropriate plugins for your application.
Projects	Select or add a project to group your run data. You can include runs in multiple projects, and remove runs from a project at any time.
Run Plan Name	Enter a name for the run.
Reference Library	Select a reference library uploaded to the Torrent Suite™ Software, if any.
BED files	Select the **Target Regions** or **HotSpot Regions** BED file in the Torrent Suite™ Software, if any.

(continued)

Step or field name	Description
Sample Tube Label (Required)	Enter or scan the barcode of the Ion Chef™ Library Sample Tube that you will use to load your sample into the Ion Chef™ Instrument. **IMPORTANT!** Each chip that is prepared in an Ion Chef™ run requires its own Planned Run. You must scan or enter the barcode of the appropriate Ion Chef™ Library Sample Tube for each Planned Run that is created for each individual chip.
Chip Barcode	Enter or scan the chip barcode.
Sample Name (Required)	Did you select a **Barcode Set** in the **Kits** step? • **No** – Enter the sample name for each sample loaded onto each chip in the **Sample Name (required)** column, then enter or scan the corresponding Ion Chef™ Library Sample Tube barcode and the chip barcode in the **Sample Tube Label** and **Chip Barcode** columns respectively. • **Yes** – Enter the sample name for each sample in the **Sample Name (required)** column, then select the barcode that is associated with each sample from the dropdown list in the **Barcode** column. Did you select the **Ion Reporter Account** and the associated Ion Reporter™ workflow? • **No** – For each sample name listed, make the appropriate selections in each column in the table. • **Yes** – For each sample name listed, also make the appropriate selections in the **Ion Reporter Workflow**, **Relation**, **Gender**, and **IR Set ID** columns.
Monitoring Thresholds	Set thresholds for **Bead Loading**, **Usable Sequence**, and **Key Signal**. In the Torrent Suite™ Software **Monitor ▸ Runs in Progress** screen, an alert is displayed if the values for a run fall below the selected thresholds.

4 Start the Ion Chef™ run

This chapter describes how to perform the following procedures:

- Set up the Ion Chef™ Instrument for use by diluting the libraries, and loading the instrument with all of the required reagents and consumables
- Start an Ion Chef™ run
- Unload the chips for sequencing

Materials required

- Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef (Cat. Nos. A34461, A34019)
- Ion 510™ Chip, Ion 520™ Chip, or Ion 530™ Chip (1 or 2)
- Library or combined library stock solution (1 or 2)
- Molecular-biology grade nuclease-free water
- P200 pipettor and filtered tips

Dilute the libraries

Sample libraries are quantified at the end of the library preparation workflow and are typically diluted to a concentration of 100 pM. The Ion Library Equalizer™ Kit procedure also normalizes library concentration to 100 pM. Barcoded libraries are typically mixed in an equimolar ratio to create a combined library. Libraries or combined libraries can require further dilution before use in Ion Chef™ template runs.

Guidelines for library dilution

- The optimal input concentration for a given library preparation depends on the library and chip type, and often needs to be empirically determined starting from the recommended concentration.
- The recommendations for diluting your sample libraries represent optimal input concentrations for control libraries.
- Recommendations are based on qPCR quantification. If libraries are quantified with an Agilent™ 2100 Bioanalyzer™ instrument, a higher calculated concentration may need to be used for equivalent input.
- Prepare a fresh dilution of each library or combined library before use with the Ion Chef™ System, and use the library dilutions within 48 hours.
- Barcoded libraries are typically combined (or pooled) before templating to allow multiple libraries to be sequenced on a single chip. We recommend that you combine equal volumes of individual barcoded libraries at the same concentration (for example, 100 pM), then dilute the combined library to the final concentration recommended in the following table.
- Follow the recommendations of your library preparation user guide if it recommends a specific final library concentration for an Ion Chef™ run.

Dilute the sample libraries

IMPORTANT! Before proceeding, dilute the two libraries or combined libraries to the optimal input concentration. The quality of your sequencing data relies greatly on achieving the correct concentration of starting library.

Dilute the individual or combined 200-bp or 400-bp stock libraries according to the following table. If needed, use polyclonality and low-quality filter results from a sequencing run performed with ISPs templated at the starting concentration, then titrate up or down to achieve optimal concentrations.

IMPORTANT! Do **NOT** mix template sizes (for example, 200-base and 400-base template run lengths) in a single templating run.

Library length[1]	Recommended concentration	Molecules per 25-µL input volume	Templating size in Planned Run setup
<300 bp	25–60 pM	600–900 × 10^6	200
≥300 bp	25–60 pM	600–900 × 10^6	400

[1] Library length is average insert length plus adapter length.

Note: For the Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef, input for all sizes is identical.

Dilute the control library (if used)

If you are running a control library for troubleshooting purposes, dilute the Human CEPH Control 200 Library or *E. coli* DH10B Control 400 Library, obtained from the Ion S5™ Controls Kit Plus (Cat. No. A30729), as described in the following table.

Control library	Volume of library	Volume of nuclease-free water
Human CEPH Control 200 Library	1 µL	49 µL
E. coli DH10B Control 400 Library	1 µL	124 µL

Prepare the consumables

1. Unbox the Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge 45 minutes before use and allow it to warm to room temperature.

   IMPORTANT! The Reagents cartridge must sit at room temperature for 45 minutes before use.

2. Remove all cartridges and consumables from their packaging, then place them on the bench next to the Ion Chef™ Instrument.

   Prepare the following cartridges and consumables:

   - Chip Adapter (2)
   - Enrichment Cartridge v2
   - Tip Cartridge v2
   - PCR Plate and Frame Seal v2
   - Recovery Station Disposable Lid v2 (2)
   - Recovery Tube v2 (12)
   - Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge (from step 1)
   - Ion S5™ Chef Solutions

   IMPORTANT!
   - Before use, gently tap the Reagents and Solutions cartridges on the bench to force the reagents to the bottoms of the tubes.
   - Briefly centrifuge the tube of NaOH (Reagents cartridge, position C) to collect the contents.

   Note: When stored under normal conditions, a precipitate can form in some tubes of the Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge. If present, load the cartridge as directed. The precipitate dissolves when the reagents are mixed during instrument operation.

Add the diluted libraries to the Ion Chef™ Library Sample Tube

1. Pipet 25 µL of each diluted library or combined library to the bottom of the appropriate Ion Chef™ Library Sample Tube (barcoded tubes). Load one individual library or combined library per tube. See "Dilute the sample libraries" on page 26.

   IMPORTANT! If you are performing a *de novo* sequencing experiment that does not include a reference BAM file, add 4 µL of the Ion S5™ Calibration Standard to your diluted libraries in each Ion Chef™ Library Sample Tube. Cap the tubes, vortex to mix, then centrifuge briefly to collect contents at the bottom of the tube.

   Note: If running the Human CEPH Control 200 Library or *E. coli* DH10B Control 400 Library, obtained from the Ion S5™ Controls Kit Plus (Cat. No. A30729), prepare the control library for use by diluting 1 µL of stock library as described in "Dilute the control library (if used)" on page 27, then add 25 µL of diluted control library to the appropriate Ion Chef™ Library Sample Tube.

2. Cap, then store the sample tubes on ice until you are ready to load them onto the Ion Chef™ Instrument.

Load the Ion Chef™ System

IMPORTANT!

- Rated centrifuge speeds are intended only for operation with the provided buckets and approved consumable chips, tubes, and sample preparation reagents.
- The Chip-loading centrifuge is rated to operate at the listed rotational frequencies with the chip buckets, chips, and adapters. The centrifuge must be load-balanced. Proper care must be taken to load the buckets properly. If excessive vibrations arise, check that items are installed properly and rotors are load-balanced.
- Use only the materials supplied in the Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef to run the centrifuges at the rated speeds. Do not remove or change the rotors. Inspect the buckets before each use to assure normal operation.
- Confirm that the instrument is powered on and was cleaned following the last use.
- Ensure that all components are clean and dry before loading them onto the Ion Chef™ Instrument.
- Ensure that the Reagents and Solutions station compartments are free of condensate before loading components.

Follow the procedures in this section to load the Ion Chef™ Instrument.

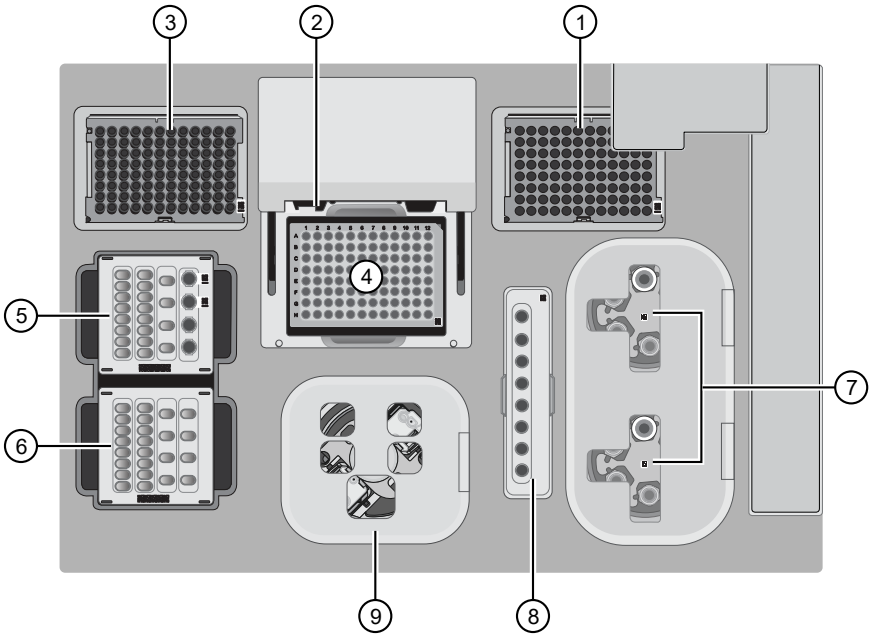


Figure 1 A schematic of a loaded Ion Chef™ Instrument

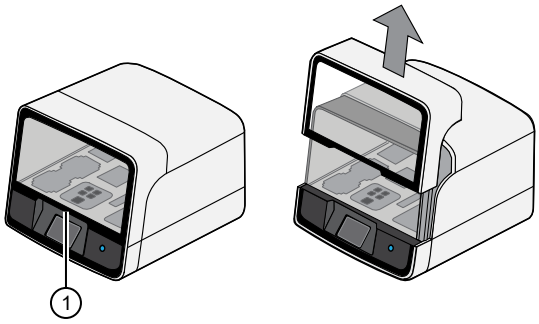
1. Empty tip rack (move from new Tip Cartridge position)
2. Frame Seal v2
3. New Tip Cartridge
4. PCR Plate
5. Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge
6. Ion S5™ Chef Solutions cartridge
7. Recovery Tubes and Recovery Station Disposable Lid v2
8. Enrichment Cartridge v2
9. Chip Adapter/Chip assemblies

Load the pipette tip racks and PCR Plate

1. Tap ⏏ **(Open Door)** in the instrument touchscreen to open the instrument door, then wait for the latch to open.

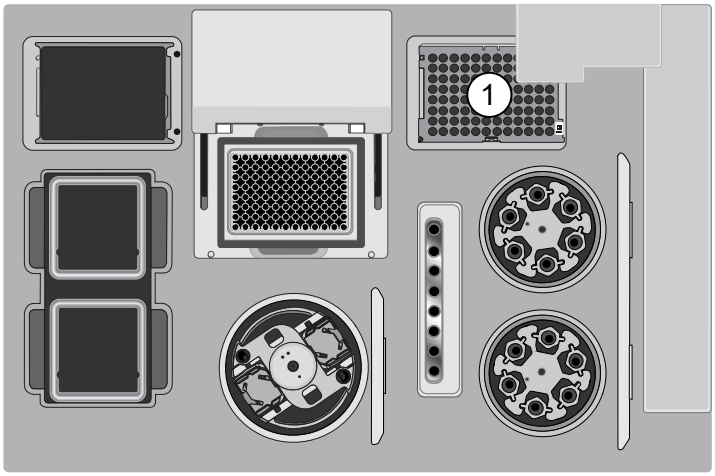
2. Lift the instrument door to the top of the travel until the latch mechanism engages.

   IMPORTANT! Lift the door from the center.



1. Center of door

3. Load an empty pipette tip rack in the *Used* (Waste) Pipette Tip Position, then change gloves.



① Used Pipette Tip Position

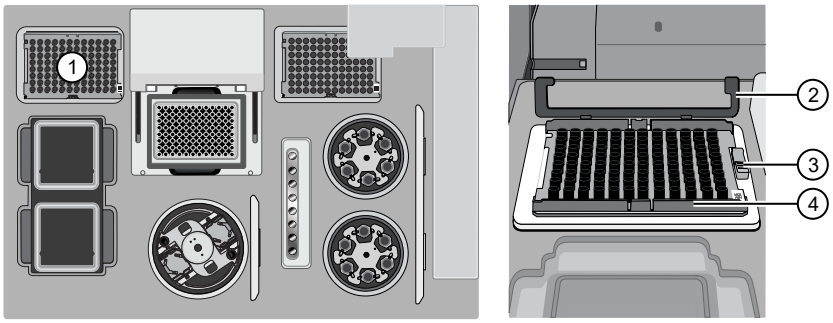
IMPORTANT!

- Ensure that the pipette tip rack in the *Used* (Waste) Pipette Tip Position does not contain any tips. The instrument aborts the run if tips are present in the *used* position.
- To prevent contamination, change gloves immediately after moving the empty pipette tip rack to the *Used* (Waste) Pipette Tip Position.

Note: A small amount of dried residue can be present in the tub of the empty pipette tip rack after a run. This residue does not affect the next run.

4. Unwrap a new Tip Cartridge v2 and remove the cover to expose the pipette tips, then load it in the *New* Pipette Tip Position. See the figure in step 5.

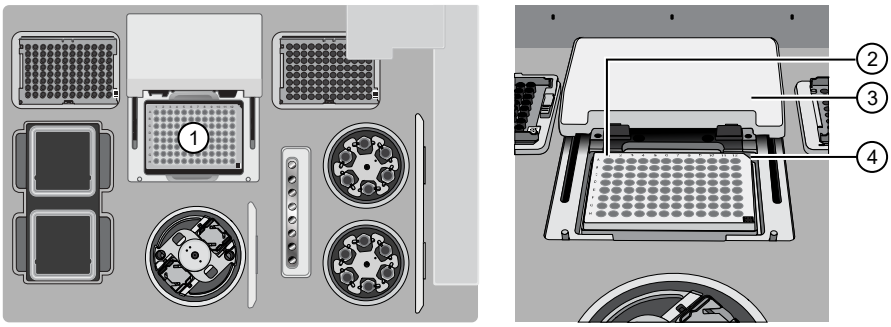
5. Slide the catch forward to allow the locking bracket to pivot upward. Load the Tip Cartridge v2 into the *New* Pipette Tip Position, pull the bracket downward, then push the catch backward to lock the bracket and cartridge in place.



① New Pipette Tip Position
② Bracket
③ Catch
④ New Tip Cartridge v2

6. Load a new PCR plate into the thermal cycler sample block, then slide a new Frame Seal v2 under the automated heated cover.

IMPORTANT! When the Frame Seal v2 is positioned correctly, its tabs project upward and contact the heated cover.



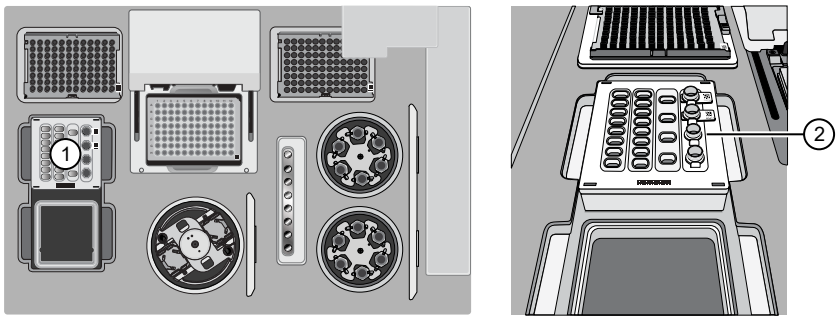
① Thermal cycler sample block
② Well A1
③ Cover
④ Keyed corner

Load the Reagents and Solutions cartridges

IMPORTANT! Thaw the Reagents cartridge at room temperature for 45 minutes before use.

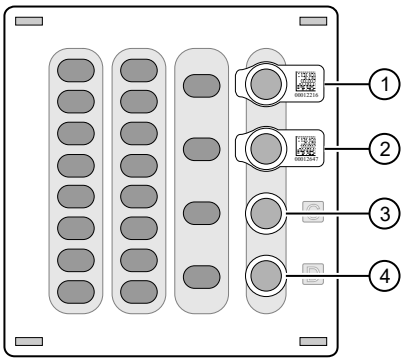
1. Gently tap the Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge on the bench to force the reagents to the bottoms of the tubes.
2. If bubbles are present below the surface of the liquid, repeat step 1.
3. Load the cartridge into the Reagents station so that it snaps into place and is level on the deck.

   IMPORTANT! Do not force the Ion Chef™ cartridges into place. Each cartridge fits only one location on the deck and in one orientation. If a cartridge does not fit, confirm that you are loading the correct cartridge in the correct orientation.



① Reagents station (4°C)
② Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge

4. Uncap, then load the two Library Sample Tubes, each containing 25 µL of diluted library or combined library pool, into Positions A and B on the Reagents cartridge.



① Position A (Library)
② Position B (Library)
③ Position C (NaOH)
④ Position D (Empty tube)

IMPORTANT!

- Orient the sample tubes so that the barcodes are visible and oriented to the right.
- Remove the caps from each Library Sample Tube before proceeding.
- Because 200- and 400-base-read libraries require different run parameters, do not load a 200-base-read library and 400-base-read library in a single Ion Chef™ run. Both libraries that are loaded in a run must have a similar read length.

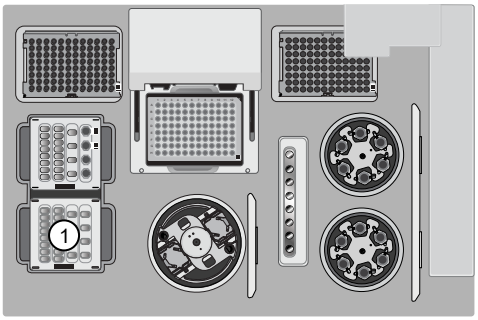
5. Uncap both the tube of NaOH in Position C and the empty tube in Position D on the Reagents cartridge.

IMPORTANT! When the Reagents cartridge is loaded:

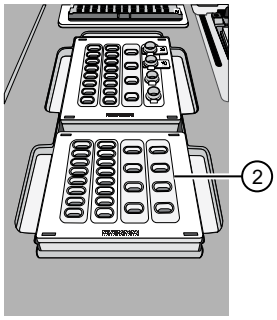
- Press down on each Library Sample Tube to ensure that they are firmly seated in the cartridge.
- **Ensure that *all* tubes are uncapped, including the tube at Position D.**

6. Gently tap the Ion S5™ Chef Solutions cartridge on the bench to force the reagents to the bottoms of the tubes.

7. Load the Solutions cartridge into the Solutions station until it snaps into place and is level on the deck.



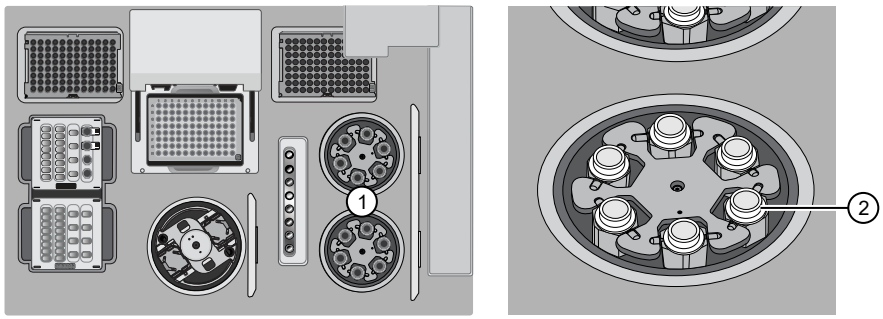
① Solutions station (room temperature)



② Ion S5™ Chef Solutions cartridge

Load the Recovery Tubes and Enrichment Cartridge v2

1. Load six Recovery Tubes into each Recovery centrifuge.



① Recovery centrifuges ② Recovery Tube v2

Before sealing each centrifuge, confirm that:

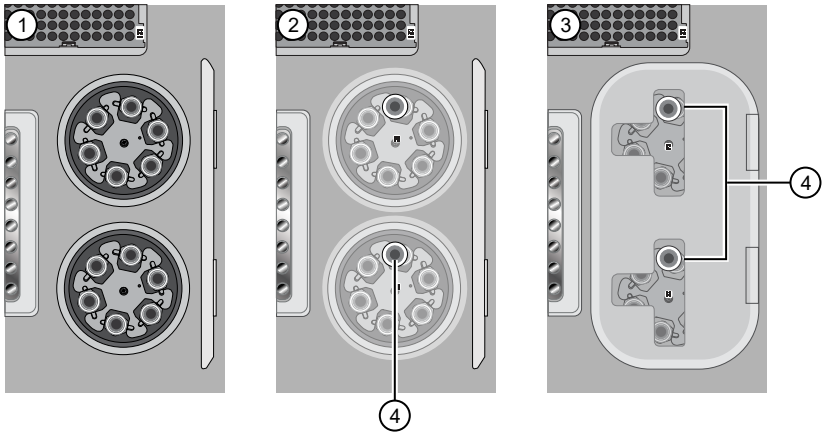
- The centrifuge is load-balanced with all required consumables.

  IMPORTANT! The centrifuge must be load-balanced.

- The buckets are securely seated in the centrifuge rotors.
- The buckets are oriented correctly in the centrifuge so that they pivot outwards.

2. Place a Recovery Station Disposable Lid v2 over each centrifuge by lining up the tab with the depression on the deck, then snap into place. Ensure that the lids snap completely into place by applying firm downward pressure along the lid perimeter.

3. Close the hinged cover of the Recovery centrifuges. Confirm that the port of each disposable lid is positioned toward the rear of the instrument.



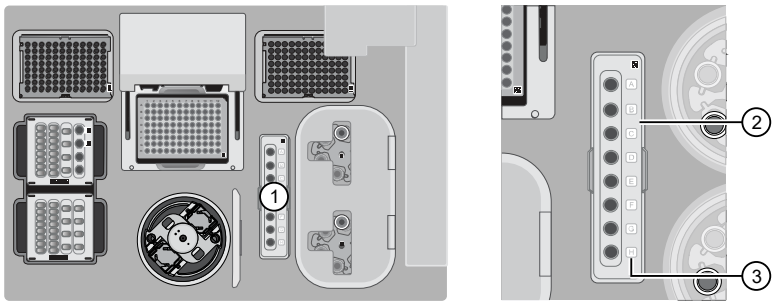
① Recovery Tubes installed
② Recovery Station Disposable Lid v2 installed
③ Recovery centrifuge cover closed
④ Port

IMPORTANT!

- Do not obstruct or place any object on top of the Recovery centrifuge cover.
- Use only the supplied materials, including buckets and disposables, to run the centrifuges at the rated speeds. Do not remove or change the rotors. To ensure normal operation, inspect the buckets before each use.

4. Load the Enrichment Cartridge v2, then press down on the cartridge to ensure that it is level with the instrument deck.

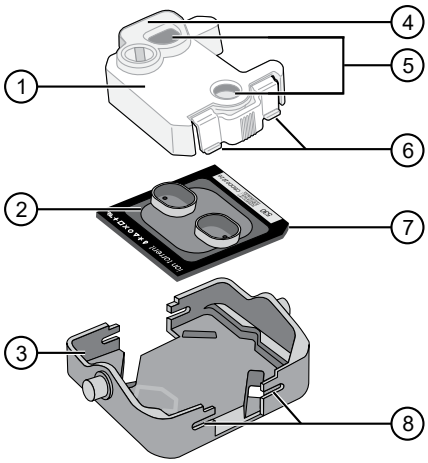
IMPORTANT! Confirm that the Enrichment Cartridge v2 is loaded so that the lettering on the cartridge is right-side-up.



① Enrichment station
② Enrichment Cartridge v2
③ Lettering

Load the Chip-loading centrifuge

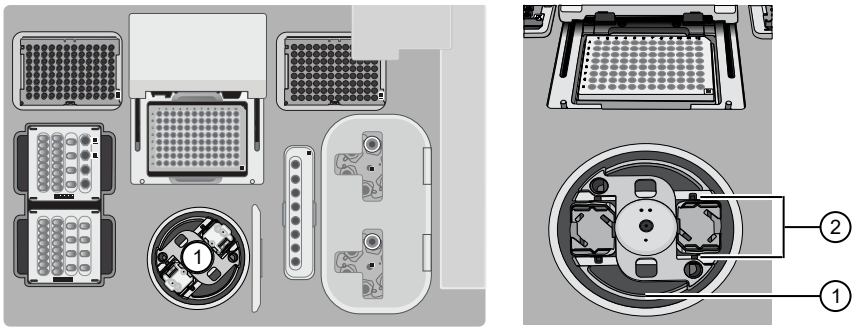
1. Load each chip that you will use for templating and sequencing into a centrifuge bucket, then attach a Chip Adapter to the assembly.
   a. Place the chip in the chip-loading bucket with the keyed corners of the chip and bucket aligned, then align the wells of the Chip Adapter to the wells of the chip, orienting the adapter onto the chip so that the chip barcode is visible.
   b. Place the adapter onto the chip, then insert the stationary tabs at the reservoir end of the adapter into the slots of the bucket.
   c. Gently squeeze the flexible tabs at the other end of the adapter into the bucket slots until the adapter locks into place.
   d. Ensure that the tabs at all four corners of the adapter are fitted into the slots in the centrifuge bucket. Loading can fail if the adapter is not attached securely.



① Chip Adapter
② Ion Chip™
③ Bucket
④ Reservoir end of Chip Adapter
⑤ Ports (align with chip)
⑥ Flexible tabs
⑦ Keyed corner (align with bucket)
⑧ Slots

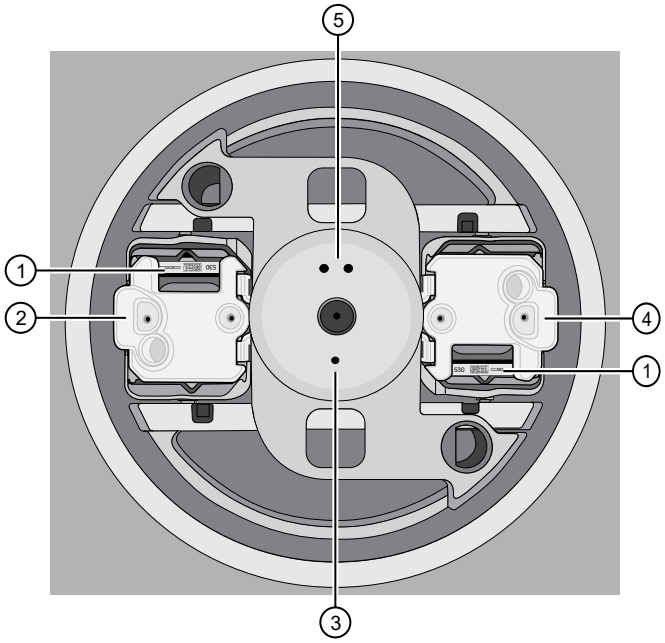
Note: If desired, you can label the tops of chips to distinguish them. Do not obstruct or overwrite the chip barcode with your label.

2. Load the adapter/chip/bucket assemblies into the Chip-loading centrifuge.



① Chip-loading centrifuge ② Mounting grooves

IMPORTANT! When the Chip-loading centrifuge is loaded, ensure that each Chip Adapter is firmly attached to a bucket, and that the buckets are securely seated in the centrifuge rotors.



① Chip barcode
② Chip position 1
③ Position 1 marker hole
④ Chip position 2
⑤ Position 2 marker hole

Note:

- Position 1 of the Chip-loading centrifuge is the position 90° clockwise from the single hole in the rotor bucket cover at rest. The chip that is loaded in Position 1 is loaded with ISPs prepared from the DNA library in the Ion Chef™ Library Sample Tube that is loaded in Position A of the Reagents cartridge.
- The chip that is loaded in Position 2 of the centrifuge is loaded with ISPs prepared from the DNA library in the Ion Chef™ Library Sample Tube that is loaded in Position B of the Reagents cartridge.
- If you are performing a mixed chip run, ensure that the chips are loaded in the appropriate positions of the centrifuge.

3. Ensure that the centrifuge is load-balanced and the chip buckets are securely seated and oriented correctly in the centrifuge so that they pivot 90° outwards when touched. Then close the lid of the Chip-loading centrifuge.

IMPORTANT! Do not obstruct or place any object on top of the lid.

Confirm that consumables are correctly installed

- Confirm that each cartridge is at the correct location and in the correct orientation.
- Press down on all cartridges to confirm that they are firmly pressed into place.
- Confirm that all tubes in the Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge, including the tube of NaOH in Position C, are uncapped and firmly pressed into place.
- Confirm that the centrifuge lids are installed correctly so that the port is oriented toward the rear of the instrument.
- Confirm that the tube and chip buckets are seated securely in the rotor arms of the Chip-loading and Recovery centrifuges, and that the consumables they contain are correctly installed.

CAUTION! To ensure correct and safe instrument operation, you must confirm that all consumables are installed correctly to the deck before you start a run. The Ion Chef™ Instrument does not verify all aspects of the consumable setup before beginning each run.

Single chip loading workflow

You can set up an Ion Chef™ run to load a single chip instead of two, using the Ion Chef™ S5 Series Chip Balance loaded opposite to the sequencing chip in the Chip-loading centrifuge. The Ion Chef™ S5 Series Chip Balance is provided in the Ion S5™ Installation Kit.

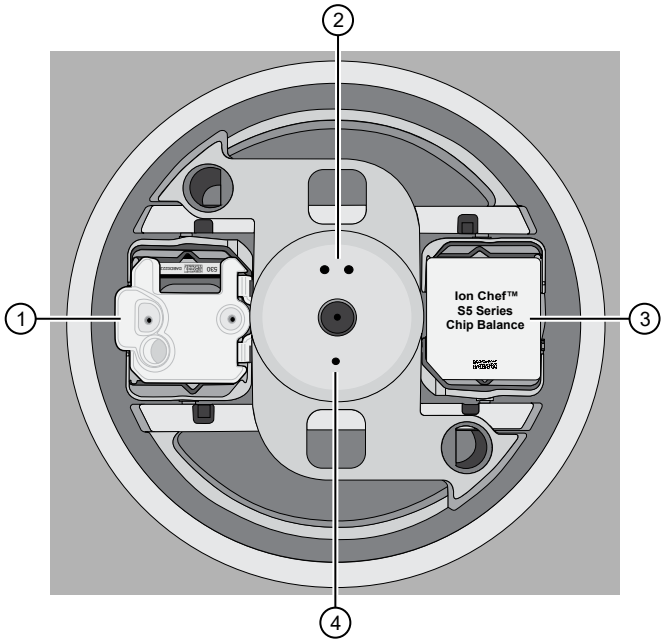
Ion Chef™ S5 Series Chip Balance

Load the Ion Chef™ Instrument as you would normally load the system. For single chip loading, perform the following steps:

1. Add the single diluted library or combined library to an Ion Chef™ Library Sample Tube, then load the tube into Position A of the Reagents cartridge.
2. Load an empty Ion Chef™ Library Sample Tube into Position B of the Reagents cartridge. Uncap both tubes.
3. Load the chip in Position 1 and the Ion Chef™ S5 Series Chip Balance in Position 2 of the Chip-loading centrifuge.

IMPORTANT! Do not use Ion Chef™ 314, 316/318, or P-Series versions of the chip balance with the Ion 510™ Chip, Ion 520™ Chip, or Ion 530™ Chip. Each chip balance is weight-matched to the chip (and corresponding chip adapter) specified on the chip balance label.

Note: Position 1 of the Chip-loading centrifuge is the position 90° clockwise from the single hole in the rotor bucket cover at rest.



① Position 1 (chip)
② Position 2 marker holes
③ Position 2 (Chip Balance)
④ Position 1 marker holes

4. Resume the normal workflow in “Load the Chip-loading centrifuge” at step 3. The Ion Chef™ Instrument detects the presence of the single chip during Deck Scan before the run starts.

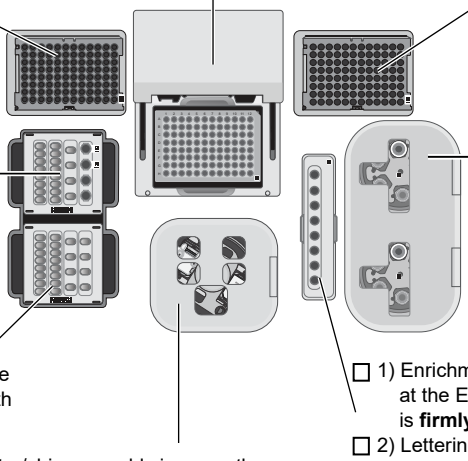
Ion Chef™ pre-run checklist

☐ 1) A new PCR Plate is loaded in the thermal cycler sample block.
☐ 2) A Frame Seal v2 is in place and oriented under the heated cover.

☐ 1) A new Ion Chef™ Tip Cartridge v2 is loaded in the New Pipette Tip position.
☐ 2) Bracket is pulled downward to lock the tip cartridge in place.

☐ 1) The tip rack from previous run is transferred from the New Pipette Tip position to the Used Pipette Tip position.

☐ 1) Reagents cartridge contents are thawed (leave at room temperature for 45 minutes).
☐ 2) **Reagents cartridge tubes are uncapped** and pressed into place at the Reagents station so that the tubes are **firmly seated** and **level** with the deck.
☐ 3) Library sample tubes are **firmly seated** and in the correct orientation with barcodes facing the PCR Plate and caps removed.



☐ 1) Recovery Tubes (v2) are securely seated in the Recovery centrifuge buckets.
☐ 2) Recovery centrifuges are load-balanced.
☐ 3) Recovery Station Disposable Lids (v2) are positioned so that the port is oriented toward the rear of the instrument.

☐ 1) Solutions cartridge is pressed into place at the Solutions station so that the cartridge is **firmly seated** and **level** with the deck.

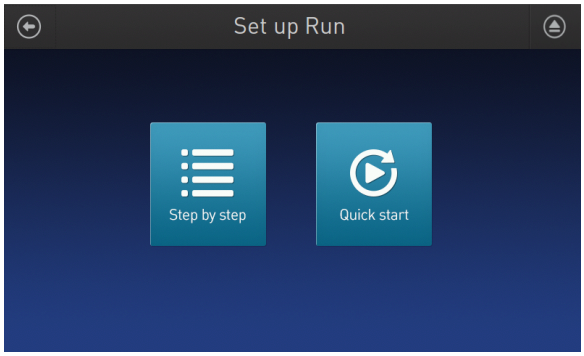
☐ 1) Enrichment Cartridge v2 is pressed into place at the Enrichment station so that the cartridge is **firmly seated** and **level** with the deck.
☐ 2) Lettering on the cartridge is right-side up and positioned to the right of the enrichment tubes.

☐ 1) Adapter/chip assembly is correctly seated in the Chip-loading centrifuge buckets, with the adaptor clips inserted into the bucket slots.
☐ 2) Centrifuge buckets containing an adapter/chip assembly or chip balance are securely seated in the centrifuge rotor, and freely pivot 90º outwards.
☐ 3) Chip-loading centrifuge is load-balanced.

Start the Ion Chef™ run

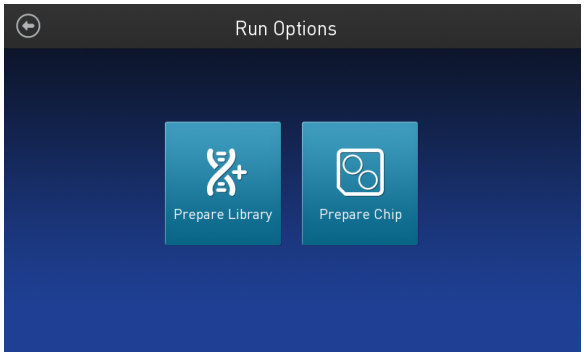
1. Ensure that you have loaded the instrument with all kits and consumables.
2. On the Ion Chef™ Instrument home touchscreen, tap **Set up run**.

3. Tap **Step by step** to have the instrument lead you through the instrument setup, or tap **Quick Start** to skip the instrument setup screens and proceed to Deck Scan.



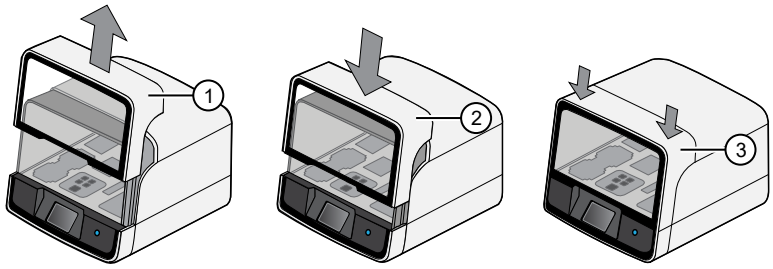
If you selected **Quick Start**, proceed to step 5, otherwise proceed to step 4.

4. *(**Step by step** setup only)* In the **Run Options** screen, tap **Prepare Chip** to select the templating run option.



5. Follow the on-screen instructions. When prompted, close the instrument door by first lifting it slightly to disengage the locking mechanism, then push down on the door until the locks engage.

IMPORTANT! Do not close the door by pulling it straight down from the open position. You must lift the door slightly before you can close it. Ensure that both sides of the door are locked after closing it.



① Open door position
② Closing door position
③ Locked door position

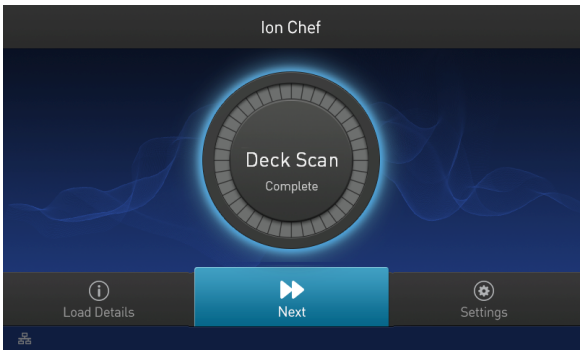
After the door closes, the instrument vision system activates.

6. When prompted, tap **Start check** to start Deck Scan. Wait while the instrument scans the barcodes of all consumables and reagents to ensure their presence and compatibility.

During Deck Scan, the touchscreen displays warnings if the Ion Chef™ Instrument detects missing or incompatible consumables. You must address all warnings before the run can start. After you address each condition, tap **Yes** to continue.

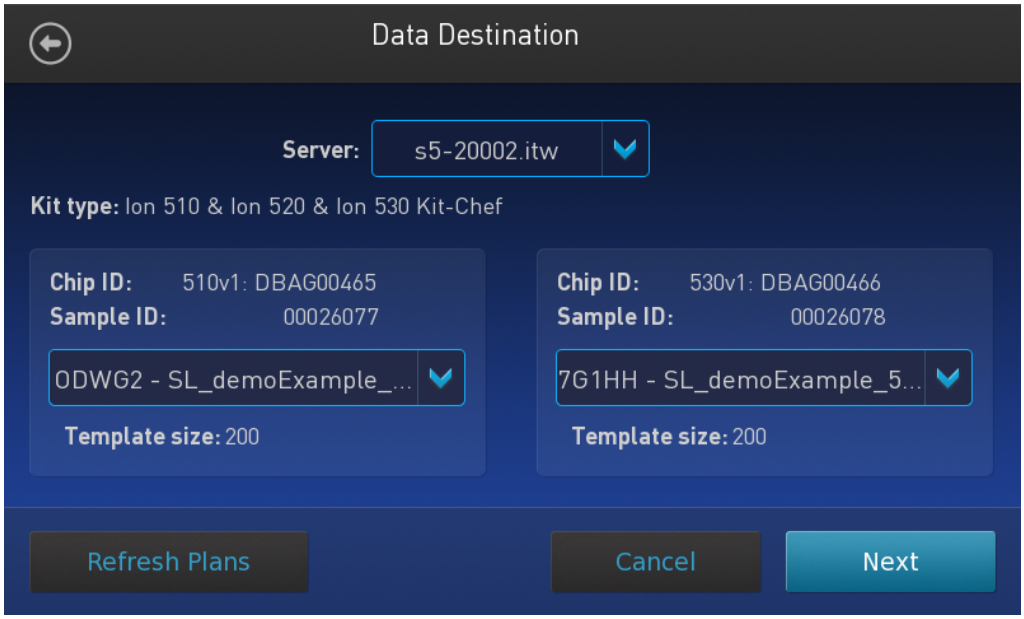
IMPORTANT! The Deck Scan function is not a substitute for manual inspection of the reagents and consumables on the Ion Chef™ Instrument before starting a run. To ensure proper and safe instrument operation, ensure that all consumables are installed correctly before you continue.

7. When Deck Scan is complete, tap **Next** to display the **Data Destination** screen.



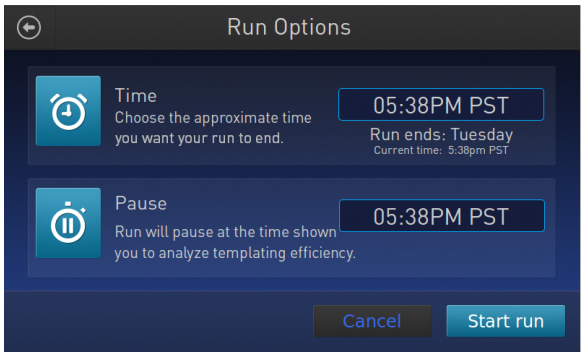
8. Ensure that the instrument displays the correct kit types, chip positions (mixed-chip run), chip type, chip barcodes, and Planned Run. If the correct Planned Runs do not display, tap the dropdown list to select the Planned Run for each chip, then tap **Next.**

Note: If the Ion Chef™ Instrument does not auto-populate the correct Planned Run, ensure that the Sample Tube Label has been entered correctly in the Planned Run.



IMPORTANT! If the kit and chip type are not correct, ensure that you are using the correct kit and chip. If you are using the correct kit and chip, and an incorrect kit or chip type is displayed on the screen, contact Technical Support.

9. On the **Run Options** screen, tap the appropriate option to complete the run, then enter the desired time of run completion if needed.



The Ion Chef™ Instrument provides two options for obtaining quality control (QC) samples that can be used to evaluate templating efficiency. Depending on your selection, the QC samples are made available either during or after the run. In either case, you can obtain unenriched samples from the corresponding Library Sample Tubes at Positions A and B on the Reagents cartridge, or enriched samples from Positions A and E on the Enrichment Cartridge v2.

By selecting	You can obtain the QC samples	Approximate time after run start
Time	immediately after the run ends, at the time you specify:	• 200–bp libraries: 14 hours 40 minutes. • 400–bp libraries: 13 hours 40 minutes.
Pause	when the instrument pauses operation before the chip loading step:	• 200–bp libraries: 13 hours 15 minutes. • 400–bp libraries: 12 hours 15 minutes.

Note:

- The library in the Ion Chef™ Library Sample Tube loaded in Position A of the Reagents cartridge is templated onto ISPs that can be sampled in Position E of Enrichment Cartridge v2 after a run. The library in the Ion Chef™ Library Sample Tube loaded in Position B is templated onto ISPs that can be sampled in Position A of Enrichment Cartridge v2.
- Select **Pause** if you are uncertain of library quality and want to evaluate templating efficiency before chips are loaded. If you do not pause the run, you can collect QC samples after the run. Save the samples until sequence analysis is complete to have them available for troubleshooting.

10. On the **Run Options** screen, tap **Start run** to start the run.

Note: If you must stop the run for any reason, tap **Cancel**, then tap **Yes** to confirm the cancellation.

If the Ion Chef™ Instrument encounters a problem during the run, it aborts the run and displays the error on the instrument touchscreen. If a run fails, perform the following tasks.

a. Remove the consumables from the deck, then clean the instrument. If possible, retain the consumables for troubleshooting.

b. Reset, then attempt to start the run again. If the run fails again, contact Technical Support to troubleshoot the problem. Record the error message for reference.

11. Initialize the sequencer at least 50 minutes before the Ion Chef™ System finishes chip loading. For more information, see Chapter 5, “Initialize the sequencer”.

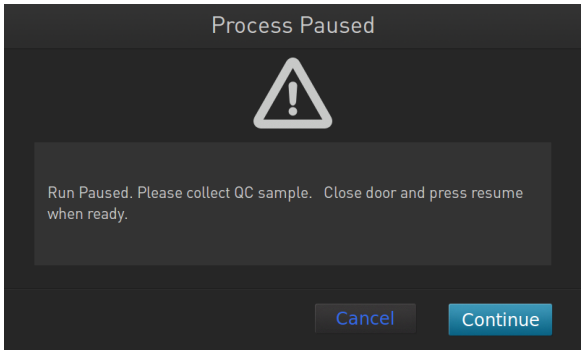
Initialization of the instrument can be performed up to 24 hours before starting a sequencing run. If the intent is to perform two sequencing runs per initialization, the first run must be completed and the second run must be started within the 24-hour period. By initializing the sequencer before completion of chip loading, you ensure that the chips can be sequenced as soon as possible after loading is complete.

12. If you chose to pause the run to analyze the templating efficiency, remove the samples for testing when prompted to do so by the Ion Chef™ Instrument.

Note: The time varies for different libraries.

- 200–bp libraries: 13 hours 15 minutes.
- 400–bp libraries: 12 hours 15 minutes.

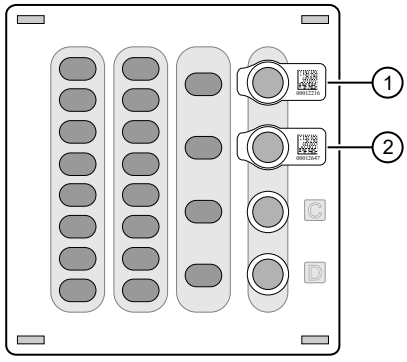
a. When prompted to remove the QC sample, open the instrument door.



IMPORTANT! If you unintentionally close the instrument door before you obtain the QC samples, you must wait until the end of the run before you can collect them. You cannot pause the run or open the door after it has been closed.

b. Transfer the entire volume of each QC sample from Positions A and B of the Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge on the instrument deck to two new labeled microcentrifuge tubes.

IMPORTANT! Do not remove the Library Sample Tubes from the Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge.



① Position A (QC Sample)
② Position B (QC Sample)

c. If you are performing quality assessment of enriched samples, transfer QC samples from positions A and E of the Enrichment Cartridge v2 to two new labeled microcentrifuge tubes.

d. Analyze the QC samples. For more information, see “Quality control of Ion 510™ ISPs, Ion 520™ ISPs, or Ion 530™ ISPs” on page 70.

e. Close the instrument door, then tap **Continue** to complete the run.

13. When the run is complete, unload the Ion Chef™ Instrument and sequence the chips immediately. You can collect QC samples from the Reagents and/or Enrichment cartridges if you have not done so already.

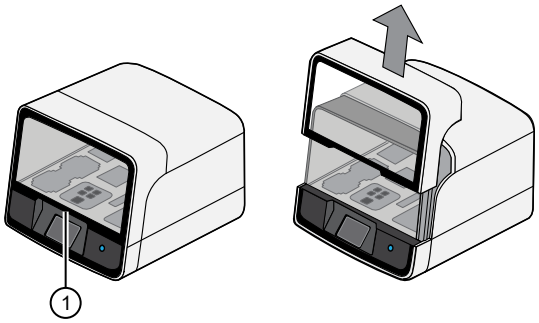
IMPORTANT! Liquid can be present in the chip wells after the Ion Chef™ run. Do NOT remove any residual liquid from the wells.

Note: If you cannot sequence a loaded chip immediately or plan to sequence two chips per initialization, place the chip into a separate chip storage container and store at 4°C until you are ready to sequence it (up to 6–8 hours maximum).

Unload the chips for sequencing

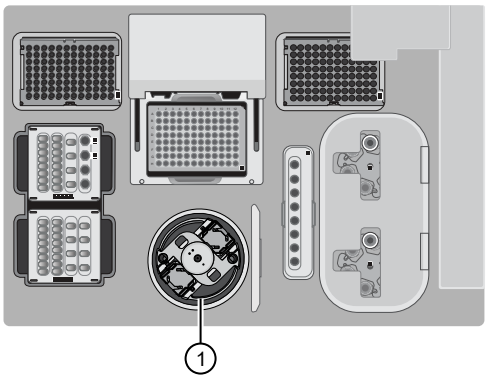
1. Open the instrument door.
   a. In the instrument touchscreen, tap ⏏ **(Open Door)**, then wait for the latch to open.
   b. Lift the instrument door to the top of the travel until the latch mechanism engages.

IMPORTANT! Lift the door from the center.

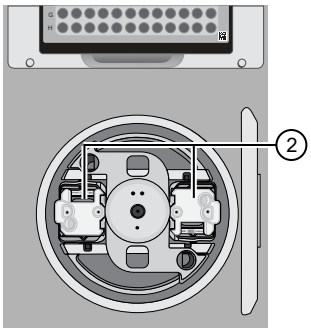


① Center of door

2. Open the lid of the Chip-loading centrifuge, then unload both adapter/chip/bucket assemblies from the instrument.

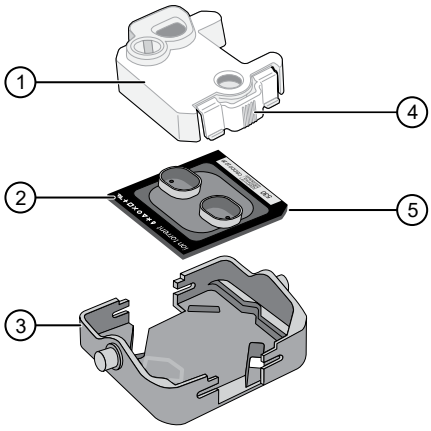


① Chip-loading centrifuge



② Adapter/chip/bucket assemblies

3. Unload each chip from the adapter/chip/bucket assembly.
   a. Apply pressure to both ends of the Chip Adapter, then remove and discard the Chip Adapter.
   b. Grasp the chip by its edges, carefully lift the chip out of the bucket, then set it aside on a clean, static-free surface. Return the bucket to the Chip-loading centrifuge.

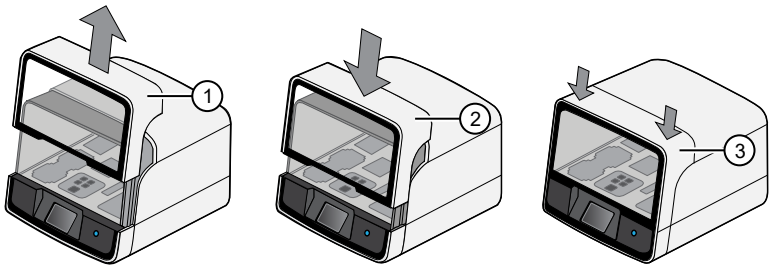


Chip Adapter

① Chip Adapter
② Chip
③ Bucket
④ Flexible tabs
⑤ Keyed corner of chip

4. Close the instrument door by first lifting it slightly to disengage the locking mechanism, then push down on the door until the locks engage.

IMPORTANT! Do not close the door by pulling it straight down from the open position. You must lift the door slightly before you can close it. Ensure that both sides of the door are locked after closing it.



① Open door position
② Closing door position
③ Locked door position

5. Load one or both chips into a sequencer, then promptly start the sequencing run or runs.

   If you cannot sequence a loaded chip immediately or plan to sequence two chips per initialization, place the chip into a separate chip storage container and store at 4°C until you are ready to sequence it (up to 6–8 hours maximum).

IMPORTANT!

- Liquid may be present in chip wells after the Ion Chef™ run. Do NOT remove any residual liquid from the wells.
- If you choose to store a loaded chip, remove the chip from 4°C storage (but keep it in the storage container) at least 20 minutes before running it, allowing the chip to warm to room temperature.

5 Initialize the sequencer

Initialization takes ~50 minutes.

Ion S5™/Ion GeneStudio™ S5 Systems component positions

1. Touchscreen
2. Power button
3. Ion S5™ Sequencing Reagents cartridge
4. Chip clamp
5. Ion S5™ Wash Solution bottle. Waste reservoir located behind the Ion S5™ Wash Solution bottle (shown on the right).
6. Ion S5™ Cleaning Solution bottle
7. Waste reservoir

Note:

- The system uses RFID technology to verify that the proper reagents are loaded in positions 3, 5, and 6. Reagents that exceed their expiration date or usage count generate an error message prompting the user to replace the reagent before performing the run.
- RFID regulatory information can be found on the main screen under **Options** ▸ **Regulatory info**.

Before you begin

The Ion S5™ Sequencer, Ion S5™ XL Sequencer, and Ion GeneStudio™ S5 Series Sequencer are equipped to verify the compatibility of each chip and consumable that is loaded during initialization and sequencing, and that these components do not exceed their expiration date. To avoid exceptions during initialization, inspect this information for each consumable before installing onto the instrument.

- Unbox the Ion S5™ Sequencing Reagents cartridge 45 minutes before use, then allow it to equilibrate to room temperature.

  Do not remove the Ion S5™ Sequencing Reagents cartridge from its packaging until immediately before loading, so that you can return the unused cartridge to storage if your sequencing run is delayed.

- Unbox the Ion S5™ Wash Solution bottle. Invert the bottle 5 times within its vacuum-sealed bag, then swirl at an angle to mix thoroughly.

- Remove the Ion S5™ Wash Solution bottle from its vacuum-sealed bag, then remove the red cap from the Ion S5™ Wash Solution and Ion S5™ Cleaning Solution bottles immediately before installing on the instrument.

When a manual cleaning of the sequencer is required

The Ion S5™ Sequencer, Ion S5™ XL Sequencer, and Ion GeneStudio™ S5 Series Sequencer require that a cleaning be performed before initialization. Cleaning is normally performed automatically at the completion of the previous sequencing run. When two sequencing runs are performed on a single initialization, the post-run cleaning is performed after the second sequencing run. However, if the "Enable post-run clean" checkbox (see “Start the sequencing run”) is deselected to allow a second run, and a second run is not performed, the instrument will not allow the subsequent initialization to proceed until a manual cleaning has been performed. For more information on how to perform a manual cleaning, see “Perform a manual cleaning of the sequencer” on page 68.

If the sequencer is initialized and a sequencing run is not started within 24 hours, or a run is not started or completed due to a power failure or an abort, do not perform a manual cleaning. An instrument reset run is required before reinitialization. For more information, see “Perform an instrument reset run with an initialized sequencer that is loaded with an unused Reagents cartridge” on page 69.

Initialize the sequencer

1. In the instrument touchscreen main menu, tap **Initialize**.

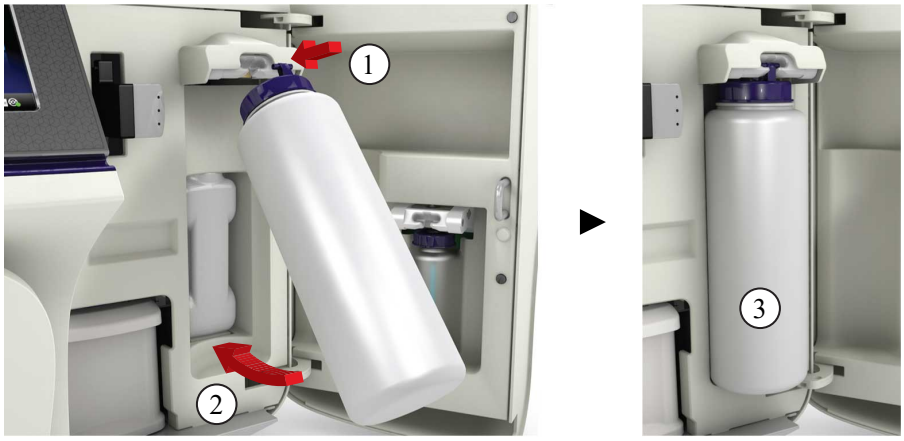
   The door, chip, and Reagent cartridge clamps unlock.

2. When prompted, remove the Ion S5™ Wash Solution bottle to access the waste reservoir, then remove and empty the waste reservoir.

3. Reinstall the empty waste reservoir.

4. Replace the expended Ion S5™ Sequencing Reagents cartridge with a new cartridge equilibrated to room temperature.

5. Ensure the new Ion S5™ Wash Solution bottle is thoroughly mixed. Then remove the red cap and install.



6. Ensure that the used sequencing chip from the previous run is properly seated in the chip clamp and the chip clamp is pushed in all the way.

7. If necessary, install a new Ion S5™ Cleaning Solution bottle.

   Note: The Ion S5™ Cleaning Solution bottle contains sufficient reagent to complete 4 cleanings.

8. Close the door, then tap **Next**.

   The instrument confirms that the consumables and chip are properly installed and that the Ion S5™ Cleaning Solution contains sufficient reagent to perform the post-run clean. Follow all on-screen recommendations to ensure proper installation of required consumables.

   IMPORTANT! If the allowed number of post-run cleans has been met, the instrument prompts the user to replace the Ion S5™ Cleaning Solution bottle.

   Note: If a **Checking Reagent: Failed** warning appears, see “Reagent Check fails” on page 65.

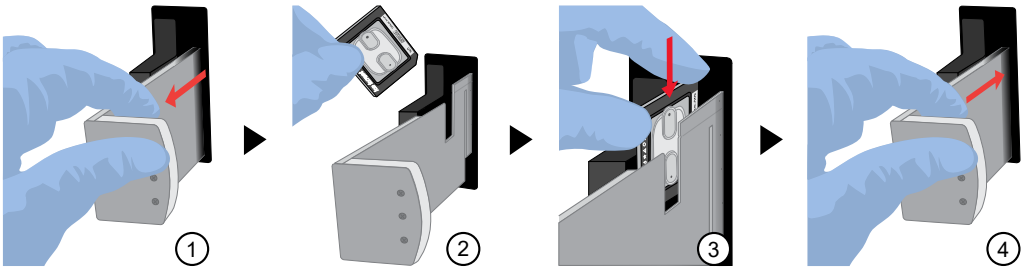
9. When initialization is complete (~50 minutes), tap **Home**.

The instrument is now ready for a sequencing run.

For information on recycling and disposal of used components, see “Reagent consumables disposal” on page 53.

6 Start the sequencing run

Place chip in chip clamp



1. Slide the chip clamp out.
2. Remove the chip currently in the clamp.
3. Place the appropriate loaded chip in the chip clamp with the chip notch in the bottom-front corner.

   Note: Do not force the chip into the clamp. If the chip does not fit easily in the clamp, ensure that the notch is oriented as shown in the drawing.

4. Slide the metal tab in fully to engage the clamp, then close the instrument door.

Start the sequencing run

We recommend that you start a sequencing run as soon as possible after chip loading and instrument initialization are complete. However, successful sequencing runs can be started up to 24 hours after instrument initialization.

IMPORTANT!

- Do *not* press the power button during a run. Interrupting power to the instrument during a run can result in sequencing run failure and loss of sample.
- We recommend that you verify the status of the Planned Run you will use as "Planned" before inserting the loaded chip. If your Planned Run does not advance to the Planned status in Torrent Suite™ Software after successful completion of an Ion Chef™ run, see “Planned Run status does not advance to "Planned"” on page 63.

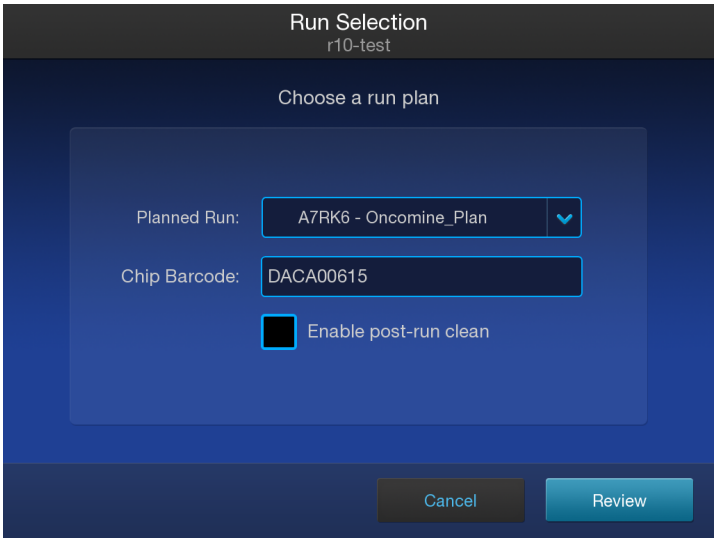
1. After completion of initialization, tap **Run** in the instrument touchscreen. The door and chip clamp unlock.
2. Remove the used sequencing chip, then secure a chip loaded with template-positive Ion Sphere™ Particles in the chip clamp.
3. Push the chip clamp all the way in to engage, close the instrument door, then tap **Next**.

   Note: Do not remove the chip from the chip clamp until completion of the run. Removing and reinserting the chip risks introducing air bubbles in the chip.

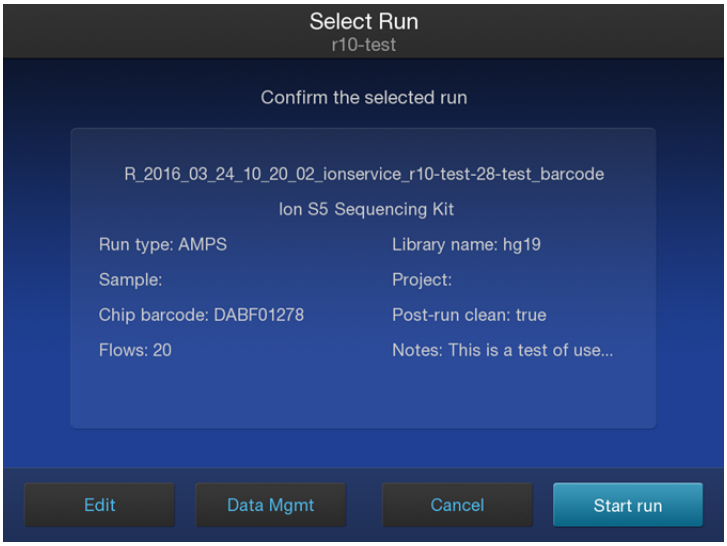
4. Confirm that the correct Planned Run has auto-populated. If this run is the first of two sequencing runs on this initialization, **deselect** the **Enable post-run clean** checkbox, then tap **Review**.

   IMPORTANT!

   - Failure to deselect the checkbox results in a cleaning performed automatically after the first run. A second run is not available.
   - When starting the second sequencing run on a single initialization, ensure that the **Enable post-run clean** checkbox is selected so that the post-run cleaning is performed automatically.



5. Confirm that the remaining pre-populated settings are correct, or tap **Edit** to make changes if needed.



6. Confirm that the instrument door is closed, then tap **Start run** to begin the sequencing run.

IMPORTANT! During a run, do not open the instrument door, and avoid touching the instrument. Touching the instrument during the sequencing run can reduce the quality of the measurements.

When the sequencing run is complete, the instrument automatically performs the cleaning procedure unless the **Enable post-run clean** checkbox was deselected. After cleaning, the touchscreen returns to the main menu. Use Torrent Suite™ Software to review the results.

If you are sequencing a second chip on a single initialization, start the second run within 24 hours of start of initialization.

Maintain the sequencer

Materials required

- Lint-free wipes
- 70% isopropanol
- *(Optional)* 10% bleach solution

Clean or decontaminate the sequencer

In the event of a spill or leak on or inside the instrument, perform the following steps.

Note: Dispose of all waste in appropriate liquid or solid waste containers.

1. Remove the Ion S5™ Wash Solution bottle, then remove and empty the waste reservoir.
2. Remove the Ion S5™ Sequencing Reagents cartridge.
3. Inspect the waste and nucleotide reagent bays for liquid.
4. Using absorbent paper soak up as much liquid as possible, then wash the affected area with 10% bleach solution.
5. Wipe the affected surfaces with 70% isopropanol, then allow to air-dry.

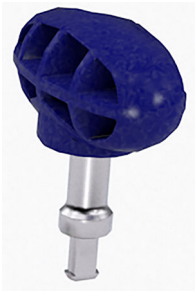
Reagent consumables disposal

IMPORTANT! Follow all applicable local, state/provincial, and/or national regulations when recycling or disposing of Ion S5™ reagent consumables.

CO_2 scrubber removal and disposal

To properly dispose of the CO_2 scrubber, you must first remove it from the Ion S5™ Sequencing Reagents cartridge.

1. Invert the Ion S5™ Sequencing Reagents cartridge over an appropriate receptacle to drain all residual liquid.
2. Wearing gloves, insert the Ion S5™ Cartridge Tool firmly into the CO_2 scrubber until the flange stops on the top of the cartridge.



Ion S5™ Cartridge Tool

3. Pull straight up on the tool while holding the nucleotide reagent cartridge down.

4. Remove the scrubber from the cartridge tool, then dispose of the scrubber according to applicable hazardous waste regulations.

The remaining nucleotide reagent cartridge should be disposed of appropriately.

Recycle Ion S5™ Wash Solution and Ion S5™ Cleaning Solution bottles

The Ion S5™ Wash Solution and Ion S5™ Cleaning Solution bottles are made of recyclable plastic.

1. Open the expended bottle by unscrewing the cap.
2. Remove the cap, sipper, and filter, then pour any residual liquid into an appropriate receptacle.
3. Rinse the empty bottle with water. Pour out the rinse water into the same liquid waste receptacle.
4. Recycle or dispose of the clean bottle, cap, sipper, and filter according to applicable regulations.

7 Clean the Ion Chef™ System

About the cleaning protocol

The Ion Chef™ System includes an automated cleaning function that must be performed following every run. The cleaning routine is initiated from the Ion Chef™ Instrument touchscreen and is designed to minimize potential contamination. During the routine, the instrument irradiates the deck with ultraviolet light for 1 minute after all consumables have been removed from the instrument.

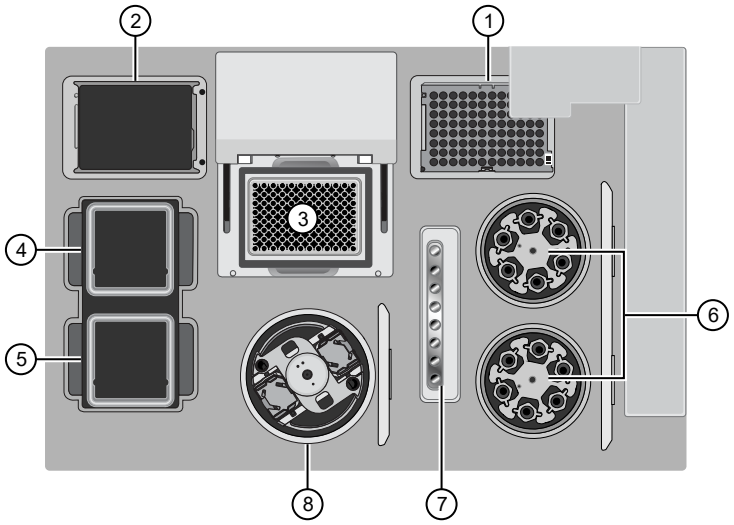
IMPORTANT! Although the Ion Chef™ Instrument cleaning routine provides some protection against contamination, it is not a substitute for good laboratory technique or precautions. When preparing DNA libraries for use or when preparing the Ion Chef™ Instrument, make certain to observe sterile laboratory procedures at all times to ensure minimal contamination.

Materials required

- Gloves, powder-free nitrile
- Isopropanol, 70% solution
- Wipes, lint-free

Clean the Ion Chef™ Instrument

IMPORTANT! Clean the Ion Chef™ Instrument as described in the following pages after every run. To prevent contamination, do not operate the instrument unless it has been recently cleaned.



Ion Chef™ Instrument stations

1. Waste pipette tip position
2. Empty Tip Cartridge v2: move to waste pipette tip station
3. Thermal cycler sample block
4. Reagents station
5. Solutions station
6. Recovery centrifuges
7. Enrichment station
8. Chip-loading centrifuge

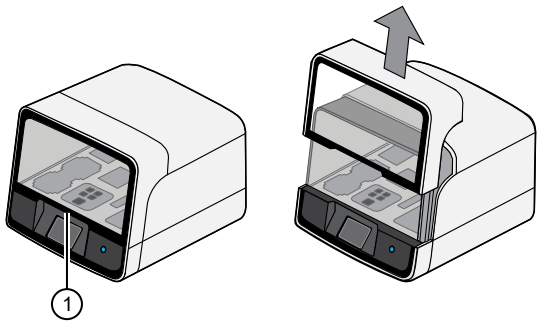
Remove and dispose of used consumables

IMPORTANT!

- Do not discard the empty Tip Cartridge v2.
- Make sure to transfer the QC samples before you remove and discard the Reagents cartridge.

1. Tap ⏏ **(Open Door)** in the instrument touchscreen, then wait for the latch to open.
2. Lift the instrument door by the door handle to the top of the travel until the latch mechanism engages.

   IMPORTANT! Lift the door from the center.

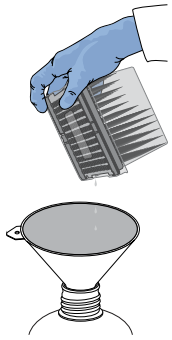


1. Center of door

3. Remove, then discard the PCR Plate from the thermal cycler sample block.

4. Remove, then discard the box of used pipette tips from the waste tip position.

IMPORTANT! Handle the disposable reservoir in the waste tip position with care. During the run, liquid waste collects in the reservoir. Dispose of the liquid waste by tipping the reservoir on one corner and pouring the waste into an appropriate waste container:



IMPORTANT! Do not reuse the waste pipette tip rack. Always move the empty Tip Cartridge v2 from the new tip position to the waste tip position.

5. Move the empty Tip Cartridge v2 to the waste tip position.

6. Remove, then discard the
   - Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge
   - Ion S5™ Chef Solutions cartridge
   - Enrichment Cartridge v2

7. Remove, then discard the consumables from the Recovery centrifuges, including the:
   - Recovery Station Disposable Lid v2
   - Recovery Tubes v2

8. Close the Chip-loading centrifuge cover.

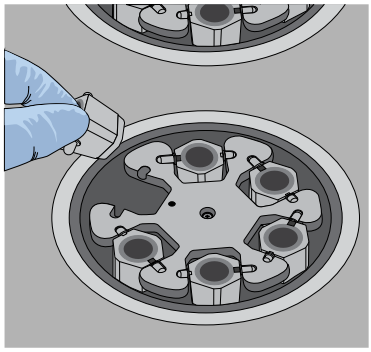
Inspect and clean the Recovery centrifuges and buckets

1. Inspect the Recovery centrifuges, then clean the components if excess liquid is present.

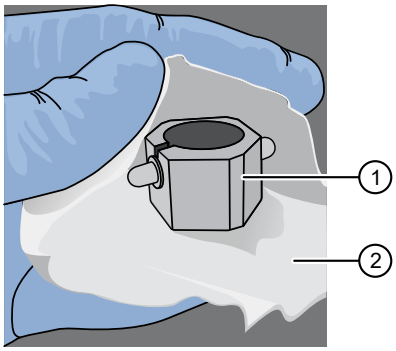
Is liquid present?	Action
No	Proceed to "Start the cleaning" on page 59.
Yes	Clean the centrifuge bowl and buckets as described below. **IMPORTANT!** Clean the Recovery centrifuges occasionally, only when excess liquid is noticeable in the bowl and/or buckets. You do *not* need to clean the centrifuges after every run.

IMPORTANT! Wear powder-free, nitrile gloves when cleaning the Recovery centrifuge.

2. Remove the buckets from the Recovery centrifuges. Clean the inside and outside of each bucket using a lint-free wipe, then place the buckets on a clean, dry surface while you clean the centrifuge.

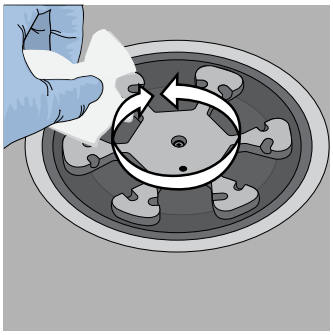


① Bucket

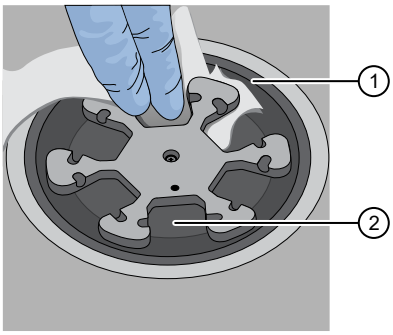


② Lint-free wipe

3. Use lint-free wipes to remove all fluid from inside the centrifuge bowl.



① Inside rim of the centrifuge

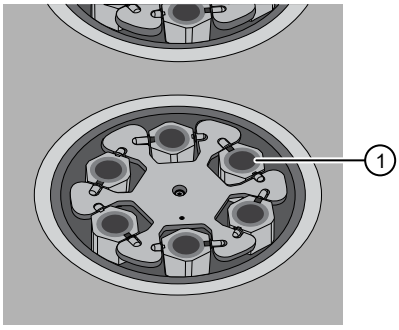


② Bottom of the centrifuge bowls

4. Use lint-free wipes treated with 70% isopropanol to clean the following parts.
   - Inside rim of the centrifuge.
   - Bottom of the centrifuge bowl.
   - Outside and inside of the centrifuge buckets.

5. Dry the centrifuge and buckets with lint-free wipes.

6. Install the centrifuge buckets, then close the Recovery centrifuge cover.



① Buckets (cleaned and installed)

Start the cleaning

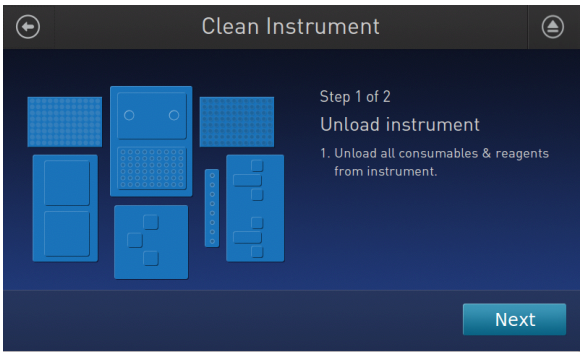
1. Close the instrument door by first lifting it up slightly to disengage the locking mechanism, then pushing down on the door until the locks engage.

IMPORTANT! Before closing the door, ensure the covers of the Chip-loading and Recovery centrifuges are closed.

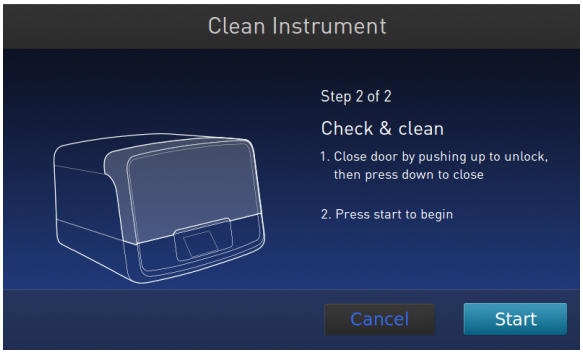
2. To start the cleaning, tap **Next** on the Ion Chef™ Instrument touchscreen that appears after run completion.

Note: You can also clean the instrument at any time starting from the home touchscreen. Tap **Settings ▸ Clean Ion Chef**.

3. Confirm that you have removed all consumables from the Ion Chef™ Instrument, except the empty pipette tip rack in the waste tip position, then tap **Next**.



4. With the door closed, tap **Start**. The instrument performs a Deck Scan before starting the cleaning routine. The Ion Chef™ Instrument stops ventilation, then illuminates the ultraviolet (UV) light in the instrument for ~1 minute.



CAUTION! The Ion Chef™ Instrument emits UV light at 254 nm. Wear appropriate eye wear, protective clothing, and gloves when working near the instrument. Do not look directly at the UV light while it is illuminated during the cleaning routine.

A Troubleshooting

View troubleshooting and FAQs online

Visit our online Support Centers and FAQ database for tips and tricks for conducting your experiment, troubleshooting information, and FAQs. The online FAQ database is frequently updated to ensure accurate and thorough content.

- For the Next–Generation Sequencing Support Center: **http://thermofisher.com/ngssupport**
- For FAQs for this product: **http://thermofisher.com/A34461faqs**
- To browse the FAQ database and search using keywords: **thermofisher.com/faqs**

Ion Chef™ System setup and operation

Observation	Possible cause	Recommended action
Instrument does not display the home screen when powered on	Multiple causes are possible.	1. Power off the instrument, wait 30 seconds, then power on the instrument. 2. If the instrument fails again, contact Technical Support.
The instrument door cannot be opened	An obstruction was present on or around the door mechanism.	Remove the obstruction blocking the door, then operate the instrument normally.
	A hardware or software error occurred.	Contact Technical Support to report the problem and for further assistance.
The instrument stops during a run	The instrument encountered an internal error.	1. Record the error displayed on the instrument display, then tap **OK**. 2. Contact Technical Support to report the problem and for further assistance.
	A consumable was improperly loaded.	Ensure that all consumables are loaded according to the instructions provided.

Observation	Possible cause	Recommended action
Liquid residue is present in the Recovery centrifuges following a run	During normal instrument operation, a noticeable coating of liquid collected on the bowl and buckets of the Recovery centrifuges after repeated runs.	Remove the residue as instructed in "Inspect and clean the Recovery centrifuges and buckets" on page 57.
Instrument will not begin a run	The instrument has encountered a Deck Scan error (one or more consumables are absent or loaded improperly).	1. Confirm that the touchscreen does not display any Deck Scan warnings. If alarms are present, note the error(s) displayed, replace the missing consumable as directed, tap **No** when prompted then tap **Next** to cancel the run. After returning to the home screen, restart the run. 2. If the error persists, confirm that: • All buckets are seated correctly in the rotors of the Recovery and Chip-Loading Centrifuges. • All cartridges are loaded correctly and are level on the instrument deck. • The barcodes of the Library Sample Tubes are visible and positioned correctly. • All tubes are both present *and* uncapped on the Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents (sample tubes, NaOH tube, and the empty tube). 3. If the error persists after you check the consumables on the instrument deck, do one of the following: • If you are confident that the Ion Chef™ Instrument is set up correctly and you are comfortable disregarding the warnings, tap **YES** following Deck Scan to proceed with the run. • If the instrument cannot begin the run, contact Technical Support for further assistance.
	The instrument has encountered an internal error.	1. Record the error displayed on the instrument display, then tap **OK**. 2. Contact Technical Support to report the problem and for further assistance.

Observation	Possible cause	Recommended action
Instrument displays one or more alerts during a run	The instrument detected one or more problems during the run.	After the instrument completes the run, contact Technical Support. If possible, capture an image of the alert or error message to help troubleshoot. **IMPORTANT!** The detected problem might impact the performance of the sequencing run.

	• Network connection to the server was interrupted.
	• User name or password was incorrect.
		1. Tap the Instrument status button to view the alert(s).
		2. In the Instrument status screen, confirm that the name of the Torrent Server connection is red.
		3. Contact your network administrator to confirm that:
		  • The Torrent Server can be accessed from the network port used by the Ion Chef™ Instrument. If not, troubleshoot the network connection.
		  • The user name and password used by the Ion Chef™ Instrument are valid. If not, contact the server administrator to renew the credentials.
		4. If the alert persists, capture an image of the alert or error message, if possible, to help troubleshoot, then contact Technical Support for further assistance.

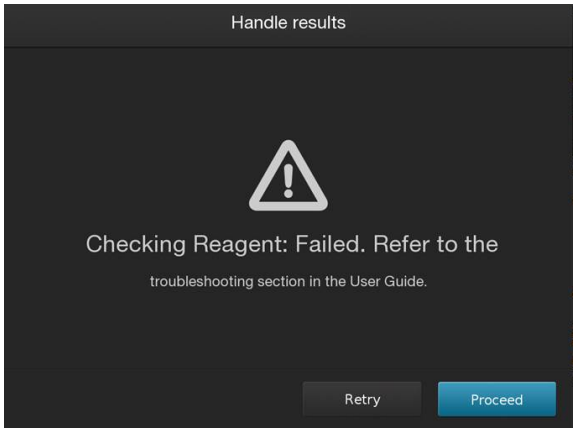
Planned Run status does not advance to "Planned" **Details:** The status of a successfully completed run remains listed as "Reserved" in Torrent Suite™ Software.	Connectivity was temporarily lost or interrupted between the Ion Chef™ Instrument and Torrent Suite™ Software.	Manually change the status of the run to "Planned". 1. Sign in to the Torrent Suite™ Software. 2. In the **Plan** tab, click **Planned Runs**. 3. For the Planned Run of interest, click ⚙ ▸ **Completed on Chef**. The status changes to "Planned".

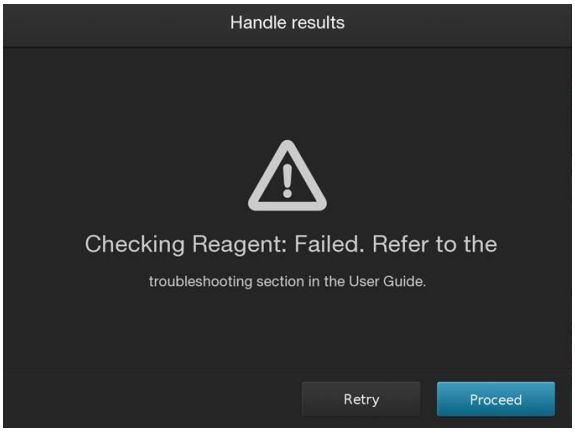
Ion S5™/Ion GeneStudio™ S5 Systems setup and operation

Instrument alarms and events

Observation	Possible cause	Recommended action
Red "Alarms" and/or "Events" message in Main Menu 	• Available software updates were detected. • Connectivity issues were detected. • Instrument did not detect required files or hardware.	Tap the red **Alarms** icon to see detailed messages. • If a message states "Newer Software Available": **IMPORTANT!** After updates are installed, the instrument must be restarted. a. In the main menu, tap **Settings** ▸ **Check for Updates**. b. Select the **Released Updates** checkbox, then tap **Update**. c. When installation is complete, follow the onscreen prompts to restart the instrument. **Note:** In some cases, the instrument restarts automatically after software installation. • If a message states "No Connectivity to Torrent Server", "No Connectivity to ftp server", or "Network Manager not connected", disconnect and re-connect the ethernet cable, confirm that the router is operational, and verify that the network is up and running. • For any other messages: a. Power off the instrument: On the home screen, tap **Settings** ▸ **System Tools** ▸ **Shut Down** ▸ **Shut Down**. b. Wait 30 seconds, then press the button on the side of the instrument to power on the instrument. • If the red "Alarms" and/or "Events" message still appears in the main menu, contact Technical Support.

Initialization—General errors

Observation	Possible cause	Recommended action
Chip Check fails	• The chip clamp was not closed. • The chip was not properly seated. • The chip was damaged.	1. Open the chip clamp, remove the chip, and look for signs of water outside the flow cell. 2. If the chip appears damaged, replace it with a new one. 3. Close the clamp, then repeat the Chip Check. 4. If the chip passes, click **Next**. If the chip fails, replace it with a new chip, then press **Chip Check**. 5. If Chip Check continues to fail, there could be a problem with the chip socket. Contact Technical Support.
Reagent Check fails 	A chip failure was detected.	1. Replace the used sequencing chip used during initialization with a different used chip. **IMPORTANT!** If you are using Torrent Suite™ Software version 5.12.1 or earlier and if a used Ion 550™ Chip was used for initialization, replace the used chip with a used Ion 510™ Chip, Ion 520™ Chip, Ion 530™ Chip, or Ion 540™ Chip for the initialization. Using an Ion 550™ Chip causes an error. 2. Press **Retry**. 3. If the initialization completes without failure, touch **Home**, then continue with your sequencing run. 4. If the Reagent Check continues to fail, contact Technical Support.

Observation	Possible cause	Recommended action
Reagent Check fails  *(continued)*	A wash failure was detected.	1. Perform a manual cleaning of the sequencer, see "Perform a manual cleaning of the sequencer" on page 68. 2. Replace the Reagents cartridge, Wash Solution bottle, and Cleaning Solution bottle, then repeat initialization of the sequencer. 3. If the initialization completes without failure, press **Home**, then continue with your sequencing run. 4. If the Reagent Check continues to fail, contact Technical Support.
		Ensure that you install the Ion S5™ Wash Solution bottle with the instrument door fully open. If you install the bottle impeded by a partially closed door, the port can be obstructed, and a faulty installation and wash failure can result.
Bottle leak check fails	• Bottle seal was not tight. • Bottle was damaged or defective.	1. Finger-tighten the bottles. 2. If the bottle continues to leak, replace the bottle. 3. If leak check continues to fail, contact Technical Support.

Ion Sphere™ Assay troubleshooting table

The following table contains troubleshooting information for unenriched ISPs tested with the Ion Sphere™ Assay on a Qubit™ Fluorometer.

Note: Successful runs that meet chip throughput specifications have been performed with a lower percentage (5–10%) of templated ISPs. Use the following recommendations as guidelines only to titrate library input for optimal results, or troubleshoot problems with library preparation.

Qubit™ Fluorometer[1] observation	Ion S5™/Ion GeneStudio™ S5 Systems observation	Possible cause	Recommended action
<10% Templated ISPs	• Lower loading • Lower % enriched • Lower key signal • Lower throughput	Too little library input into template preparation.	• Increase library input to target 10–25% templated ISPs. *or* • Continue with sequencing; expect lower throughput.
>25% Templated ISPs, but <70%	Increased number of filtered out reads	Too much library input into template preparation.	• Decrease library input to target 10–25% templated ISPs. *or* • Continue with sequencing; expect lower throughput.
>70% Templated ISPs	• Increased % primer dimer filtered out reads • Lower throughput	Adapter dimer contaminating library, more likely in short amplicon libraries, Ion AmpliSeq™ libraries, or miRNA libraries.	• Check Agilent™ 2100 Bioanalyzer™ traces for adapter dimer peak (Amplicon library or Ion AmpliSeq™ library peak around 70 bp; miRNA library peak around 60bp). • Re-purify library using Agencourt™ AMPure™ XP Kit clean-up steps as outlined in the appropriate user guides.
	• Low loading • Low % enriched • Lower throughput • High % filtered out reads	Ion Chef™ System underperformance	Troubleshoot with Technical Support or a Field Application Scientist.

[1] Qubit™ 2.0 Fluorometer or later.

B

Supplemental procedures

Perform a manual cleaning of the sequencer

A cleaning protocol is normally performed automatically at the completion of each sequencing run. If a cleaning is necessary, perform the following procedure.

1. On the home screen, select **Settings** ▸ **Clean Instrument**.
   The instrument door unlocks allowing access to the consumables.

2. Remove the Ion S5™ Wash Solution bottle to access the waste reservoir, then remove and empty the waste reservoir.

3. Reinstall the empty waste reservoir and a *used* Ion S5™ Wash Solution bottle.

4. Ensure that the Ion S5™ Sequencing Reagents cartridge and Ion S5™ Wash Solution bottle are properly installed.

IMPORTANT! Perform the cleaning with a used reagent cartridge and wash solution bottle installed. The cleaning procedure pumps cleaning solution into the wash solution bottle and reagent cartridge making them unsuitable for sequencing.

5. Place a used sequencing chip in the chip clamp, then push the chip clamp in all the way to engage.

6. Close the instrument door, then tap **Next**.

   Cleaning takes ~35 minutes to complete. On completion the instrument door automatically unlocks and the chip and cartridge clamps disengage.

7. Proceed to Chapter 5, “Initialize the sequencer”.

Perform an instrument reset run with an initialized sequencer that is loaded with an unused Reagents cartridge

Cleaning is normally automatically performed at completion of a sequencing run. If an Ion S5™ Sequencer, Ion S5™ XL Sequencer, or Ion GeneStudio™ S5 Series Sequencer is initialized, an instrument reset run is required to ensure proper cleaning before reinitialization in one of the following situations.

- A sequencing run is not started within 24 hours after initialization.
- A sequencing run is not completed due to a power failure or an abort, and <200 flows occurred before the stoppage.

Do NOT perform a manual cleaning with an unused, initialized Ion S5™ Sequencing Reagents cartridge.

Note:

- If a power failure or abort occurs during the second of two runs started after a single initialization, a manual cleaning is sufficient (see “Perform a manual cleaning of the sequencer” on page 68).
- If the number of flows that occurred before a power failure or abort is unknown, perform an instrument reset run.

To perform an instrument reset run, use the following procedure before reinitialization.

1. In the instrument touchscreen main menu, tap **Run**.
   The instrument door unlocks and the chip clamp disengages.

2. Ensure that a used sequencing chip is in the chip clamp, then push the chip clamp in all the way to engage.

3. Close the instrument door, then tap **Next**.

4. When prompted, select **Planned Run (none)**. Ensure that the **Enable post-run clean** checkbox is selected, then tap **Review**.

5. In the **Select Run** screen, tap **Edit**, then in the **Detail** screen set the number of flows to **200**. Ensure that the **Post-Run/Clean** checkbox is selected, then tap **Close**.

6. Tap **Start run**, then tap **Accept** to confirm that Post-Run Clean is enabled, and to start the run.

When the instrument reset run completes, the instrument automatically performs the cleaning procedure. After cleaning, the touchscreen returns to the main menu.

Quality control of Ion 510™ ISPs, Ion 520™ ISPs, or Ion 530™ ISPs

Quality control using the Ion Sphere™ Assay on the Qubit™ Fluorometer

The Qubit™ Fluorometer can be used with the Ion Sphere™ Quality Control Kit (Cat. No. 4468656) to perform a quality assessment of unenriched Ion Sphere™ Particles generated for up to 400-base-read sequencing on an Ion S5™ Sequencer, Ion S5™ XL Sequencer, or Ion GeneStudio™ S5 Series Sequencer.

For detailed protocols, see the *Ion Sphere™ Quality Control Kit User Guide* (Pub. No. MAN0017531) available at **thermofisher.com**.

Note: This protocol supports the Qubit™ 2.0 Fluorometer or later.

Acceptance criteria for unenriched Ion 510™ ISPs, Ion 520™ ISPs, or Ion 530™ ISPs

The optimal amount of library corresponds to the library dilution point that gives Percent Templated ISPs between 10–25%.

Samples that fall within the recommended range generally produce the most data; however, samples that fall outside of the recommended range can still meet the throughput specifications on the Ion Chips™.

The recommended optimal range is not intended to be a pass/fail criterion. The range provides guidance for the quality of the sample.

Note: If the results are outside the desired Percent Templated ISPs range, then increase or decrease the library input appropriately. For more information, see the "Ion Sphere™ Assay troubleshooting table" on page 67.

Percent Templated ISPs	Description
<10%	Sample contains an insufficient number of templated ISPs to achieve optimal loading density on the Ion Chip™.
10–25%	Optimal amount of library.
>25%	Sample will yield multi-templated ISPs (mixed reads).

Quality control using the Guava™ easyCyte 5 Benchtop Flow Cytometer

The Guava™ easyCyte 5 Benchtop Flow Cytometer can be used for quality assessment of unenriched and enriched Ion Sphere™ Particles generated for up to 400-base-read sequencing on an Ion S5™ Sequencer, Ion S5™ XL Sequencer, or Ion GeneStudio™ S5 Series Sequencer.

- **Unenriched samples** – Obtain the QC samples from the corresponding Ion Chef™ Library Sample Tubes on the Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge (positions A and B).
- **Enriched samples** – Obtain sample 1 from position E and sample 2 from position A on the Enrichment Cartridge v2.

For details, see the *Ion Sphere™ Particles Quality Assessment for the Ion Proton™ and Ion S5™ Systems Using the Guava™ easyCyte 5 Benchtop Flow Cytometer User Bulletin* (Pub. No. MAN0007496), available at **thermofisher.com**.

Maintain the Ion Chef™ System

For further information on the following Ion Chef™ System maintenance procedures, see the *Ion Chef™ Instrument User Guide* (Pub. No. MAN0018668), available at **thermofisher.com**.

- Install a firmware update
- Change the instrument name
- Replace the ultraviolet lamp
- Perform an XYΘ calibration
- Perform a Z calibration

Note: You must install the Ion Chef™ Instrument firmware update in Torrent Suite™ Software 5.12 or later to access the XYΘ calibration and Z calibration routines.

C

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words:

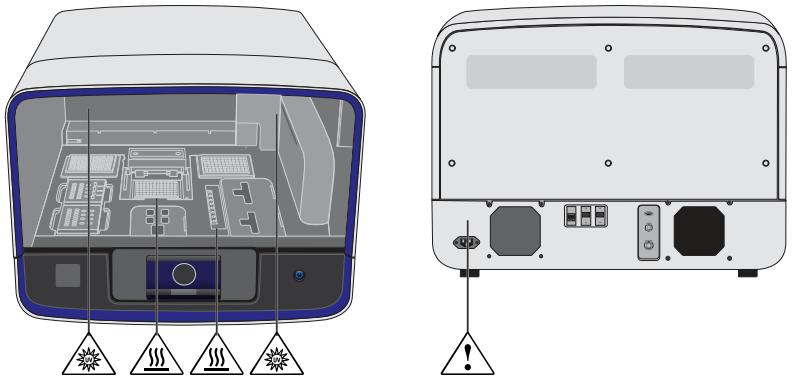
- **CAUTION!** – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!** – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!** – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Symbol	English	Français
	Caution, risk of danger Consult the manual for further safety information.	Attention, risque de danger Consulter le manuel pour d'autres renseignements de sécurité.
	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)
	Do not dispose of this product in unsorted municipal waste  **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.	Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif.  **MISE EN GARDE !** Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable.

Conformity symbols

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with European Union Low Voltage Directive 2006/95/EC, EMC Directive 2014/30/EU, and R&TTE Directive 1999/5/EC.
	Indicates conformity with Australian standards for electromagnetic compatibility.

Location of safety labels on this instrument



Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

Physical injury

CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Electrical safety

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.

WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

Cleaning and decontamination

CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment.

Ultraviolet (UV) Safety

The Ion Chef™ System uses a UV lamp which emits light at 254 nm. Under normal operating conditions, the UV lamp is powered on when performing the cleaning protocol. Safety interlocks are used to ensure that the UV lamp is not powered when the door is open.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety compliance

Reference	Description
EU Directive 2006/95/EC	European Union “Low Voltage Directive”
IEC 61010-1 EN 61010-1 UL 61010-1 CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-010 EN 61010-2-010	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials*
IEC/EN 61010-2-020	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-020: Particular requirements for laboratory centrifuges*
IEC 61010-2-081 EN 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*

EMC

Reference	Description
Directive 2014/30/EU	*European Union “EMC Directive"*
FCC Part 15	*U.S. Standard “Industrial, Scientific, and Medical Equipment"*
AS/NZS 2064	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-001, Issue 3	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*
US FCC CFR Title 47 Part 15.225, Subpart C	*Operation within the band 13.110–14.010 MHz.*
Industry Canada RSS 210, Issue 8, Annex 2 EN 302 291-1/2 V1.1.1	*Licence-Exempt Radio Apparatus: Category I Equipment*

Environmental design

Reference	Description
Directive 2012/19/EU	European Union “WEEE Directive” – Waste electrical and electronic equipment
Directive 2011/65/EU	European Union “RoHS Directive” – Restriction of hazardous substances in electrical and electronic equipment

Instrument safety — Ion S5™/Ion GeneStudio™ S5 Systems

For detailed information on Ion S5™/Ion GeneStudio™ S5 Systems instrument safety symbols and alerts, safety and electromagnetic compatibility standards, and general instrument safety, see the Safety appendix of the *Ion S5™ and Ion S5™ XL Instrument User Guide* (Pub. No. MAN0010811), or the *Ion GeneStudio™ S5 Instrument User Guide* (Pub. No. MAN0017528), available at **thermofisher.com**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/publications/i/item/9789240011311

Documentation and support

Related documentation

Document	Pub. No.
Ion Chef™ Instrument User Guide	MAN0018668
Ion GeneStudio™ S5 Instrument User Guide	MAN0017528
Ion S5™ and Ion S5™ XL Instrument User Guide	MAN0010811

Note: For additional documentation, see "Customer and technical support".

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

invitrogen

Qubit™ 4 Fluorometer

Catalog Number Q33226

Publication Number MAN0017209

Revision D.0

ThermoFisher SCIENTIFIC

Manufacturer: Life Technologies Holdings Pte Ltd | Block 33 | Marsiling Industrial Estate Road 3 | #07-06, Singapore 739256

Information in this document is subject to change without notice.

Revision history: MAN0017209

Revision	Date	Description
D.0	20 October 2021	Adding various edits, including deleting "Register your instrument" topic and product edits in "Ordering Information".
C.0	09 April 2018	Add note about the requirement for driver installation in "Export data directly to computer".
B.0	30 January 2018	Update Safety and EMC standards and Results screen images for quality assays.
A.0	05 October 2017	New user guide.

Contents

About this guide

Overview

This user guide describes how to operate the Qubit™ 4 Fluorometer.

User documentation

The guides listed below are available with the Qubit™ 4 Fluorometer.

Guide	Pub. No.
Qubit™ 4 Fluorometer User Guide	MAN0017209
Qubit™ 4 Fluorometer Quick Reference Card (QRC)	MAN0017210

Additional resources are available on the Qubit™ Technical Resources page. Go to **thermofisher.com/qubit** to access protocols, application notes, and tutorials.

Text and keyboard conventions

Text and keyboard conventions used in the *Qubit™ 4 Fluorometer User Guide* are listed below. For safety alert words and symbols used in Thermo Fisher Scientific user documentation, see page 4.

Convention	Use
Bold	**Bold** text indicates user action. For example: Click **Run**.
▶	Right arrow symbol (▶) indicates a menu choice and separates successive commands you execute or select from a drop-down or shortcut menu. For example: Select **Settings**▶**Instrument settings**.

User attention words

Two user attention words appear in Thermo Fisher Scientific user documentation. Each word implies a particular level of observation or action as described below.

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation, accurate installation, or safe use of a chemical.

Safety alert words

Four safety alert words appear in Thermo Fisher Scientific user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate installation, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Except for **IMPORTANT!** safety alerts, each safety alert word in a Thermo Fisher Scientific document appears with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to Thermo Fisher Scientific instruments (see "**Safety symbols**" in Appendix C).

SDSs

The Safety Data Sheets (SDSs) for any chemicals supplied by Thermo Fisher Scientific are available to you free 24 hours a day. For instructions on obtaining SDSs, see "**Safety Data Sheets (SDS)**".

IMPORTANT! For the SDSs of chemicals not distributed by Thermo Fisher Scientific contact the chemical manufacturer.

1. Product information

Product contents

The Qubit™ 4 Fluorometer is shipped with the components listed below:

Component	Quantity
Qubit™ 4 Fluorometer	1 each
Universal Power Cord with 4 adaptor plugs (for U.S./E.U./U.K./Australia)	1 each
USB drive (includes the user guide)	1 each
Qubit™ 4 USB cable	1 each
Qubit™ 4 Fluorometer Quick Reference Card (QRC)	1 each
Certificate of Conformity (COC)	1 each
Qubit™ screen cleaning cloth	1 each

The complete user guide is also available for download at **thermofisher.com/qubit.**

See page 6 for the description and specifications of the Qubit™ 4 Fluorometer.

Upon receiving the instrument

Examine the instrument carefully for damage incurred during transit. Ensure that all parts of the instrument, including the accessories listed above, are included with the product. Damage claims must be filed with the carrier; the warranty does not cover in-transit damage.

See page 9 for instructions to set up the Qubit™ 4 Fluorometer.

Product description

Qubit™ 4 Fluorometer

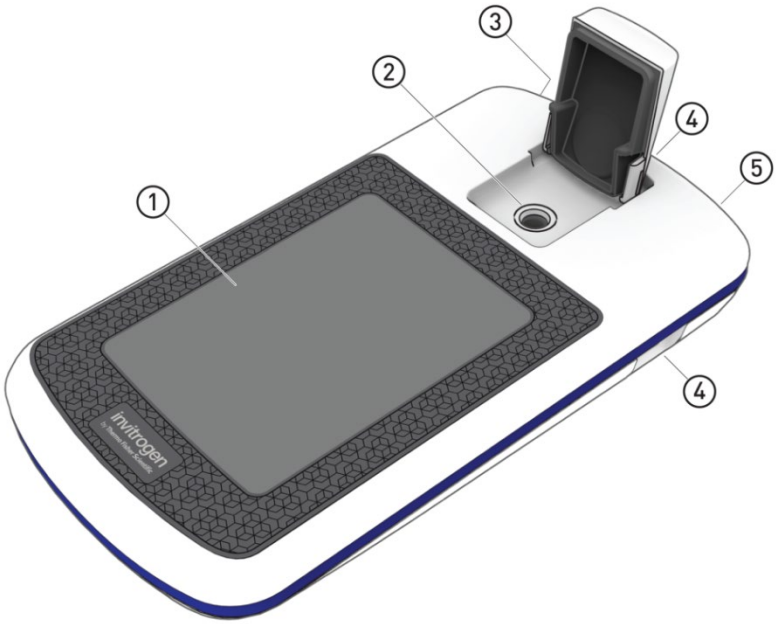
The Qubit™ 4 Fluorometer is a benchtop fluorometer that can be used for the quantitation of DNA, RNA, microRNA, and protein, as well as for the measurement of RNA integrity and quality using the highly sensitive and accurate fluorescence-based Qubit™ assays. Additionally, Ion Sphere™ Particle quality can be evaluated on the Qubit™ 4 Fluorometer using the Ion Sphere™ Quality Control Kit prior to performing a sequencing run on the Ion Personal Genome Machine™ (PGM™) Sequencer. You can also use the Qubit™ 4 Fluorometer to directly measure the fluorescence of samples or to create new assays using the MyQubit software pre-programmed into the instrument.

Features

- Fast and highly accurate quantitation of DNA, RNA, and protein in less than 3 seconds per sample.
- Measure percentage of intact and degraded RNA in the sample in less than 5 seconds per sample.
- High levels of accuracy using only 1–20 µL of sample, even with very dilute samples.
- Use of dyes selective for dsDNA, RNA, or protein minimizes the effects of contaminants in the sample.
- Stores results from up to 1000 samples.
- Large, state-of-the-art color touchscreen for easy workflow navigation.
- Graphically displays up to 20 data points for quantitation assays.
- Instrument indicates samples that are in the extended range or out of range in the graph, as well as in the simple sample output screen.
- Saves sample data as a CSV (comma separated value) or PDF file.
- Exports data to a USB drive or directly to your computer via a USB cable.
- On-board Reagent Calculator provides instructions to prepare Qubit™ working solution using your sample and standard inputs.
- Allows easy definition and saving of assay preferences.
- Instrument user interface can be personalized to show only the frequently used assays, to add new assays, including user-defined assays created with the MyQubit software and web tool, and to display in the language of your choice including English, French, Spanish, Italian, German, simplified Chinese and Japanese.

Instrument exterior components

Top view



Back view

① **Touchscreen** is the user interface containing the controls for all the functions needed and displays data from the assays.

② **Sample chamber** is used to load the assay tube containing the sample into the fluorometer for analysis.

③ **USB cable port (type mini-B)** allows you to transfer your data directly to your computer using the USB cable supplied with the instrument or any other similar USB cable.

④ **USB drive ports (Type A)** allow you to transfer and save data to your computer using the USB flash drive supplied with the instrument (or any other similar USB drive) for record keeping and printing purposes.

⑤ **Power inlet** connects the Qubit™ 4 Fluorometer to an electrical outlet using the supplied power cord and the appropriate plug, based on the electrical outlet configuration in your country.

Product specifications

Physical characteristics	**Instrument type:**	Benchtop fluorometer
	Instrument dimensions:	5.4 in (w) × 10 in (l) × 2.2 in (h) (13.6 cm × 25 cm × 5.5 cm); rectangular shape
	Weight:	26.2 oz (743 g)
	Operating power:	100–240 VAC, 1.0 A/1.2 A
	Frequency:	50/60 Hz
	Electrical input:	12 VDC, 2.5 A
	Installation site:	Indoor use only
	Operating temperature:	10–40°C
	Operating humidity:	20–80% (non-condensing)
Technical specifications	**Dynamic range:**	5 orders of magnitude
	Processing time:	≤5 seconds/sample
	Light sources:	Blue LED (max ~470 nm) Red LED (max ~635 nm)
	Excitation filters:	Blue 430–495 nm Red 600–645 nm
	Emission filters:	Green 510–580 nm Red 665–720 nm
	Detectors:	Photodiodes; measurement capability from 300–1000 nm
	Calibration type:	2- or 3-point standard
	Tube type:	0.5-mL Real Time PCR (polypropylene) tubes
	Warm-up time:	<35 seconds
USB drive	**Capacity:**	4 Gigabyte

2. Getting started

Set up the Qubit™ 4 Fluorometer

Set up the instrument

The Qubit™ 4 Fluorometer is a stand-alone instrument that does not require connection to a computer.

1. After unpacking the instrument, place the instrument on a flat, level, dry surface.
2. Plug one end of the supplied power cord into the Qubit™ 4 Fluorometer.
3. Attach the appropriate plug adaptor to the other end of the power cord.
4. Plug the power cord into the electrical outlet.

> **Note:** Ensure the plug of power adaptor remains accessible to allow disconnection.

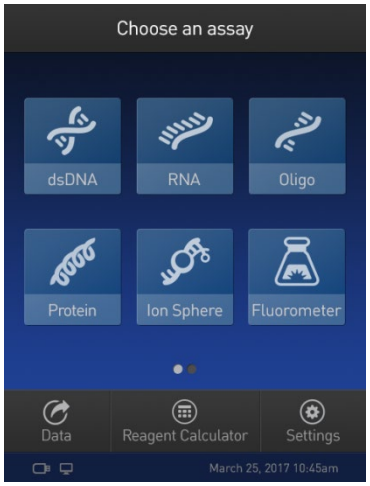
5. The instrument automatically powers on, first displaying the splash screen, and then the Home screen.
6. To power down the Qubit™ 4 Fluorometer, unplug it.

> **IMPORTANT!** Use the power cord plug adapter supplied with the instrument that is appropriate for the electrical outlet configuration in your country. Powering the instrument with an unapproved power cord may damage the instrument.

Home screen

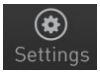
When the instrument is powered on, the *Home* screen is automatically displayed.
From the Home screen, you can:

- Select one of the assays: dsDNA, RNA, oligo (ssDNA), protein, or Ion Sphere™.
- Select the Fluorometer mode (the instrument behaves like a mini-fluorometer).
- Access saved data.
- Configure instrument settings.
- Use the Reagent Calculator to determine the exact volumes of Qubit™ buffer and reagent required to prepare the Qubit™ working solution.

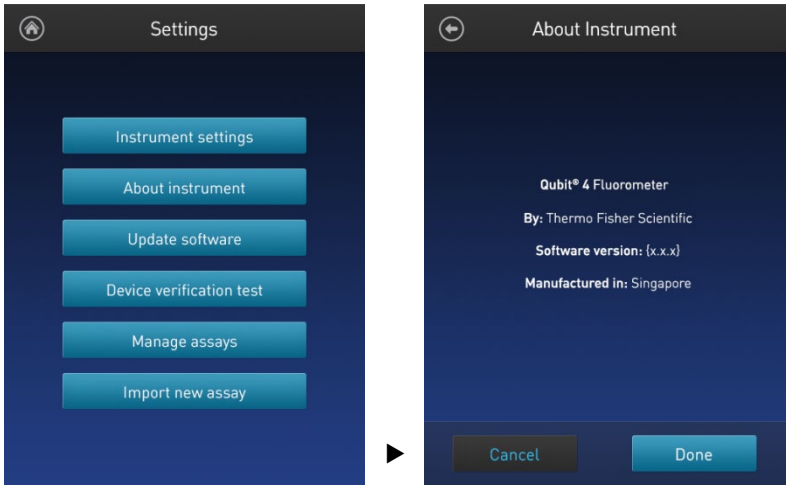


About Instrument screen

The *About Instrument* screen displays information about your Qubit™ 4 Fluorometer, including the currently installed software version. To access the About Instrument screen:

1. On the Home screen, press **Settings.** 

2. On the Settings screen, press **About Instrument** to display the About Instrument screen.



3. Press **Done**, **Cancel,** or **Back** () to return to the Settings screen.

Optional: Configure instrument settings

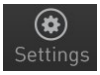
Introduction

You can configure the following instrument settings for the Qubit™ 4 Fluorometer:

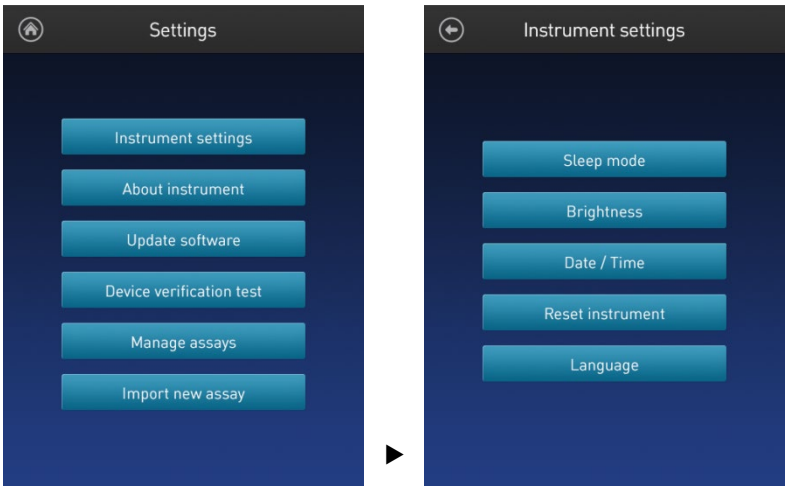
- Sleep mode (page 12)
- Brightness (page 12)
- Date/Time (page 13)
- Reset instrument (page 14)
- Language (page 15)

Access the Instrument settings screen

1. On the Home screen, press **Settings**.



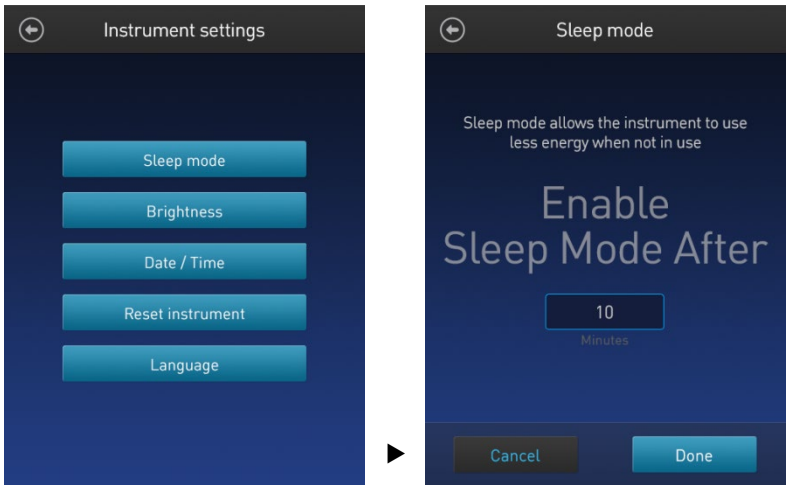
2. On the *Settings* screen, press **Instrument settings** to display the *Instrument settings screen*.



Adjust the sleep mode

The Qubit™ 4 Fluorometer has a sleep mode (i.e., automatic standby) that is triggered by inactivity. The system default is 10 minutes of inactivity before the instrument goes into sleep mode. To adjust this time:

1. On the *Instrument settings screen* (page 11), press **Sleep mode** to display the *Sleep mode* screen.

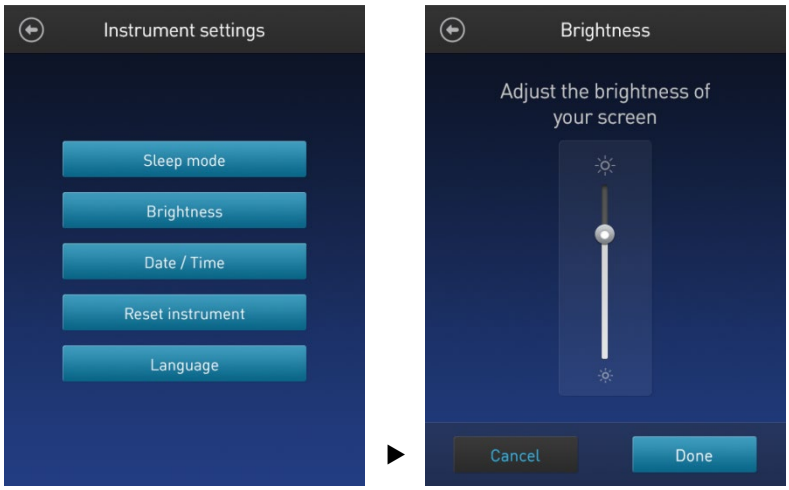


2. Enter the time in minutes allowed before the instrument goes into sleep mode by pressing the **minutes** field, then using the number pad to select a value. The software requires a minimum of 1 minute and a maximum of 60 minutes.
3. Press **Done** to save the changes and return to the Instrument settings screen.

   Press **Cancel** or **Back** ( ) to return to the Instrument settings screen without saving the changes.

Adjust screen brightness

1. On the *Instrument settings screen* (page 11), press **Brightness** to display the *Brightness* screen.



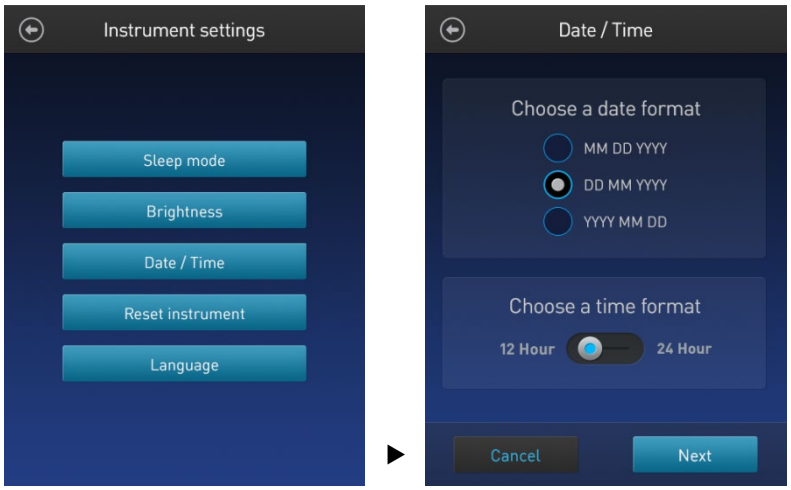
2. Move the slider button up or down to adjust the brightness of the display.
3. Press **Done** to save the changes and return to the Instrument settings screen.

   Press **Cancel** or **Back** ( ) to return to the Instrument settings screen without saving the changes.

Adjust the date and time

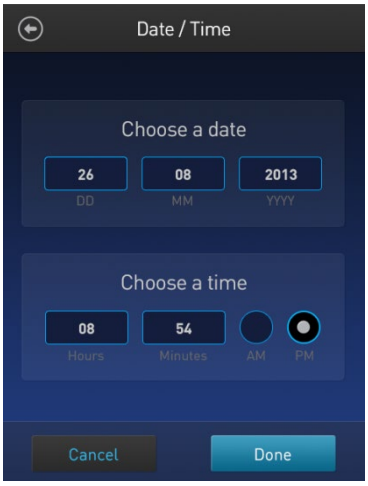
The date and time are preset when you receive the instrument. To adjust the date and time:

1. On the *Instrument settings screen* (page 11), press **Date/Time**.



2. Select a date format, select a time format, then press **Next**.
3. Enter the date and time:
   a. Press a date field (**day**, **month**, or **year**), then use the number pad to select a value.
   b. Press a time field (**hours** or **minutes**), then use the number pad to select a value.
   c. Select **AM** or **PM**.
4. Press **Done** to save the changes and return to the Instrument settings screen.

   Press **Cancel** or **Back** ( ) to return to the Instrument settings screen without saving the changes.



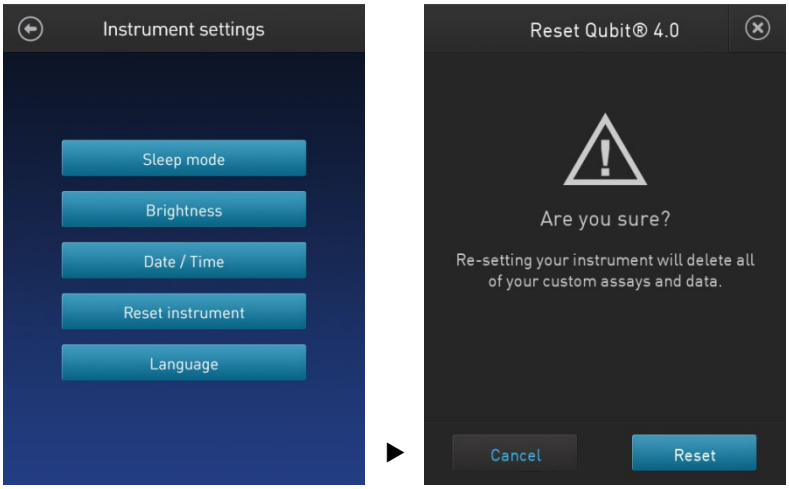
Reset function

The Reset function returns the Qubit™ 4 Fluorometer to its default factory settings, and **erases all saved data, user–defined instrument settings, and custom assays.**

IMPORTANT! The reset function is not reversible.

To return the instrument to its default factory settings:

1. On the *Instrument settings screen* (page 11), press **Reset instrument** to display the *Reset Qubit™ 4* screen.



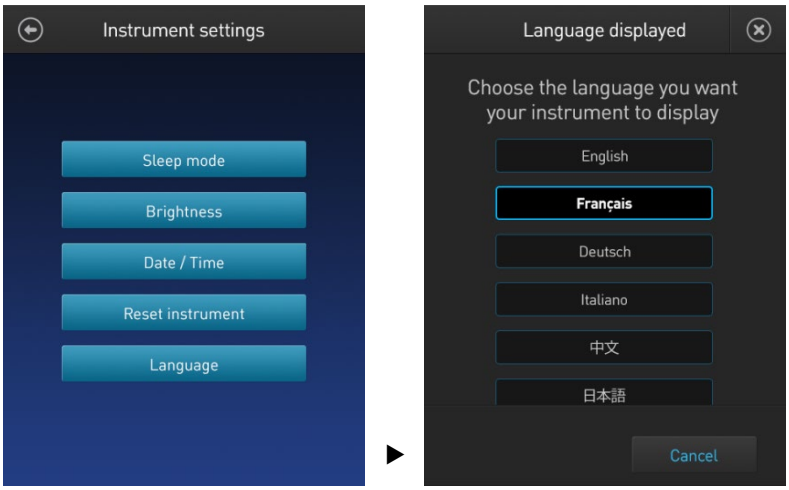
2. Press **Reset**. After the reset is complete, the Home screen is displayed. All data, user–defined instrument settings, and custom assays are removed.

   Press **Cancel** or **Exit** ( ) to return to the Instrument settings screen without saving the changes.

Change the display language

You can change the language that the Qubit™ 4 Fluorometer displays to English (default), French, German, Italian, Spanish, simplified Chinese, and Japanese.

1. On the *Instrument settings screen* (page 11), press **Language** to display the *Language* screen.



2. Select a language, then press **Next**.
3. Press **Yes** to confirm the change and return to the Instrument settings screen.

   Press **Cancel** or **Exit** ( ) to return to the Instrument settings screen without saving the changes.

Guidelines for using the Qubit™ 4 Fluorometer

Recommendations

To obtain the best results, follow the recommendations below. For more information, see "Critical Qubit™ Assay considerations", page 59.

- Do **not** operate the instrument in direct sunlight.
- Wear gloves during sample handling.
- Use the instrument at room temperature only (22–28ºC).
- Bring all kit reagents to room temperature and insert all assay tubes into the instrument only for as much time as it takes for the instrument to measure the fluorescence.
- Do not hold the assay tubes in your hand before performing a measurement.
- Make sure that you have calibrated the Qubit™ 4 Fluorometer using the appropriate standards.
- Incubate the tubes for the Qubit™ DNA and RNA assays for 2 minutes after mixing the sample or standard with the working solution.
- Incubate the tubes for the Qubit™ protein assays for 15 minutes after mixing the sample or standard with the working solution.
- Incubate the tubes for the Qubit™ Protein Broad Range Assay for 10 minutes after mixing the sample or standard with the Protein BR Assay Reagent.
- If you are performing multiple readings of a single tube, remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

  Note: Multiple readings of RNA samples is not recommended.
- Visit **thermofisher.com/qubit** for additional application notes, technical notes, citations, software updates, and a list of validated Qubit™ assays that have been tested using the Qubit™ 4 Fluorometer.

Assay tubes for the Qubit™ 4 Fluorometer

Only thin-wall, clear 0.5-mL PCR tubes are appropriate for use in the Qubit™ 4 Fluorometer. Acceptable tubes include Qubit™ assay tubes (Cat. No. Q32856, 500 tubes). The minimum assay volume must be 200 µL for an accurate read.

3. Perform assays

Overview

This section provides instructions for using the Reagent Calculator, running new calibration standards or for using the last calibration, and for reading the samples using a Qubit™ assay kit.

Materials needed

- A Qubit™ assay kit appropriate for quantifying your samples (see page 61 for available Qubit™ assay kits and ordering information)
- DNA, RNA, or protein samples
- Qubit™ assay tubes or other appropriate 0.5-mL assay tubes
- Appropriate standards for the assay you are performing

  Note: For instructions on the preparation of these standards, see the instructions that accompany the assay you are using or the *Qubit™ 4 Fluorometer Quick Reference Card (QRC)* (Pub. No. MAN0017210).

- *(Optional)* USB drive or USB cable for data transfer, supplied with the instrument or available separately

Optional: Use Reagent Calculator to prepare Qubit™ Working Solution

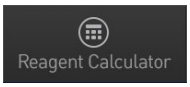
Introduction

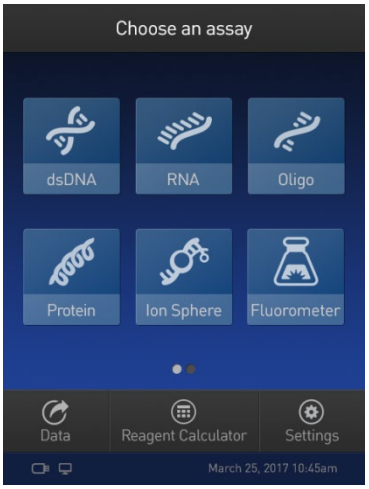
Use the on-board Reagent Calculator to quickly determine the correct amount of Qubit™ dye and buffer required to make the appropriate amount of Qubit™ Working Solution required to prepare your samples and standards.

Note: The Reagent Calculator is not intended for use with the Qubit™ Protein Broad Range Assay.

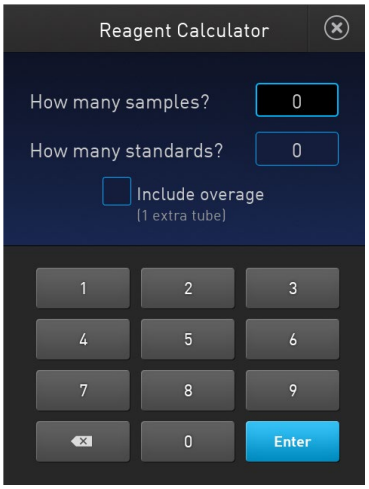
Use the Reagent Calculator

1. On the **Home** screen, press **Reagent Calculator**.



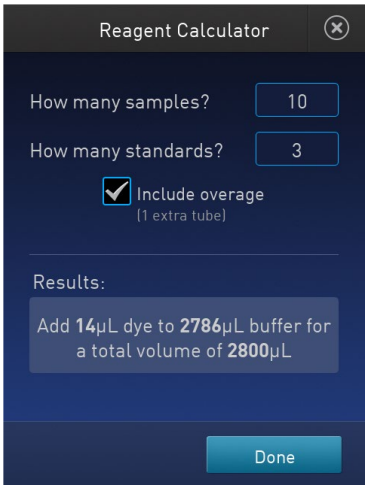


2. Enter the total number of samples and standards that you will be running on the Qubit™ 4 Fluorometer.



3. *Optional:* Select **Include overage** if you want to include an additional tube in the total calculated volume.

4. Press **Enter** to calculate the amount of Qubit™ dye and buffer required to prepare the Qubit™ Working Solution with these inputs.



Note: You can change the total number of tubes that you plan to run or the overage selection on this screen.

5. Press **Done** to return to the **Home** screen to run your Qubit™ assay.

Calibrate assays on the Qubit™ 4 Fluorometer

Introduction

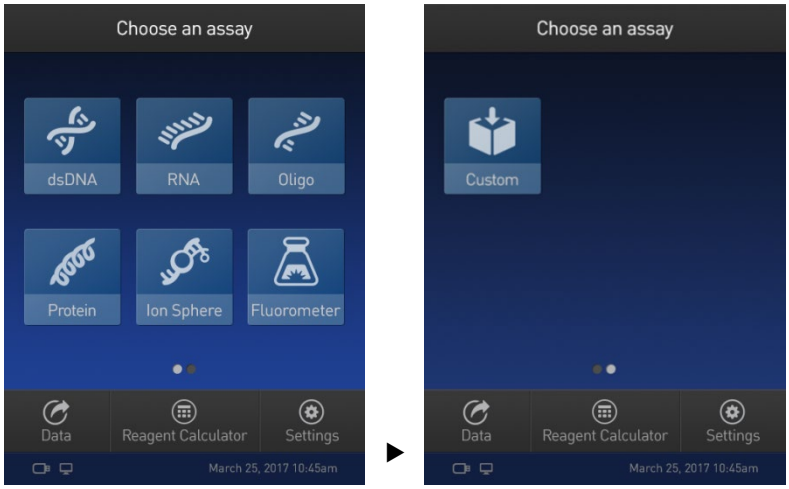
For each assay, you have the choice to run new standards for calibrating the assay on the Qubit™ 4 Fluorometer or to use the values from the previous calibration (for more information, see "Qubit™ 4 Fluorometer", page 60).

Note: The Reagent Calculator is not intended for use with the Qubit™ Protein Broad Range Assay.

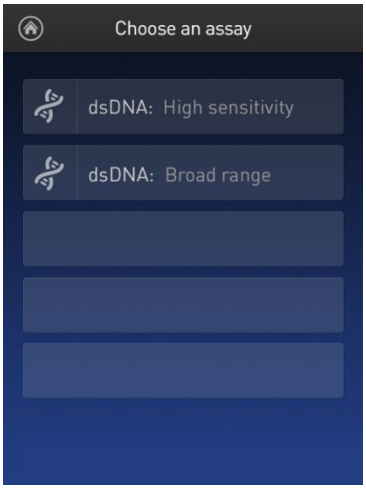
Read new standards for calibration

1. On the **Home** screen, select the **assay type** for which you wish to read new standards.

   To go to the second page of available assays, swipe the screen to the right. To return to the first page, swipe the screen to the left.

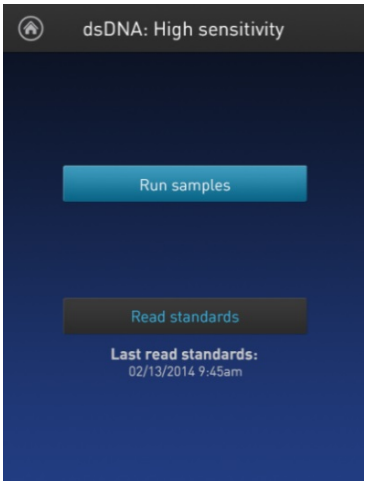


2. Select the desired **assay**.



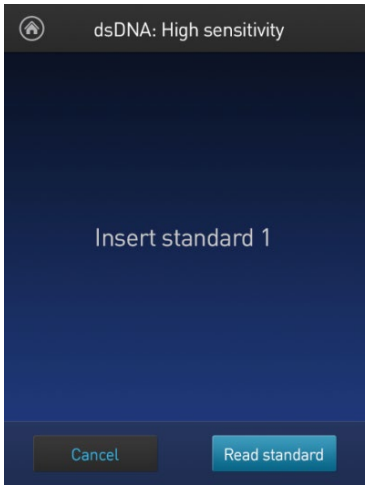
Note: The software displays the available assays for the assay type you selected in step 1. The example in step 2 shows the available assays for the dsDNA assay type.

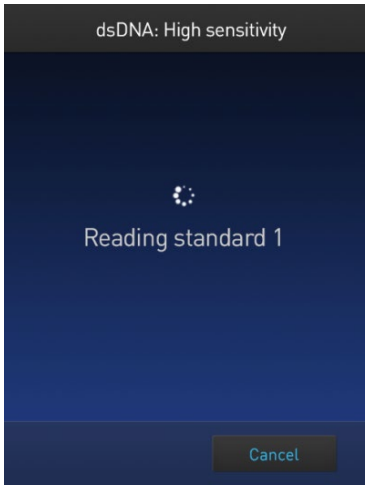
3. If you have already performed a calibration for the selected assay, the software prompts you to choose between reading new standards and running samples using the previous calibration. Press **Read standards**.



Note: To apply the previous calibration to your sample readings, press **Run samples**. The software prompts you to insert the assay tube containing sample. See "Read samples", page 24.

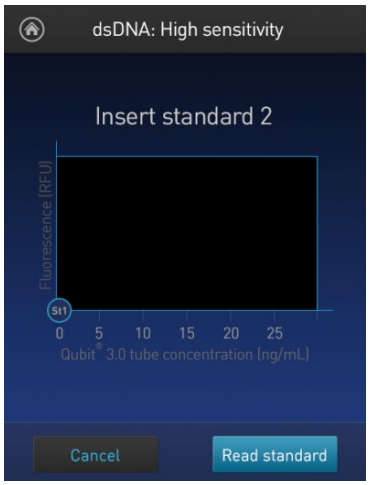
4. At the prompt, insert Standard #1 into the sample chamber and press **Read standard**. Be sure to use the Standard #1 appropriate for the assay you are performing. The reading takes approximately 3 seconds.





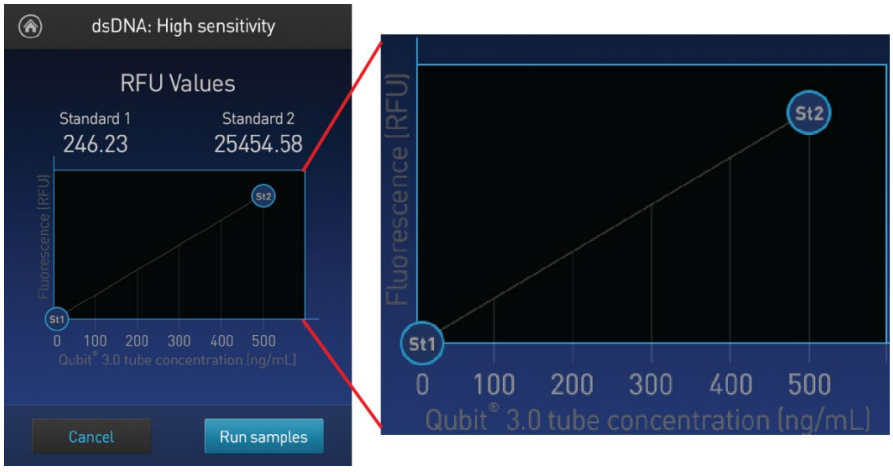
5. At the prompt, insert Standard #2 into the sample chamber and press **Read standard**.
   Be sure to use the Standard #2 appropriate for the assay you are performing.
6. *For Qubit™ protein and RNA IQ assays only:* At the prompt, insert Standard #3 into the sample chamber and press **Read standard**.

7. The calibration is complete after Standard #2 is read (or after Standard #3 is read for the Qubit™ protein or RNA IQ assay). The software displays the results.



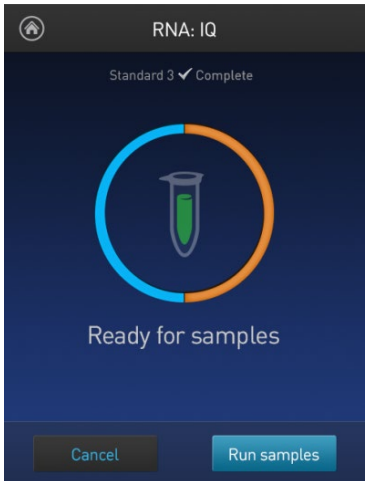
Calibration results

- **For Qubit™ quantitation assays:** If the calibration is successful, the software displays the *Read standard* screen with the *Fluorescence vs. Concentration* graph.



In the Fluorescence vs. Concentration graph, the standard data points are connected by a line and open circles represent correct standards.

- **For the RNA IQ assay:** If the calibration is successful, the software displays the *Ready for samples* message.



- If the calibration is not successful, the software displays the *Calibration error* message (page 23).

Note: The Read standard screen displays the raw fluorescence values for Standard #1 and Standard #2 (and Standard #3, if applicable). These values can assist you in making a judgment regarding the calibration results.

For the Qubit™ dsDNA BR, Qubit™ dsDNA HS, Qubit™ ssDNA, Qubit™ microRNA, Qubit™ RNA HS, Qubit™ RNA BR, Qubit™ RNA XR, and Qubit™ protein assays, the reading given by Standard #2 should be at least ten times higher than that of Standard #1.

For the Qubit™ protein assay, the reading given by Standard #3 should be at least 40% higher than that of Standard #2.

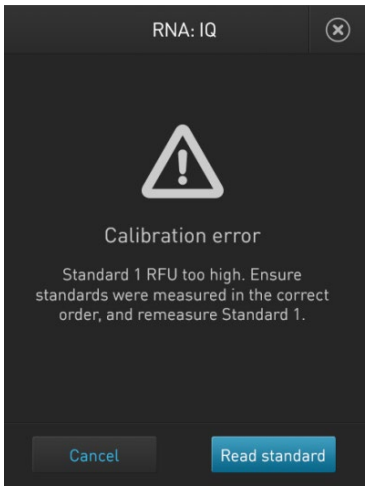
For the Qubit™ RNA IQ assay, the instrument verifies the calibration parameters at each stage of the three point calibration process, confirming the appropriate response of the assay to the standards provided in the kit. If any issues arise during calibration, the instrument will guide you to appropriate actions to correct the issue.

Calibration error

If you receive the "Calibration error" message, you can re-run the standards:

1. In the Error screen, press **OK**.
2. Review the *Read standard* screen (see **Note** on page 23).
3. If you wish to re-run the standards, or run new standards, press **Read standards**, then repeat the calibration procedure (beginning on page 18).

Note: For the RNA IQ assay, specific standards can be re-run depending on the calibration error. The error message provides guidance on how to correct the calibration error.



Read samples

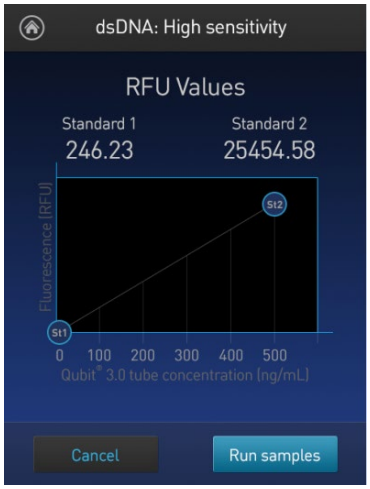
Before you begin

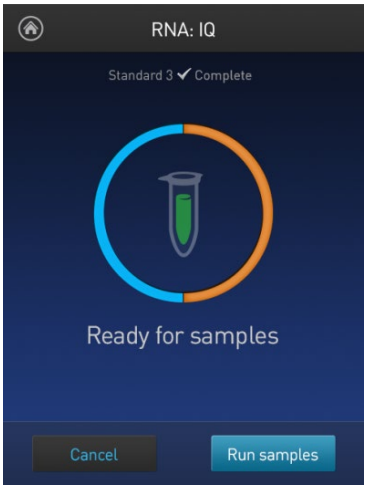
- Calibrate the Qubit™ 4 Fluorometer as described on page 18. (Run the appropriate standards or accept the values from the previous calibration.)
- Prepare the samples. Refer to the instructions provided with the assay.

Note: Incubate the samples for the appropriate amount of time after mixing them with the working solution (2 minutes for the Qubit™ DNA and RNA assays, 15 minutes for the Qubit™ protein assay, or 10 minutes for the Qubit™ Protein Broad Range Assay).

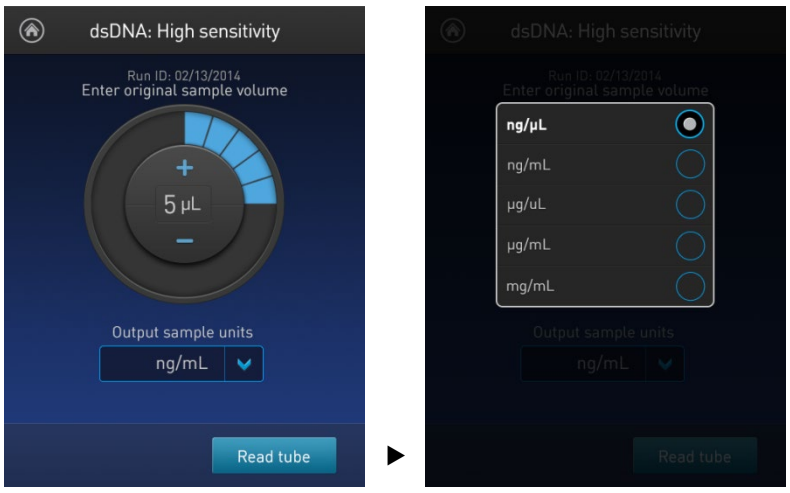
Read samples

1. In the *Read standard* (for Qubit™ quantitation assays) or *Ready for samples* screen (for Qubit™ RNA IQ assay), press **Run samples**.



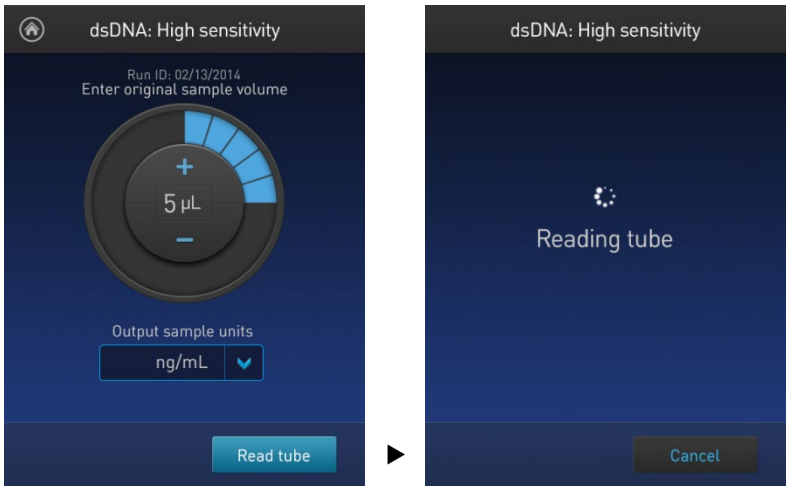


2. In the *Sample volume* screen, select the sample volume and units for the Qubit™ quantitation assays or the Qubit™ RNA IQ assay:
   a. Press the + or – buttons on the wheel to select the sample volume added to the assay tube (between 1 and 20 µL).
   b. For the Qubit™ quantitation assays, select the units for the output sample concentration from the dropdown menu.



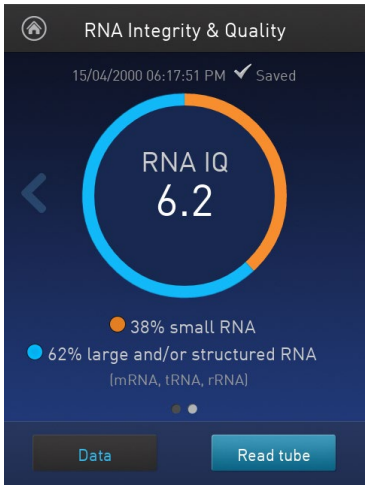
Note: In the RNA IQ assay, there are no units associated with the RNA IQ value (i.e., RNA IQ#) and only sample volume is required.

3. Insert a sample tube into the sample chamber, close the lid, and then press **Read tube**. The reading takes approximately 3 seconds for the quantitation assays and approximately 5 seconds for the quality assays.



4. The software displays the results. See "Results screen" on page 26.

Results screen for quantitation assays



Results screen for quality assays

Read multiple samples

To read multiple samples for the same assay:

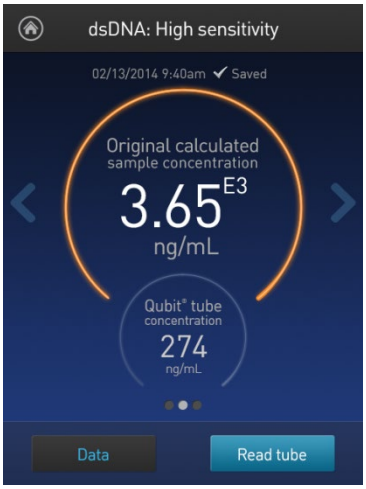
1. Remove the current sample, and insert a new sample.
2. To change the sample volume, swipe **right** or press the **left arrow**.
3. Press **Read tube**.
4. Repeat step 2 (page 24) and step 3 (page 25).

Results

Results screen – Sample quantitation

The Results screen displays the results of the sample run.

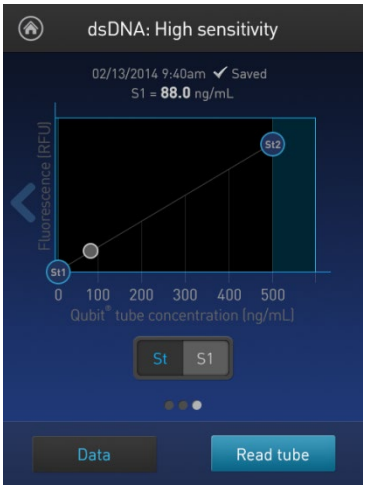
- If the results are within the assay's range, the concentration values are displayed. The top value (in large font) is the concentration of the original sample. The bottom value is the dilution concentration (the concentration of the sample in the tube inserted into the Qubit™ 4 fluorometer).



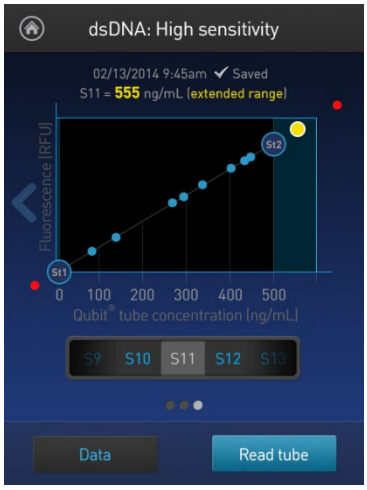
- If the results are outside of the assay's range, an "Out of Range" message is displayed.

- To return to the sample volume screen, **swipe right** or press the **left arrow**.

- To view the Fluorescence vs. Concentration graph, **swipe left** or press the **right arrow**. In the graph:
  - Open circles represent correct standards.
  - The large gray circle represents the most recent sample.
  - Blue circles represent samples that fall within the assay's core range.
  - Yellow circles represent samples that fall within the assay's extended range.
  - Red circles represent samples or standards that fall outside the assay's range.



Single sample



Multiple samples

Out of range high

Out of range low

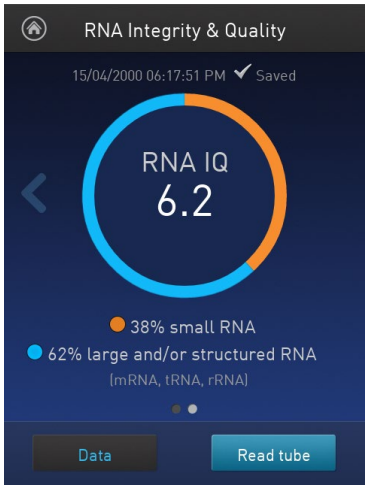
- To return to the previous screen, **swipe right** or press the **left arrow**.

Note: The Qubit™ 4 Fluorometer automatically stores numeric data from all reads, but does not store graphic data.

Results screen – Sample quality

The Results screen displays the results of the sample run.

- If the results are within the assay's range, the sample quality values are displayed. The result presented in the circle (i.e., RNA IQ#) is the total value for the RNA sample integrity and quality, which is the calculated percentage of large and small RNA in the sample.

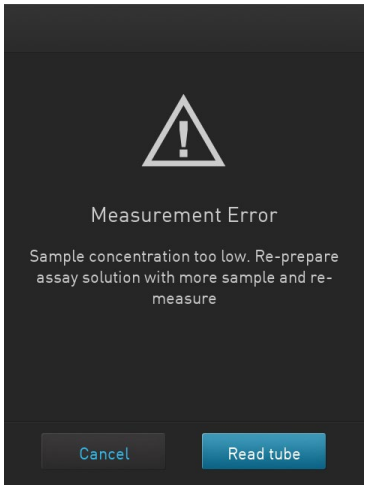


The RNA IQ# is based on a scale of 1–10, similar to other RNA quality scores. A small RNA IQ# in the assay indicates that the sample is comprised of mainly small RNA and a larger RNA IQ# indicates that the sample consists of mainly large RNA or structured RNA (mRNA, tRNA, rRNA).

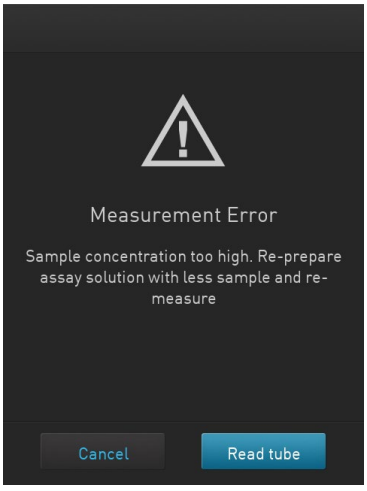
The actual percentages of small or degraded RNA and large or intact RNA in the sample are displayed below the RNA IQ#.

Measurement error

If the results are outside of the assay's range, a "Measurement error" message is displayed.



Measurement error – Sample concentration too low



Measurement error – Sample concentration too high

Fluorometer mode

Introduction

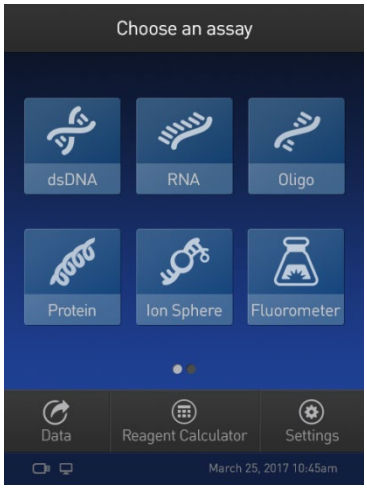
You can use the Qubit™ 4 Fluorometer as a mini-fluorometer by selecting the **Fluorometer mode**.

The Fluorometer mode allows you to choose the excitation light source (blue LED or red LED) while reading fluorescence in both the green and far-red emission channels (for the blue LED) or in the far-red emission channel only (for the red LED).

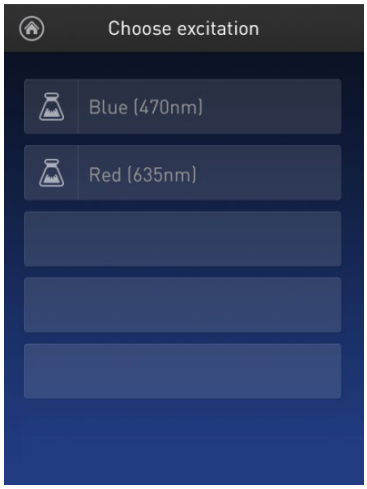
The reading is in raw fluorescence units (RFU).

Read sample in Fluorometer mode

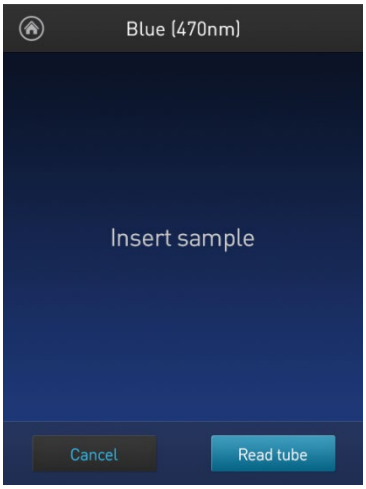
1. On the *Home* screen, press **Fluorometer**.



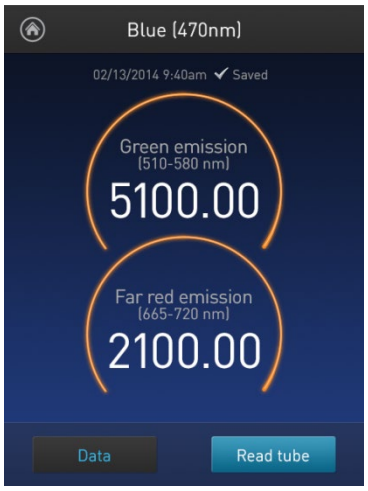
2. On the *Choose excitation* screen, select **Blue (470 nm**) or **Red (635 nm)**.



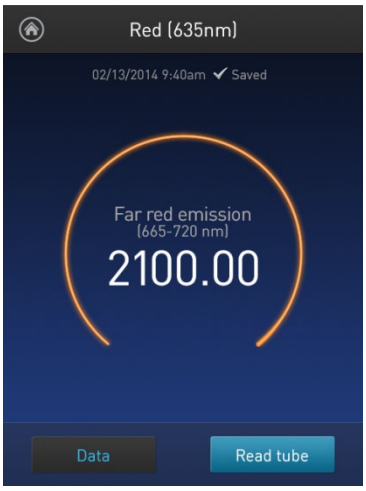
3. Insert the sample, then press **Read tube**.



4. For Blue excitation, the Qubit™ 4 Fluorometer displays both the green emission and the far red emission of your sample. For Red excitation, the Qubit™ 4 Fluorometer displays only the far red emission of your sample.



Blue (470 nm) excitation



Red (635 nm) excitation

Read multiple samples in Fluorometer mode

To read multiple samples in the fluorometer mode:

1. Remove the current sample, and insert a new sample.
2. Press **Read tube.**

Ion Sphere™ Assay

Ion Sphere™ Assay

The Ion Sphere™ assay on the Qubit™ 4 Fluorometer enables quick and easy assessment of Ion Sphere™ Particles (ISPs) prior to performing a sequencing run on the Ion Personal Genome Machine™ (PGM™) Sequencer using the Ion Sphere™ Quality Control Kit.

All ISPs bind a complimentary, green-fluorescent Alexa Fluor™ 488 dye-labeled oligonucleotide, whereas only those beads that are template positive bind a complementary red-fluorescent Alexa Fluor™ 647 dye-labeled oligonucleotide. The Qubit™ 4 Fluorometer measures the fluorescence emission of each dye conjugate and a Microsoft Excel™ file template is used to calculate the percentage of template-positive ISPs from the ratio of the Alexa Fluor™ 647 dye signal to the Alexa Fluor™ 488 dye signal. This quality-control step using the Qubit™ Fluorometer determines the likelihood of a successful sequencing run, ultimately saving time and expense during subsequent sequencing.

Note: For more information about the Ion Sphere™ Quality Control Kit (Cat. No. 4468656), go to **thermofisher.com** and search for the catalog number.

4. Manage data

Introduction

The Qubit™ 4 Fluorometer can save data for up to 1000 samples.

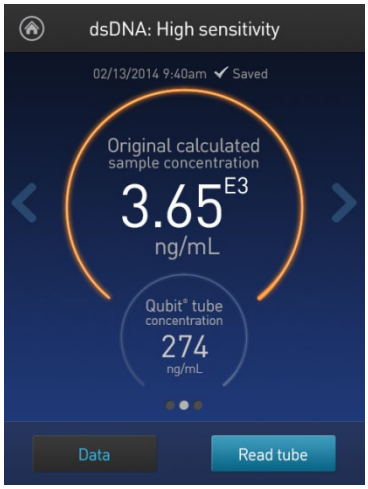
For the saved data, the instrument allows you to:

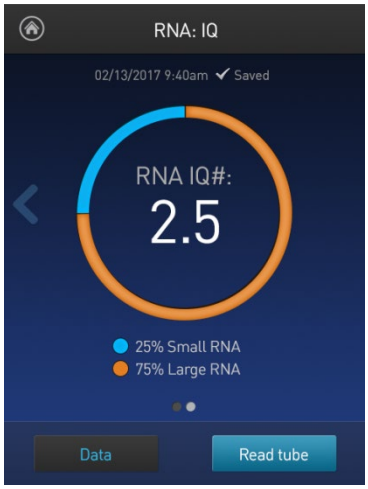
- View detailed data for each sample (page 32).
- Rename data files (page 35).
- Save data as a CSV (comma separated value) or PDF file, and export the file to a USB drive or directly to your computer (page 36).
- Delete data files (page 40)

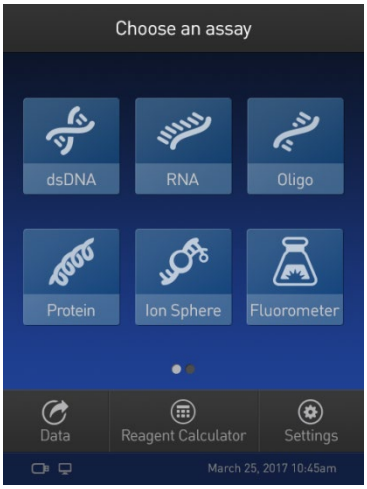
View detailed sample data

View detailed sample data

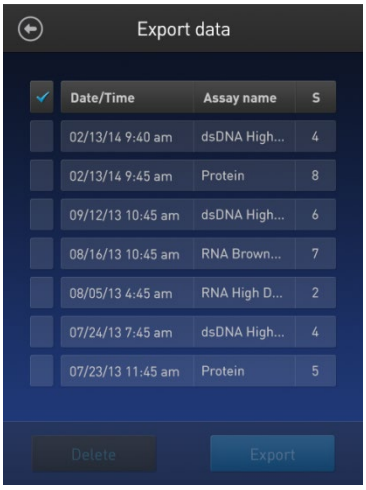
1. From the **Concentration**, **Graph**, **RNA IQ results**, or **Home** screen, press **Data**.





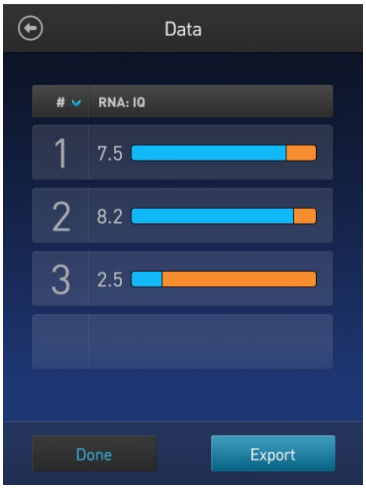


2. On the Export data screen, press the **data set of interest**.



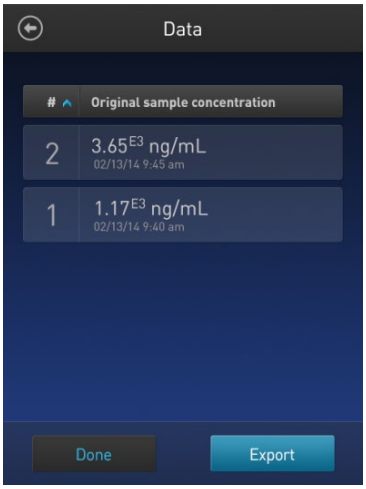
A *Data* screen displaying a list of data entries for that data set appears. To view additional data entries that do not fit in the screen, scroll up or down.

Data screen for quantitation assays

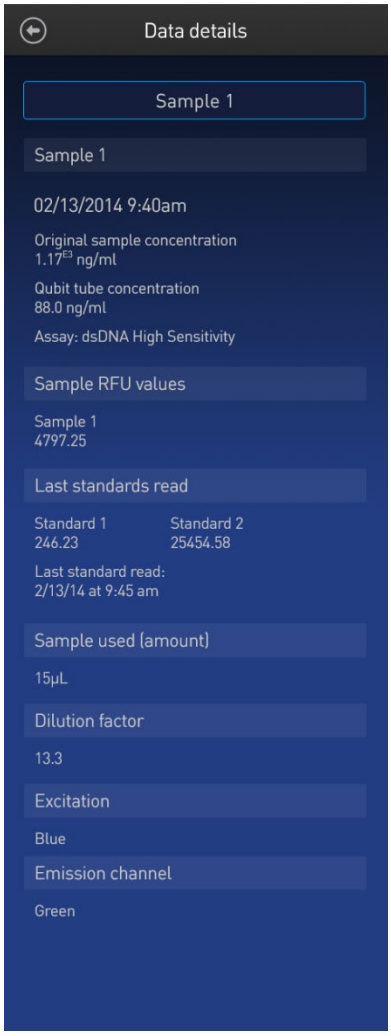


Data screen for quality assays

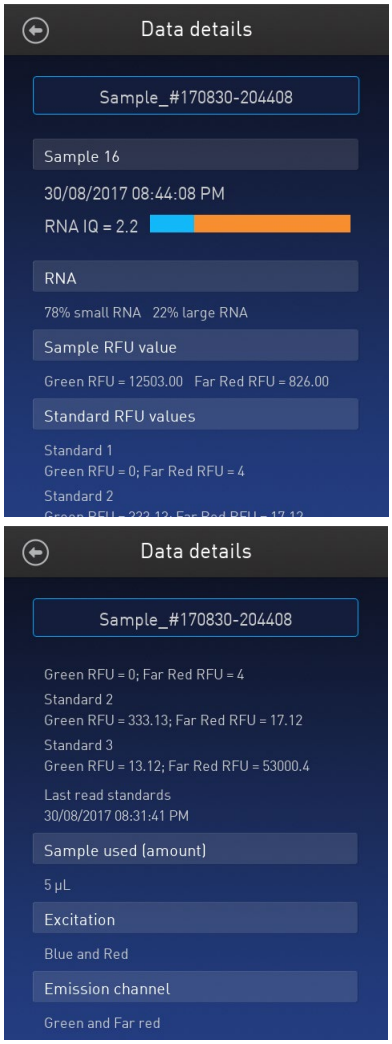
3. To reverse the order of the data entries, press the **blue chevron** above the sample number column.



4. To view the sample details, press the **sample of interest**. A *Data details* screen opens.



Data details for quantitation assays

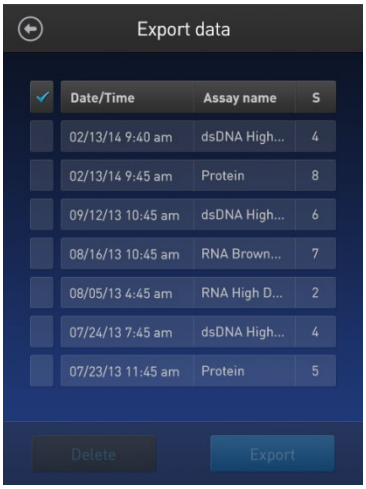


Data details for quality assays

Rename data files

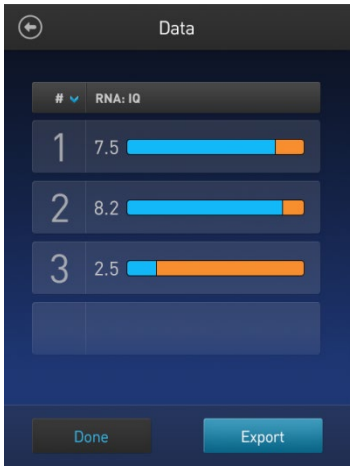
Rename data files

1. On the **Concentration**, **Graph**, **RNA IQ results**, or **Home** screen, press **Data**.
2. On the Export data screen, select the **data set of interest**.



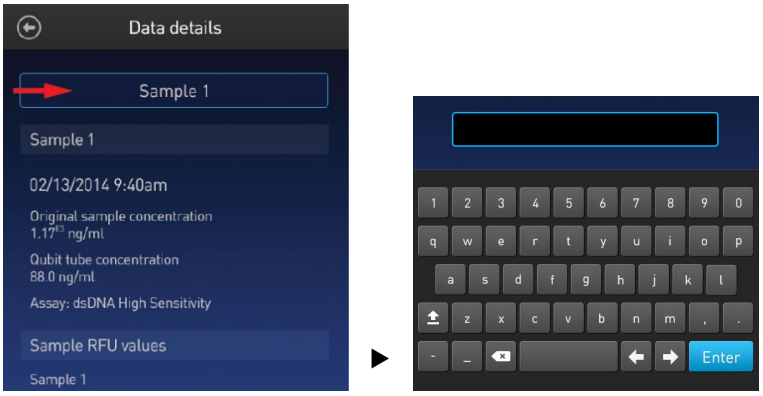
3. From the list of data entries for the selected data set, press the **sample of interest** to open the *Data details* screen.

Data screen for quantitation assays



Data screen for quality assays

4. On the Data details screen, press the **Sample #** field (indicated by the red arrow), enter the new name using the keyboard that appears, then press **Enter**.



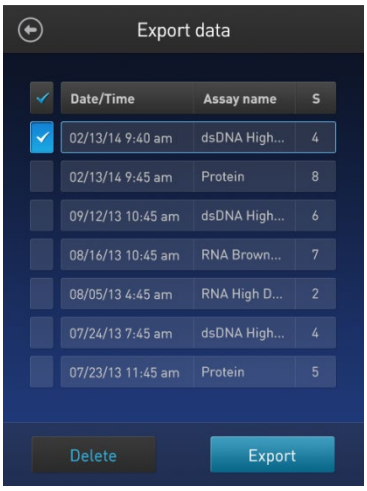
Export data

Introduction

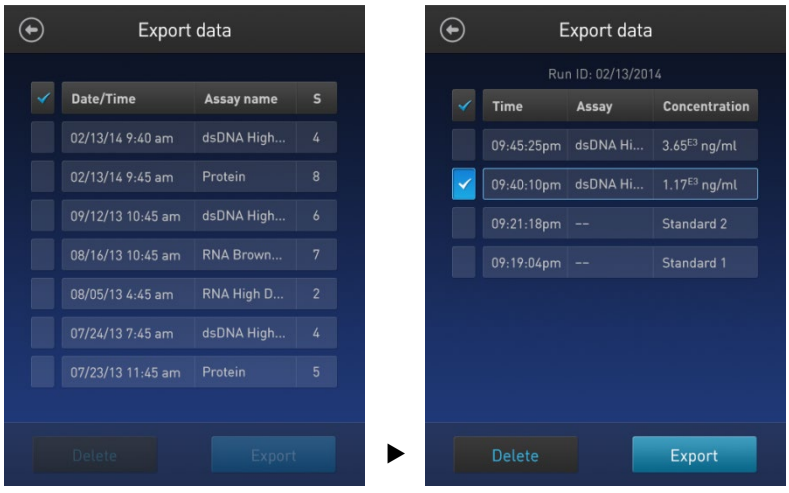
The Qubit™ 4 Fluorometer is designed for standalone use; it does not require an external computer. However, to archive data and generate reports, you can export the numeric data stored in the CSV or PDF file to a computer either by using the USB drive or by saving directly to the computer using the USB cable. You can then import the file into any spreadsheet program.

Export data using the USB drive

1. Insert the USB drive into the Qubit™ 4 Fluorometer.
2. On the **Concentration**, **Graph**, **RNA IQ results**, or **Home** screen, press **Data**.
3. On the *Export data* screen, check the **selection box** to the left of each data set you wish to export.



4. To save only individual data entries from a data set, press the **data set of interest**, and then check the **selection box** to the left of the samples you wish to export.



5. Select CSV or PDF and press **Export** to export the data.

6. Transfer the USB drive to the USB drive port on your computer.

Export data directly to a computer

Data files may also be exported from the Qubit™ instrument to a computer using a USB cable such as the one provided with the Qubit™ instrument. The USB cable must remain connected to both the Qubit™ instrument and the computer during this process.

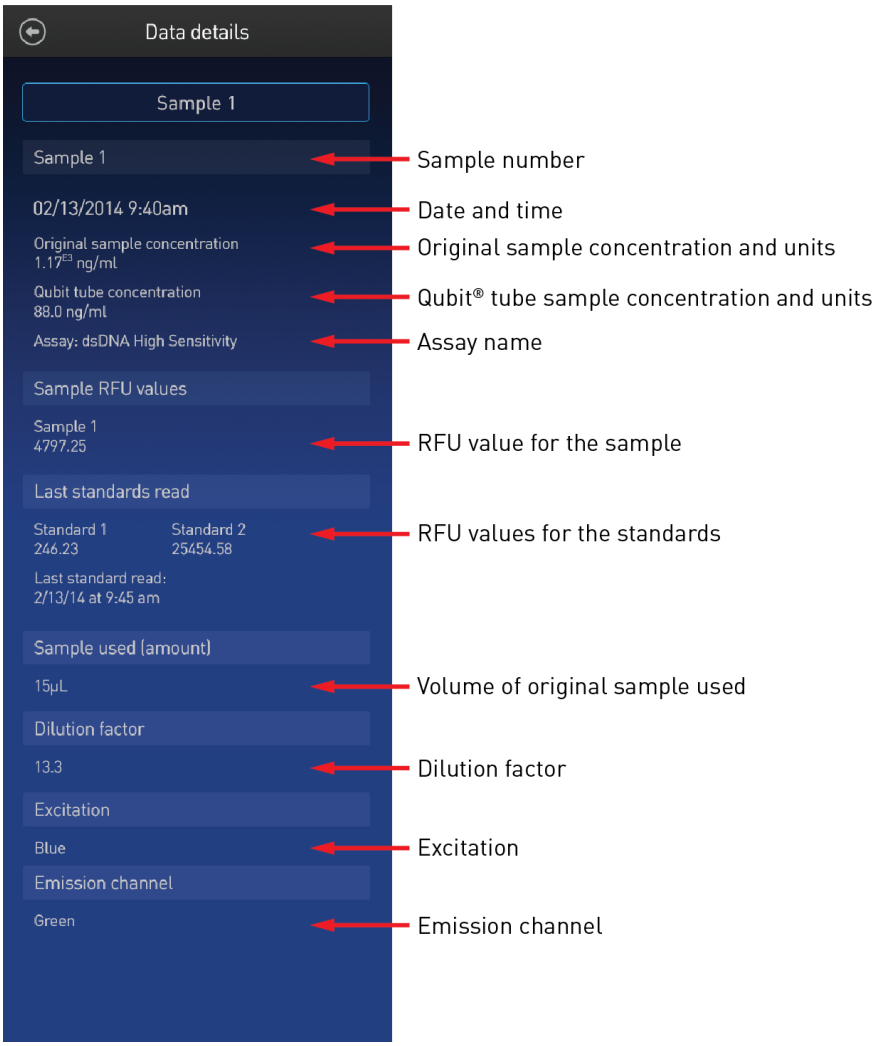
Note: The Windows® XP operating system requires the installation of a driver. The driver and installation instructions are available for download from Qubit Technical Resources at **thermofisher.com/qubit**.

To export data directly to a computer:

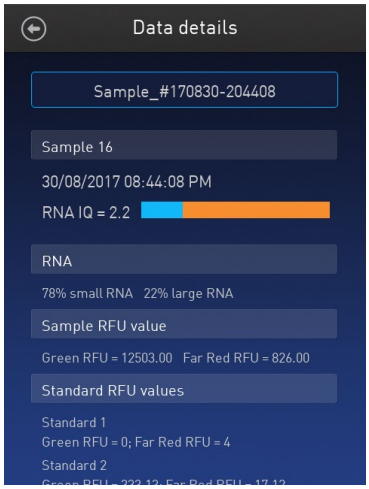
1. Connect the Qubit™ instrument to the computer using the USB cable.
2. From the computer, access the Qubit™ 4 Fluorometer as an external device.
3. Access the files saved on the Qubit™ instrument using the operating system's normal file browsing menus.
4. Copy the desired files to the computer.

Note: Files cannot be exported directly to an external computer using the Export workflow on the Qubit™ instrument.

Information saved to the Export file



Note: For the RNA IQ assay, there is no original sample concentration, Qubit™ tube concentration or dilution factor. Instead, the results file contains % large and % small RNA information.



Export data to the cloud using Wi-Fi

Data from the Qubit™ instrument can also be exported to the cloud using a Wi-Fi connection.

Note: Confirm the Qubit™ 4 instrument has the latest firmware (under Settings > About Instrument) and correct date and time.

The lastest firmware is available at: https://www.thermofisher.com/us/en/home/industrial/spectroscopy-elemental-isotope-analysis/molecular-spectroscopy/fluorometers/qubit/qubit-technical-resources.html.

1. Insert the Wi-Fi dongle into the USB port, then power on the instrument.
2. From the **Home** screen, press **Settings** > **Instrument Settings**, then select **Network Connection**.
3. On the **Choose Network** screen, select the network to join.
    a. On the **Configure & Join Network** screen, enter the password into the PSK box.
    b. Press **Join**.

    The network will be highlighted in a brighter blue color once connected.
4. On the **Home** screen, press **Data**.
5. On the **Data** screen, check the selection box to the left of each data set you wish to export.
    a. To select all data sets, press the blue check icon in the header row.
    b. To export only individual data entries from a data set, press the data set of interest, then check the selection box to the left of the samples desired for export.
6. After selecting the data sets or sample runs, press **Actions**, then **Export**.
7. In the **Export data** screen, select the **Export method**.
8. Press **Export**.

Export data to the cloud using your Connect account

Data from the Qubit™ instrument can also be exported to the cloud using a Wi-Fi connection.

1. Register or sign in to your account at https://www.thermofisher.com/us/en/home/digital-science.html.
2. Click the **Instrument Connect** icon on the left side toolbar, then click on the blue **Add Instrument** icon in the upper right corner.
3. Select **Qubit_4** from the drop-down menu and click **Next**. You are then instructed to "**Insert linking code: Get the code from your instrument.**"
4. On the Qubit™ 4 instrument **Home** screen, click on the profile (person) icon on in the top left corner to go to the **Sign In** screen.
5. Under **New to this instrument?**, select **Get Started**.
6. Under **Connect to the Thermo Fisher Cloud**, select **Connect**.
7. From the **Connect to the Cloud** screen on the Qubit™ 4 instrument, select **Dashboard on PC**.
8. On the **Home** screen, press **Data**.

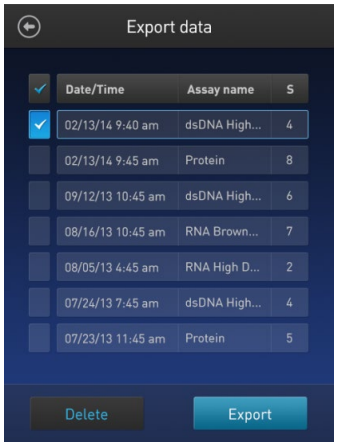
9. On the **Data** screen, check the selection box to the left of each data set you wish to export.
    a. To select all data sets, press the blue check icon in the header row.
    b. To export only individual data entries from a data set, press the data set of interest, then check the selection box to the left of the samples desired for export.
10. After selecting the data sets or sample runs, press **Actions**, then **Export**.
11. In the **Export data** screen, select the **Export method**.
12. Press **Export**.

Delete data

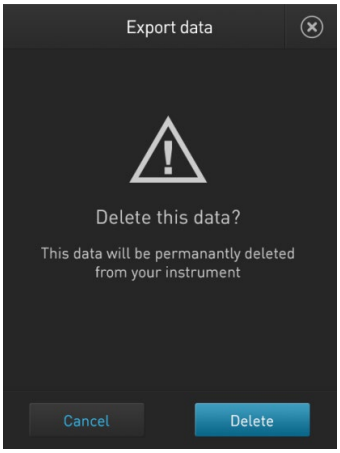
Delete data files

1. On the **Concentration**, **Graph**, **RNA IQ results**, or **Home** screen, press **Data**.
2. On the *Export data* screen, check the **selection box** to the left of each data set you wish to delete.

   To delete only individual data entries from a data set, press the **data set of interest**, and then check the **selection box** to the left of the samples you wish to delete.



3. Press **Delete**. A warning screen appears.



4. Press **Delete** to permanently delete the sample data or data set.

   Press **Cancel** to return to the screen previously viewed without deleting any data.

5. Manage, import, and create assays

Overview

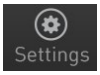
The Qubit™ 4 Fluorometer allows you to personalize the instrument's user interface to hide and show (unhide) assays or folders and to move them to different locations. In addition, you can add custom MyQubit assays by importing them from our website at **thermofisher.com/qubit** or create new assays using the MyQubit software and web tool.

Manage assays

Manage assays function

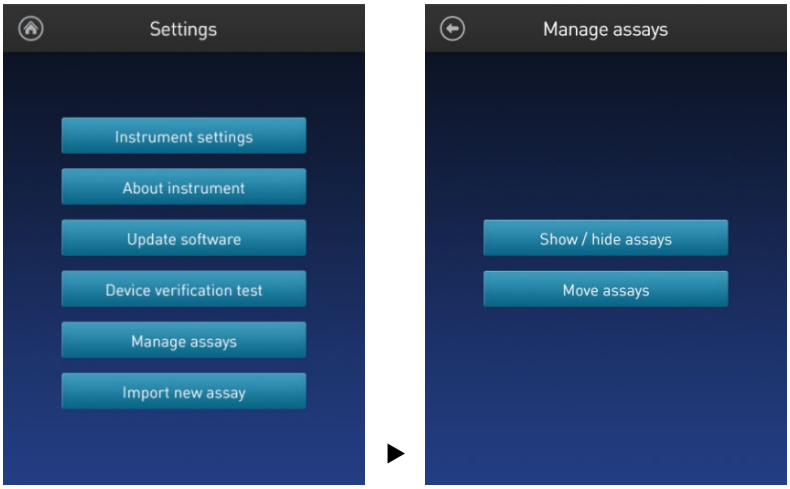
The *Manage assays* function allows you to hide and show (unhide) assays or folders and to move them to different locations.

1. To access the Manage assays function, press **Settings** on the Home screen.



2. On the Settings screen, press **Manage assays** to open the *Manage assays* screen.

   From the Manage assays screen, you can select **Show/hide assays** (page 42) or **Move assays** (page 44).

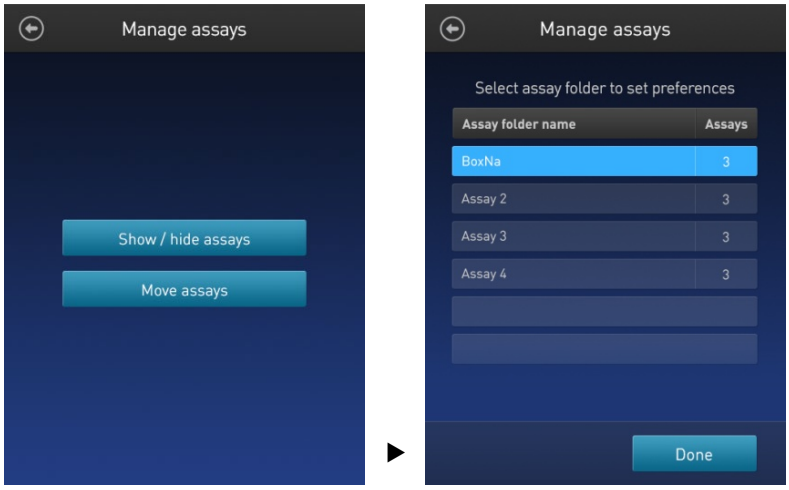


Show/Hide assays

Show/Hide all assays within the assay folder

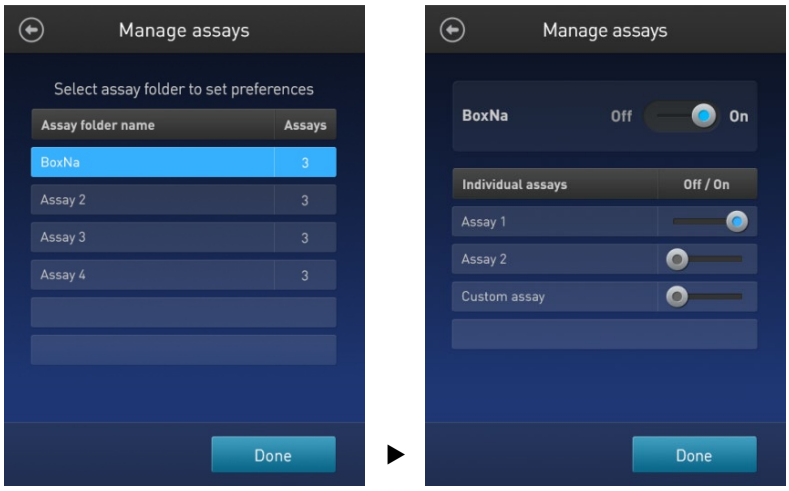
1. From the **Settings** screen, press **Manage assays**.
2. On the *Manage assays* screen, press **Show/hide assays**.

   The software displays a list of the existing *assay folders* available on the instrument. Each assay folder contains individual assays.



3. Select the **assay folder** containing the assays you wish to show or hide.

   The software displays a list of existing individual assays within the selected assay folder and the controls for showing and hiding them.



4. To hide all of the assays in the assay folder, swipe the **Assay folder On/Off** switch located to the right of the assay folder name to the **Off** position (to the **left**).

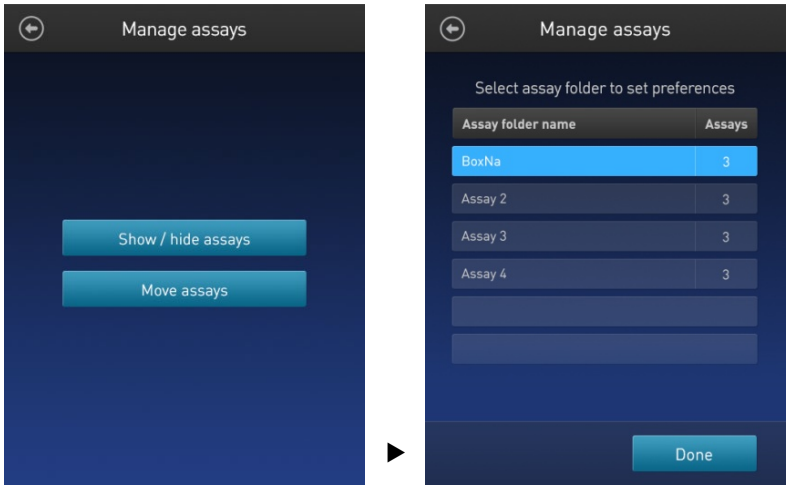
   To show all of the assays in the assay folder, swipe the **Assay folder On/Off** switch to the **On** position (to the **right**).

5. Press the **Back** button ( ) to save the changes and return to the previous screen, or press **Done** to save the changes and return to the Settings screen.

Show/Hide individual assays

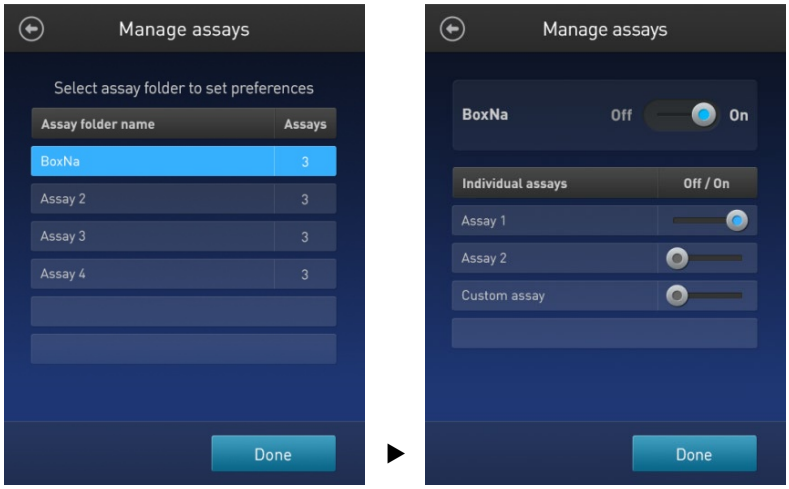
1. From the **Settings** screen, press **Manage assays**.
2. On the *Manage assays* screen, press **Show/hide assays**.

   The software displays a list of the existing assay folders available on the instrument. Each assay folder contains individual assays.



3. Select the **assay folder** containing the assays you wish to show or hide.

   The software displays a list of existing individual assays within the selected assay folder and the controls for showing and hiding them.



4. To hide an **individual assay**, swipe the **Assay On/Off** switch located to the right of the assay to the **Off** position while keeping the **Folder On/Off** switch in the **On** position.

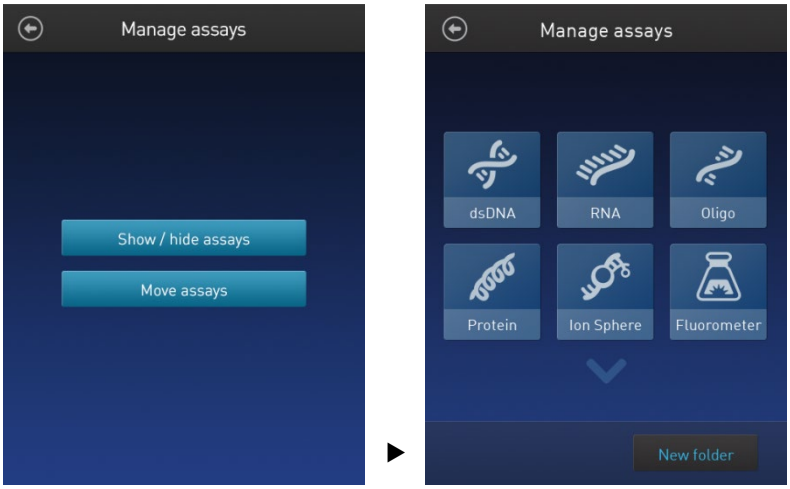
   To show an **individual assay**, swipe the respective **Assay On/Off** switch to the **On** position while keeping the **Folder On/Off** switch in the **On** position.
5. Press the **Back** button ( ) to save the changes and return to the previous screen, or press **Done** to save the changes and return to the Settings screen.

Move assays

Move assay folders/ individual assays on the Home screen

1. From the **Settings** screen, press **Manage assays**.
2. On the *Manage assays* screen, press **Move assays**.

   The software displays the Manage assays screen with available assay folders and individual assays, and activates the move function.



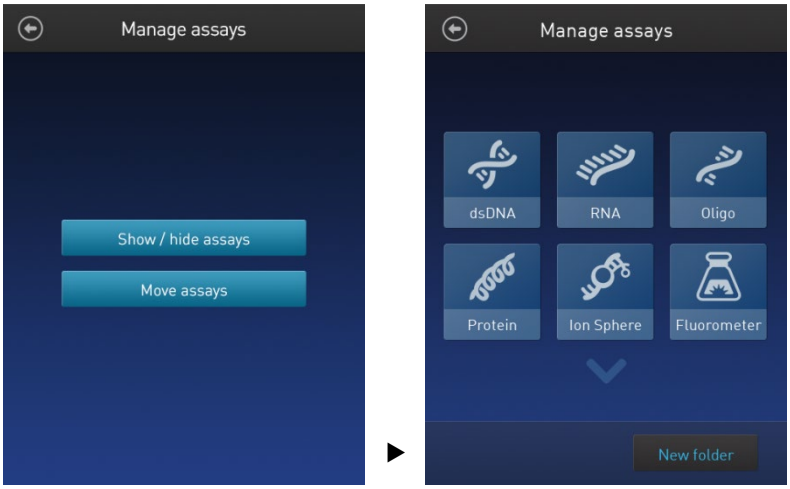
3. Press and hold on the desired assay folder or individual assay to detach it from its current position, then drag it to its new location.

   To move the selected object to a different Home screen page, drag it up or down to the desired screen.
4. Release the drag to drop the selected assay folder or individual assay in its new location.
5. Press the **Back** button ( ) to save the changes and return to the Manage assays screen.

Move assays between folders

1. From the **Settings** screen, press **Manage assays**.
2. On the *Manage assays* screen, press **Move assays**.

   The software displays the Manage assays screen with available assay folders and individual assays, and activates the move function.



3. Press the desired **assay folder** to open the folder's assay screen.
4. Press and hold the **individual assay** to detach it and drag it over the Home screen icon ( ).
5. Release the drag to drop the selected assay to the Home screen.
6. Press the **Home** button ( ) to return to the Home screen.

   In the Home screen, you can press and drag the selected assay to another assay folder or location as described in "Move assay folders/ individual assays on the Home screen", page 44.
7. Press the **Back** button ( ) to save the changes and return to the Manage assays screen.

Move assays within folders

1. From the **Settings** screen, press **Manage assays**.
2. On the *Manage assays* screen, press **Move assays**.
3. Press the desired **assay folder** to open the folder's assay screen.
4. Press and hold the **individual assay** to detach it, and drag and drop it to its new location in the folder.
5. Press the **Back** button ( ) to save the changes and return to the Manage assays screen.

Import assays

MyQubit assay files

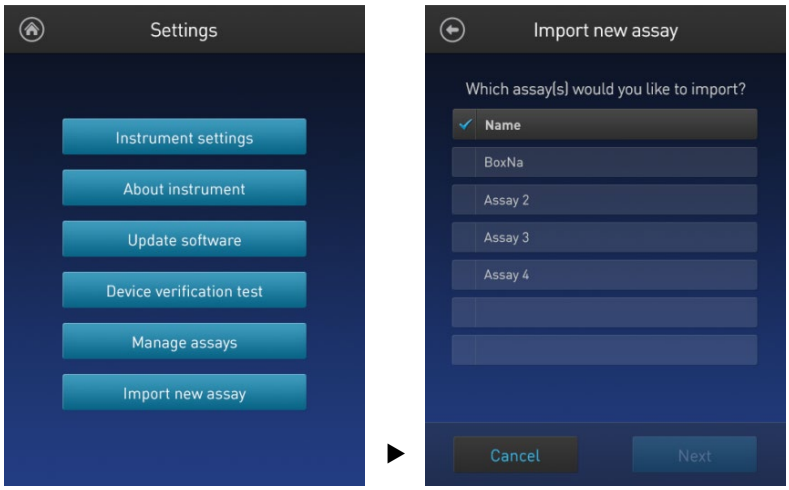
You can add pre-existing MyQubit assays to your Qubit™ 4 Fluorometer by downloading the assay files from **thermofisher.com/qubit**, then importing the files to the instrument from a USB drive or from an external computer via a USB cable.

Note: MyQubit assay files have the file extension .qbt.

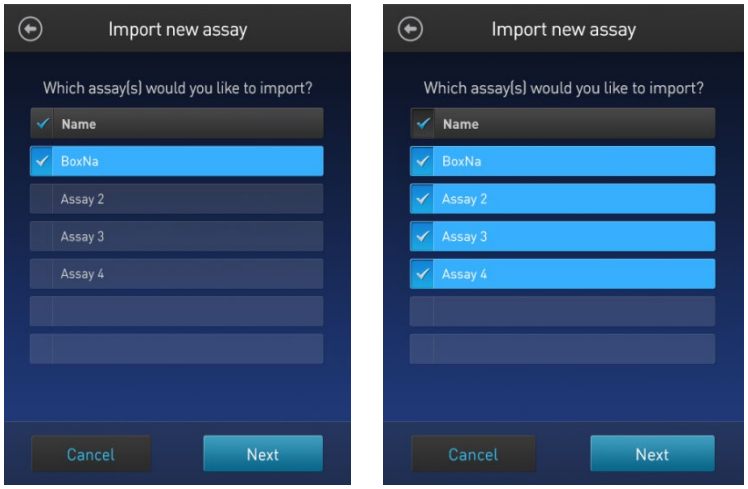
Import assays from a USB drive

1. To import one or more new assays, go to **thermofisher.com/qubit** and download the MyQubit assay files (.qbt file) you wish to install to the **root directory** of a USB drive.

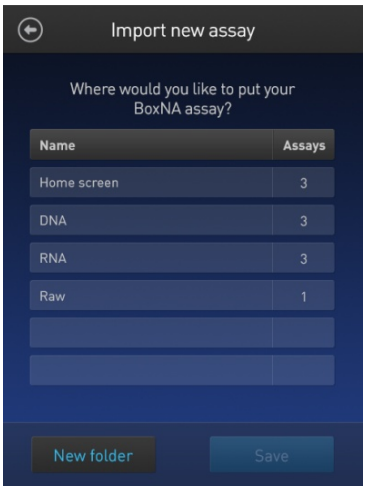
   Do **not** download the .qbt files to a folder in the USB drive.
2. Insert the USB drive containing the new assay files into the USB drive port of the Qubit™ 4 Fluorometer.
3. Go to the Settings screen and press **Import new assay**. The instrument searches the USB drive for assay files and the *Import new assay* screen displays the names of the assays found in the USB drive.



4. Press the assays you wish to import to select. Touching the selected assay a second time deselects it. You can select multiple assays for import.



5. Press **Next**. The *Import new assay* screen displays the name of the assay being imported (or the first assay, if multiple assays were selected) and lists the destination folders where you can save it.

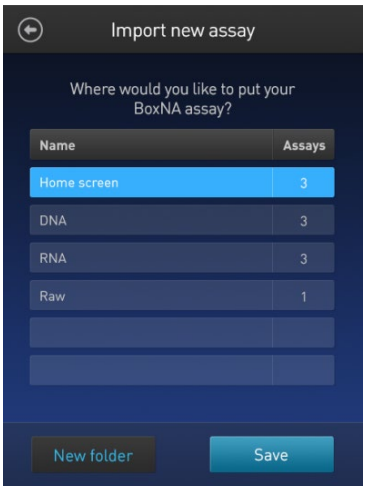


Press the **Back** button ( ) or **Cancel** to return to the Settings screen without importing the assays.

Note: Each assay folder can contain up to 9 individual assays. The Home screen can contain up to 36 assays or folders.

6. Select the destination folder for the new assay and press **Save**. The *Verification* screen opens (see Step 9, page 48).

   Alternatively, press **New folder** to create a new destination folder (see Step 8, page 48).



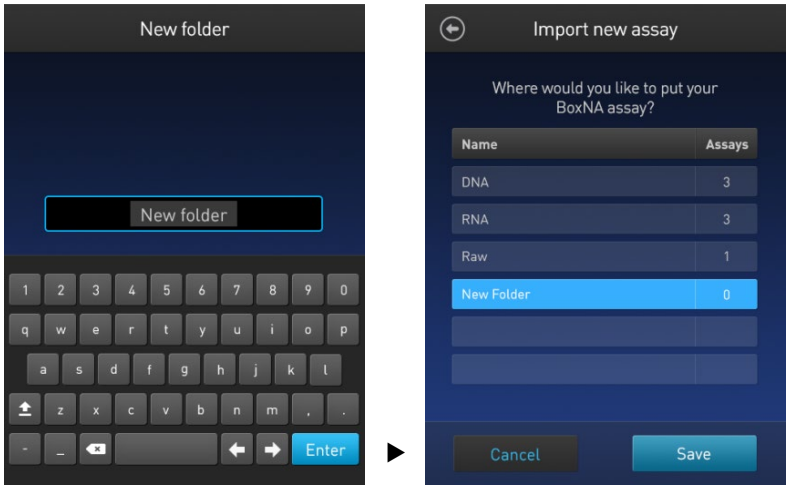
Press the **Back** button ( ) or **Cancel** to abort the import process and return to the Settings screen.

7. In the *New folder* screen, press the **New folder** text field. The keyboard opens.

   Using the **keyboard**, enter the name of the new destination folder. You can use up to 11 characters; spaces at the beginning or the end of the name are not recognized.

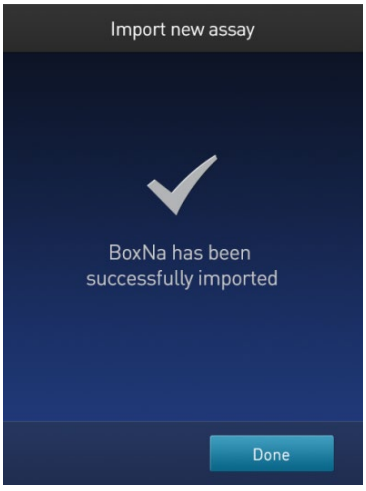
   Press **Enter**, then press **Done** to return to the *Import new assay – Where* screen. The newly created folder will appear on the list of folders.

   Select the new destination folder, then press **Save**. The *Verification* screen opens (see Step 9, page 49).



8. In the Verification screen, press **Done** to complete the import process.

   If importing only one assay, you will be returned to the Home screen.

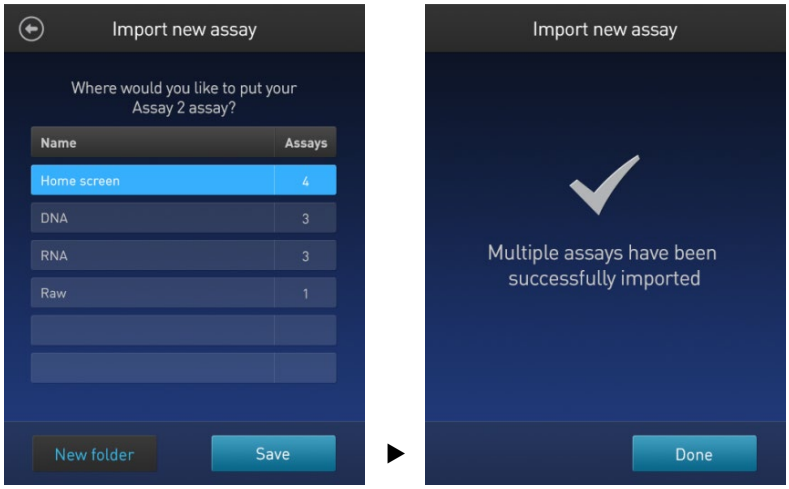


   If importing multiple assays, you will be returned to the *Import new assay* screen and prompted to select the destination folder for the next assay in your import queue (see Step 10, page 49).

9. Select the destination folder for the next assay in your import queue, then press **Save**.

   Repeat this process until you have imported all the assays you have selected in Step 4 (page 46).

   Once all the assays you have selected are imported successfully, the *Verification* screen opens.



10. Press **Done** to complete the import process and return to the *Home screen*.

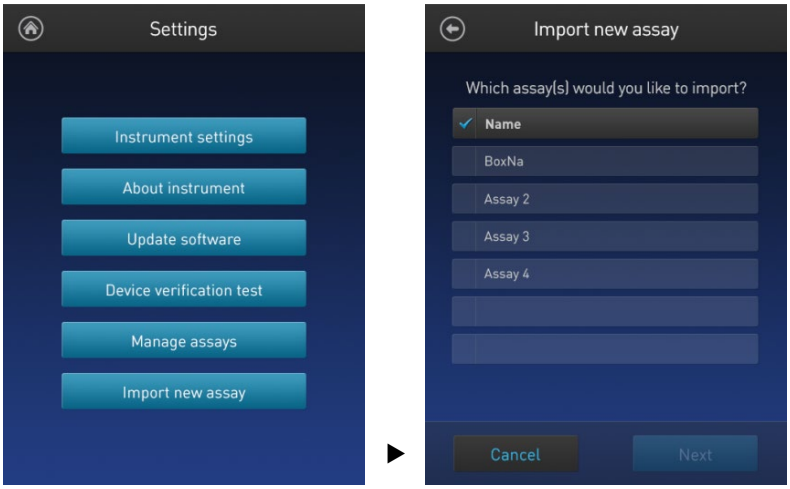
Import assays from a computer

When the Qubit™ 4 Fluorometer is connected to a computer via a USB cable, MyQubit .qbt assay files can be transferred directly from the computer to the instrument.

1. Go to **thermofisher.com/qubit** and download the MyQubit assay files (.qbt file) you wish to install.
2. Connect the Qubit™ 4 Fluorometer from the USB cable port to the computer using a mini-B USB cable. The Qubit™ 4 Fluorometer should be recognized as an external device by the computer.
3. **Copy** and **paste** or **click-drag** the .qbt files you wish to import to the Qubit™ instrument window on your computer.

> **Note:** The assays will not be visible on the Qubit™ 4 Fluorometer until you perform the next step.

4. Go to the Settings screen on the Qubit™ 4 Fluorometer and press **Import new assay**. The *Import new assay* screen displays the names of the assays found on the computer.



5. Follow Steps 4–11 of the import procedure described in "Import assays from a USB drive", page 46.

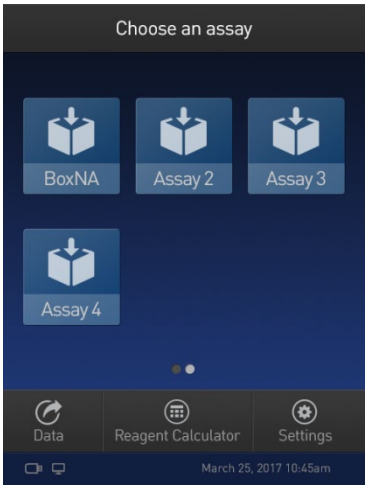
> **Note:** The USB cable must remain connected to both the Qubit™ 4 Fluorometer and the computer for the assay to be imported.

Create your own assay for the Qubit™ 4 Fluorometer

Create a MyQubit assay

All Qubit™ 4 Fluorometers are pre-loaded with MyQubit software, which allows you to create new applications for the Qubit™ 4 Fluorometer using parameters uploaded to the instrument via a USB drive, without changing the existing assays.

Fluorometer mode allows you to use the Qubit™ 4 Fluorometer with any reagent or assay compatible with the optical settings of the instrument. In this way, the device can be used as a mini-fluorometer. With the data gathered in the Fluorometer mode, personalized, custom assays can be created using the guided webtool and the MyQubit EZGuide available at **thermofisher.com/qubit**.



Note: For complete instructions on creating your own assay and access to the web tool, go to **thermofisher.com/qubit**.

6. Instrument maintenance

Maintenance and cleaning

Maintenance

The Qubit™ 4 Fluorometer does not need regular maintenance. To troubleshoot problems with the instrument, contact Technical Support (page 71).

- **Do not** perform any repairs or service on the Qubit™ 4 Fluorometer to avoid damaging the instrument.
- **Do not expose the Qubit™ 4 Fluorometer to direct sunlight.**

CAUTION! Never disassemble or service the instrument yourself. Do not remove any covers or parts that require the use of a tool. Unauthorized repairs may damage the instrument or alter its functionality, which may void your warranty. Contact your local distributor to arrange for service.

Clean the Qubit™ 4 Fluorometer

We recommend that you clean the Qubit™ 4 Fluorometer periodically to prevent the buildup of dust and dirt that might reduce its performance and cause contamination.

CAUTION! To avoid electrical shock, always disconnect the power cable before cleaning or decontaminating the instrument.

IMPORTANT! Using a cleaning or decontaminating method other than that specified by the manufacturer may result in damage to the instrument.

- Clean the surface of the Qubit™ 4 Fluorometer with a damp cloth.
- To clean the touchscreen, disconnect the power cable, and clean the touchscreen with a soft cloth lightly moistened with LCD (liquid crystal display) cleansing detergent.
- Cleaning the screen with excessive force can damage the touchscreen. Wipe the screen dry immediately.
- Do not use abrasive cleaning solutions or material to prevent the touchscreen from getting scratched.
- To disinfect the instrument, disconnect the power cable from the Qubit™ 4 Fluorometer and clean the instrument, including the touchscreen, with a soft cloth lightly moistened with 70% ethanol, 70% isopropanol, or 10% bleach (0.6% sodium hypochlorite).
- The cloth included with the instrument is not recommended for use with ethanol or isopropanol.
- Ensure that the cleaning solution does not enter the power button, the power inlet, the sample port, or the USB drive ports.
- Never pour or spray any liquids directly on the instrument to avoid electrical shock when the instrument is plugged in.

Software updates

Update the software

To update the Qubit™ 4 Fluorometer with the latest software from Thermo Fisher Scientific:

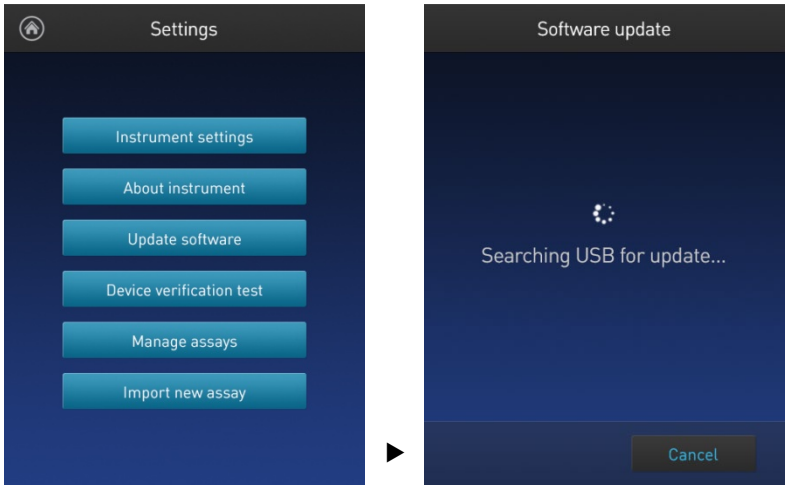
1. Download the latest software to a USB drive or to your computer from **thermofisher.com/qubit**.
2. If using a USB drive, insert the USB drive into the instrument.

   If using a computer, connect the Qubit™ instrument to the computer using a USB cable. The Qubit™ Fluorometer will be recognized as an external device by the computer.

   Note: The connection between the computer and the Qubit™ instrument must be maintained until the transfer and update (described in the next steps) are complete.

   a. On your computer, find the Qubit™ software update file that you wish to upload.

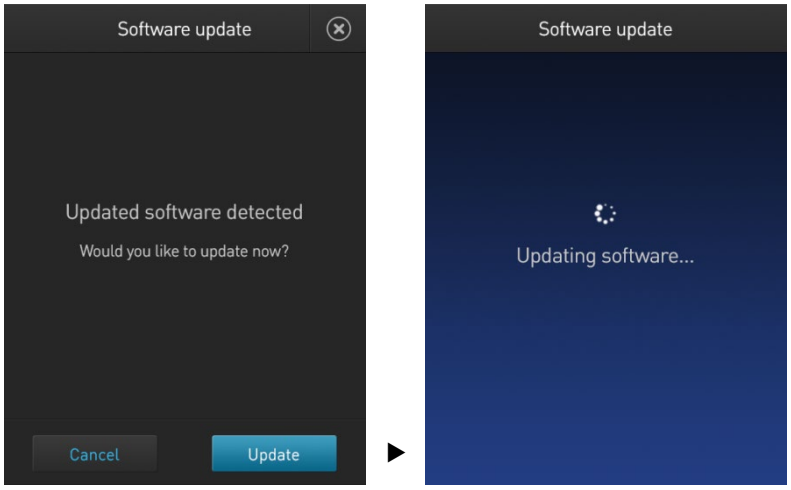
   b. Copy and paste or click-drag the software file to the Qubit™ instrument drive. This saves the software file to the root directory of your Qubit™ instrument; however, the software file transfer will not be visible on the Qubit™ instrument.
3. On the Home screen, press **Settings**.
4. On the Settings screen, press **Update software**. The instrument searches the USB drive or the computer for the update.



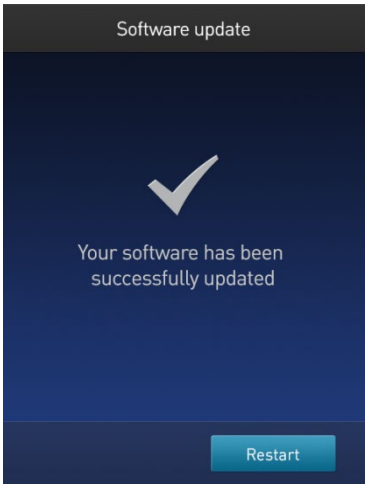
Note: If the USB drive is not inserted into the USB drive port or the instrument does not recognize the USB drive, a warning message is displayed.

To proceed with the software update, insert the correct USB drive into the instrument, then press **Close**.

5. When the appropriate files are detected, the Software update screen is displayed. Press **Update** to update the software.



6. When prompted, press **Restart** to complete the software update.



System verification test

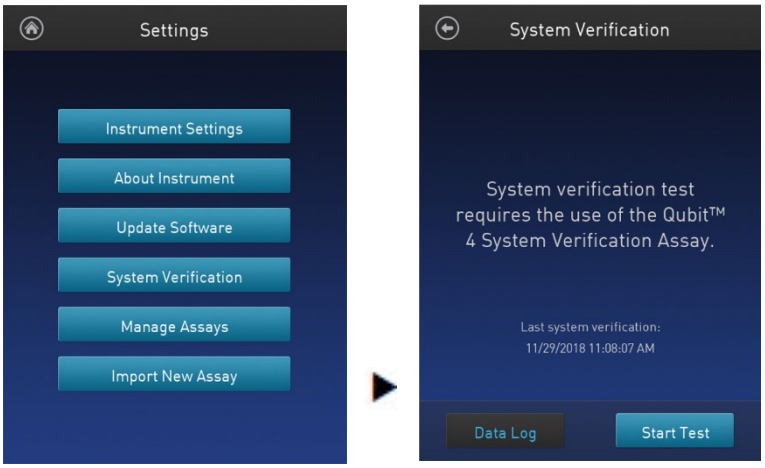
Perform System verification test

The system verification test checks the internal components of the Qubit™ 4 Fluorometer and requires the use of the Qubit™ 4 System Verification Assay Kit (Cat. No. Q33237). Perform the system verification when a problem with the instrument is suspected. It is not necessary to regularly perform the verification.

Required materials not provided:

- Qubit™ 4 System Verification Assay Kit (Cat. No. Q33237)
- Qubit™ assay tubes (500 tubes, Cat. No. Q32856)

1. On the Home screen, press **Settings** then **System Verification Test**. Press **Start Test** to proceed.



2. Set up 3 Qubit™ assay tubes (Cat. No. Q32856).
3. Label the tube lids 1, 2, and 3.

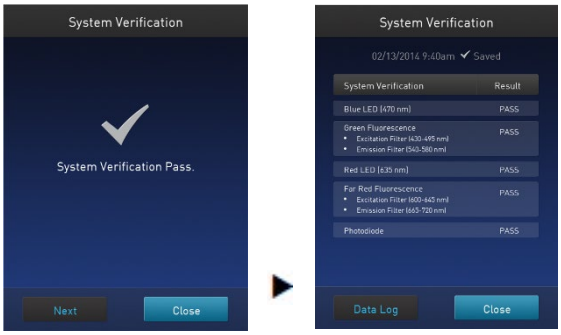
> **Note:** Do not label the side of the tube as this could interfere with the sample read.

4. Add 200 µL of the Blank Reagent (Component A) to Tube 1.
5. Add 200 µL of the Green Fluorescence Reagent (Component B) solution to Tube 2.
6. Add 200 µL of the Far Red Fluorescence Reagent (Component C) solution to Tube 3.
7. Insert Tube 1 containing the Blank Reagent (Component A) into the sample chamber, close the lid, then press **Read tube**. When the reading is complete (~3 seconds), remove Tube 1.
8. Insert Tube 2 containing the Green Fluorescence Reagent (Component B) into the Qubit™ sample chamber, close the lid, then press **Read tube**. When the reading is complete (~3 seconds), remove Tube 2.

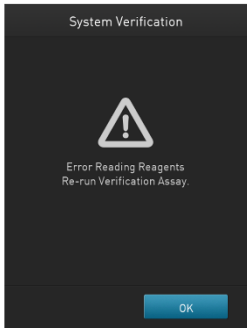
9. Insert Tube 3 containing the Far Red Fluorescence Reagent (Component C) into the sample chamber, close the lid, then press **Read tube**. When the reading is complete (~3 seconds), remove Tube 3.

> **Note:** Always insert tubes in the order 1, 2, 3.

10. Upon read completion, the instrument displays the overall result of the system verification.
    - If no errors are found, the System Verification Pass message appears. Press **Close** to return to the Settings screen or press **Next** to view the System Verification Report.

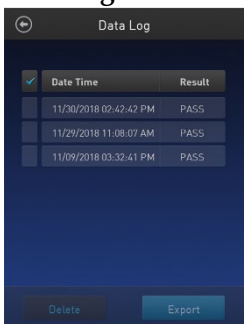


    - If errors are found, the Error Reading Reagents message appears. Verify that the test was run with the lid closed, then press **OK** to re-run the test with the tube strips in the correct order.



• If the System Verification Failed message persists after re-running the tube strips with the lid closed, contact Technical Support for help (page 70).

    - Press **Next** to view system verification test results details or press **Data Log** to access and export system verification test results.



Appendix A: Troubleshooting

Troubleshooting

Handling samples

- The calibration standards included in the Qubit™ microRNA, Qubit™ RNA HS, Qubit™ RNA XR, Qubit™ RNA IQ, and Qubit™ RNA BR Assay Kits are high-quality RNA standards. The integrity and concentration of these standards is critical to the optimal performance of the Qubit™ RNA assays. We highly recommend treating the rRNA standards as you would any other precious RNA. Use appropriate RNAse-free handling techniques, including RNAse-free gloves, pipette tips, and tubes. Keep the tube lids closed whenever possible; do not press the pipet to the inside wall of the tube when withdrawing a sample. Return the RNA standards to –80°C as soon as possible after use.
- Ensure that the assay tubes are at room temperature at the time the reading is taken. Do not hold assay tubes in your hand and do not leave assay tubes in the Qubit™ 4 Fluorometer for longer than it takes to read the fluorescence. See "Assay temperature", page 60.
- Be careful not to spill sample into the sample chamber. Promptly wipe any spills.
- The Qubit™ assays are very sensitive and even small amounts of material from a previous sample may result in errors. Use a clean 0.5-mL PCR tube for each reading.
- The tube **must be clean and dry** on the outside when taking readings. Moisture and condensation on the tube surface can lead to reading errors.
- Minute bubbles in samples will cause errors in readings. Be sure not to introduce bubbles into samples. Slight tapping on the tube wall or brief centrifugation will often help dissipate bubbles.

High reading

- The sample is out of range. Use a sample that is less concentrated.
- For Qubit™ quantitation assays, view the Fluorescence vs. Concentration graph in the Results screen to confirm that the values for the samples fall between the values of the standards (page 27).
- Ensure that the lid is closed while reading standards and samples.
- Prepare samples and standards according to the instructions in the Qubit™ assay kit you are using.
- Ensure that the assay is performed entirely at room temperature.

Low reading

- The sample is out of range. Use a sample that is more concentrated or use a lower dilution (for example, 20 µL in 180 µL instead of 10 µL in 190 µL).
- For Qubit™ quantitation assays, view the Fluorescence vs. Concentration graph in the Results screen to confirm that the values for the samples fall between the values of the standards (page 27).
- Ensure that you have prepared the Qubit™ working solution correctly (1:200 dilution using the buffer provided in the kit).
- Ensure that you have prepared the standard tubes correctly (10 µL of each standard in 190 µL of Qubit™ working solution).
- Ensure that the standard and sample tubes are filled to 200 µL.
- Protect the Qubit™ reagent and working solutions from light.

- Select the correct Qubit™ 4 Fluorometer assay for the Qubit™ assay you are performing and calibrate the fluorometer correctly. Standards must be used in the correct order.
- Ensure that the assay is performed entirely at room temperature.

Critical Qubit™ Assay considerations

How the Qubit™ 4 Fluorometer calculates concentration

The Qubit™ 4 Fluorometer generates concentration data based on the relationship between the two standards used in calibration (three for the Qubit™ protein assay). The plot below shows the line corresponding to the curve-fitting algorithm (a modified Hill plot) used in the calculation of concentration data for the Qubit™ RNA HS assay. For reference, the positions of the standards and a set of data points from an actual experiment are shown superimposed onto the line. This plot demonstrates that the curve-fitting algorithm gives accurate values for quantitation.

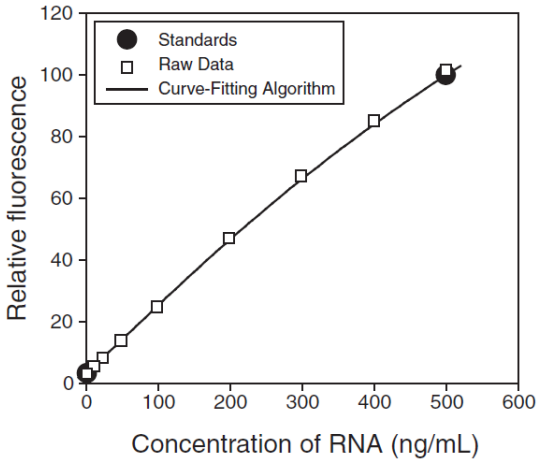


Figure 1. The curve-fitting algorithm used to determine concentration in the Qubit™ RNA HS assay. Data for other Qubit™ quantitation assays are generated by similar algorithms.

Incubation time

To allow the Qubit™ assay to reach maximum fluorescence, incubate the tubes for the DNA and RNA assays for 2 minutes after mixing the sample or standard with the working solution. After this incubation period, the fluorescence signal is stable for 3 hours at room temperature for all nucleic acid assays except the Qubit™ ssDNA assay, which is stable for up to 30 minutes.

The Qubit™ protein assay requires 15 minutes of incubation for a stable signal. For greatest accuracy in the protein assay, the incubation time of the samples should be within 10 minutes of the incubation time of the standards. The Qubit™ Protein Broad Range Assay requires 10 minutes of incubation time for best results.

Photobleaching of Qubit™ reagents

The Qubit™ DNA and protein exhibit high photostability in the Qubit™ 4 Fluorometer, showing <0.3% drop in fluorescence after 9 readings and <2.5% drop in fluorescence after 40 readings. It is important to remember, however, that if the assay tube remains in the Qubit™ 4 Fluorometer for multiple readings, a temporary reduction in fluorescence will be observed as the solution increases in temperature (see Figure 2 in "Assay temperature", page 60). The RNA assays including the RNA IQ assay should only be read once.

Note that the temperature inside the Qubit™ 4 Fluorometer may be as much as 3°C above room temperature after 1 hour. For this reason, if you want to perform multiple readings of a single tube, remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

Assay temperature

The Qubit™ assays were designed to be performed at room temperature (22–28°C), and temperature fluctuations can influence the accuracy of the assay.

To minimize temperature fluctuations, store all kit reagents at room temperature and insert all assay tubes into the Qubit™ 4 Fluorometer only for as much time as it takes for the instrument to measure the fluorescence, because the Qubit™ 4 Fluorometer can raise the temperature of the assay solution significantly, even over a period of a few minutes. Do not hold the assay tubes in your hand before a measurement, because holding the tubes warms the solution and results in a low reading.

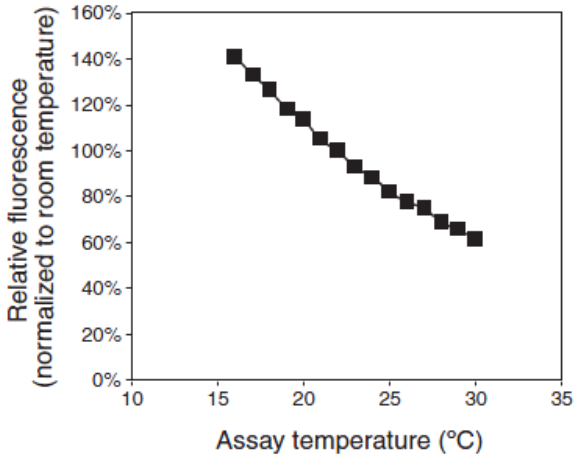


Figure 2. Effect of temperature on the Qubit™ dsDNA BR assay. Qubit™ dsDNA HS, Qubit™ ssDNA, Qubit™ RNA HS, Qubit™ RNA XR, Qubit™ RNA IQ, and Qubit™ protein assays show similar sensitivities over the same range.

Qubit™ 4 Fluorometer calibration

For each assay, you have the choice to run a new calibration or to use the values from the previous calibration. As you first use the instrument, you perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, you can determine the level of comfort you have using the calibration data stored from the last time the instrument was calibrated. Remember also that the fluorescence signal in the tubes containing the standards and the samples is stable for not longer than 3 hours. See Figure 1 in "How the Qubit™ 4 Fluorometer calculates concentration" (page 59) for an example of the calibration curve used to generate the quantitation results.

Appendix B: Ordering information

Qubit™ 4 Fluorometer and accessories

The following products can be used with the Qubit™ 4 Fluorometer and are available separately from Thermo Fisher Scientific. For more information, visit **thermofisher.com** or contact Technical Support (page 71).

Product	Quantity	Cat. No.
Qubit™ 4 Fluorometer, with Wi-Fi	1 kit	Q33238
Qubit™ 4 Quantitation Starter Kit, with Wi-Fi	1 kit	Q33239
Qubit™ 4 NGS Starter Kit, with Wi-Fi	1 kit	Q33240
Qubit™ RNA IQ Starter Kit, with Wi-Fi	1 kit	Q33241
Qubit™ 4 USB Flash Drive	1 each	Q46009
Qubit™ 4 Fluorometer International Power Supply (replacement)	1 each	A36204
Qubit™ Assay Tubes	500 tubes	Q32856
Qubit™ Protein BR Assay Kit *0.1–20 mg/mL*	100 assays 500 assays	A50668 A50669
Qubit™ Protein Assay Kit *0.25–5 µg*	100 assays 500 assays	Q33211 Q33212
Qubit™ 1X dsDNA HS Assay Kit	100 assays 500 assays	Q33230 Q33231
Qubit™ 1X dsDNA BR Assay Kit	100 assays 500 assays	Q33265 Q33266
Qubit™ ssDNA Assay Kit *1–200 ng*	100 assays	Q10212
Qubit™ RNA IQ Assay Kit	75 assays 275 assays	Q33221 Q33222
Qubit™ RNA HS Assay Kit	100 assays 500 assays	Q32852 Q32855
Qubit™ RNA BR Assay Kit	100 assays 500 assays	Q10210 Q10211
Qubit™ RNA XR Assay Kit	100 assays 500 assays	Q33223 Q33224
Qubit™ microRNA Assay Kit *0.5–100 ng*	100 assays 500 assays	Q32880 Q32881

Appendix C: Safety

Symbols on instruments

Electrical symbols

The following table describes the electrical symbols that may be displayed.

Symbol	Description
	Indicates a terminal that can receive or supply alternating current or voltage.

Safety symbols

The following table describes the safety symbols that may be displayed. Each symbol may appear by itself or in combination with text that explains the relevant hazard (see "Safety labels on instruments"). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.

Environmental symbols

The following symbol applies to all Thermo Fisher Scientific electrical and electronic products placed on the European market after August 13, 2005.

Symbol	Description
	**Do not dispose of this product as unsorted municipal waste.** Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). **European Union customers:** Call your Customer Service representative for equipment pick-up and recycling. See **thermofisher.com** for a list of customer service offices in the European Union.

Safety labels on instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on Thermo Fisher Scientific instruments in combination with the safety symbols described in the preceding section.

Hazard symbol	English	Français
	**CAUTION!** Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.	**ATTENTION!** Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant toute manipulation de produits.
	CAUTION! Hazardous waste. Refer to SDS(s) and local regulations for handling and disposal.	**ATTENTION!** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
	**DANGER!** High voltage.	**DANGER!** Haute tension.
	WARNING! To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Thermo Fisher Scientific qualified service personnel.	**AVERTISSEMENT!** Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié venant de chez Thermo Fisher Scientific.

General instrument safety

WARNING! PHYSICAL INJURY HAZARD. Use this product only as specified in this document. Using this instrument in a manner not specified may result in personal injury or damage to the instrument.

Operating the instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Safety Data Sheets (SDSs). See "Safety Data Sheets (SDS)".

Safety precautions

Do not install the instrument in heavy humidity such as a greenhouse or an incubator to avoid a danger of electric shock. If water or other material enters the instrument, the adaptor, or power inlet, disconnect the power cord and contact a service person. For operating environment, refer to "Product specifications" (page 8).

- Do not press the main plug or power cord with wet hands.
- Always ensure that the power supply input voltage matches the voltage available in your location.
- Do not install the instrument on a slant or a place prone to vibrations, which induces the risk of instrument malfunction or damage of the instrument.
- Plug the power cord firmly into the wall outlet and the instrument.
- To avoid potential shock hazard, make sure that the power cord is properly grounded.
- Be sure to position the equipment such that it is easy to disconnect the instrument.
- If the instrument is broken or dropped, disconnect the power cord and contact technical services. Do not disassemble the instrument.
- Use only authorized accessories (adaptor, power cord, and USB drive).
- For operating environment, see "Product specifications" (page 8).
- If the instrument emits smoke, disconnect the power cord from the wall outlet and contact technical services.

Cleaning or decontaminating the instrument

CAUTION! Using cleaning or decontamination methods other than those recommended by the manufacturer may compromise the safety or quality of the instrument.

Removing covers or parts of the instrument

CAUTION! PHYSICAL INJURY HAZARD The instrument is to be serviced only by trained personnel or vendor specified in the user guide. Do not remove any covers or parts that require the use of a tool to obtain access to moving parts.

Chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

General safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "Safety Data Sheets (SDS)", page 71)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical waste safety

Chemical waste hazard

CAUTION! HAZARDOUS WASTE. Refer to Safety Data Sheets (SDSs) and local regulations for handling and disposal.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste. (See "Safety Data Sheets (SDS)", page 71)
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis, if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Electrical safety

DANGER! ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the Qubit™ 4 Fluorometer without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Power

DANGER! ELECTRICAL HAZARD. Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.

DANGER! ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.

DANGER! ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage rating

The Qubit™ 4 Fluorometer has an installation (overvoltage) category of II, and is classified as portable equipment.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in these publications.

ATTENTION! BIOHAZARD. Les échantillons biologiques tels que les tissus, les fluides corporels et le sang des humains et d'autres animaux ont la possibilité de transmettre des maladies infectieuses. Suivre tous les règlements municipaux, provinciaux/provincial et / ou nationales en vigueur. Porter des lunettes de protection approprié, des vêtements et des gants.

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories*

  (stock no. 017-040-00547-4;
  www.cdc.gov/OD/ohs/biosfty/bmbl4/bmbl4toc.htm)
- Occupational Safety and Health Standards, Bloodborne Pathogens

  (29 CFR§1910.1030;
  www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html)
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at:

  www.cdc.gov

In the EU:

- Check your local guidelines and legislation on biohazard and biosafety precaution, and the best practices published in the World Health Organisation (WHO) Laboratory Biosafety Manual, third edition

  www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

Safety, Electromagnetic compatibility (EMC), and Environmental standards

This section provides information on:

- U.S. and Canadian safety and EMC standards
- European safety, EMC, and Environmental standards
- Australian EMC standards
- China RoHS Standards

U.S. and Canadian Safety standards

The Qubit™ 4 Fluorometer has been tested to and complies with standard:

UL 61010-1/CAN/CSA-C22.2 No. 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."

UL 61010-2-081/CAN/CSA-C22.2 No. 61010-2-081, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 2-081: Particular Requirements for Automatic and Semi-Automatic Laboratory Equipment for Analysis and Other Purposes."

U.S. EMC standard

This instrument has been tested to and complies with standard 47 CFR FCC Part 15 "Radio Frequency Devices"; Subpart B "Unintentional Radiators".

Note: This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/TV technician for help.

Canadian EMC standard

This instrument has been tested to and complies with standard ICES-001, "Industrial, Scientific and Medical (ISM) Radio Frequency Generators."

European Safety, EMC, and Environmental standards

Safety

This instrument meets European requirements for safety (Low Voltage Directive 2014/35/EU). This instrument has been tested to and complies with standards:

IEC/EN 61010-1:2010, “Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements.”

IEC/EN 61010-2-081:2015, "Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes."

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 2014/30/EU). This instrument has been tested to and complies with standards:

EN 61326-1:2013 Class B, “Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements.”

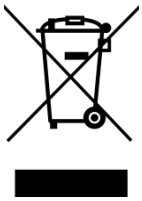
EN 61000-3-2:2014, “Electromagnetic compatibility (EMC) — Part 3 – 2: Limits — Limits for harmonic current emissions (equipment input current ≤16 A per phase).”

EN 61000-3-3:2013, “Electromagnetic compatibitity (EMC) — Part 3 – 3: Limits – Limitation of voltage changes, voltage fluctuations and flicker in public low-voltage supply systems, for equipment with rated current ≤ 16 A per phase and not subject to conditional connection (IEC 61000–3–3:2008).”

Restriction of Hazardous Substance (RoHS)

This instrument meets European requirements RoHS Directive 2011/65/EU.

This instrument has been tested to and complies with standard EN 50581:2012, “Technical documentation for the evaluation of electrical and electronic products with respect to restriction of hazardous substances.”



Waste of Electrical and Electronic Equipment (WEEE)

This instrument meets European requirement WEEE Directive 2012/19/EU.

Australian EMC standards

This instrument has been tested to and complies with standard AS/NZS CISPR 11, “Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment.”

China RoHS standards

This instrument does not contain any hazardous substances above the limit and complies with China RoHS standards:

GB/T 26572-2011, “Requirements on Concentration Limits for Certain Restricted Substances in Electrical and Electronic Products”

SJ/T 11364-2014, "Marking for the Restricted Use of Hazardous Substances in Electronic and Electrical Products"

Documentation and support

Obtaining support

Technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **thermofisher.com/support**.

IMPORTANT! For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

IMPORTANT! Wiping the Qubit™ 4 Fluorometer computer (i.e., erasing the hard drive to remove all programs, files, and the operating system) voids the product warranty.

iontorrent

Ion GeneStudio™ S5 Instrument

USER GUIDE

for use with:
Ion GeneStudio™ S5 Instrument
Ion GeneStudio™ S5 Plus Instrument
Ion GeneStudio™ S5 Prime Instrument

Catalog Numbers A38194, A38195, A38196
Publication Number MAN0017528
Revision C.0

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

Life Technologies Holdings Pte Ltd | Block 33 | Marsiling Industrial Estate Road 3 | #07-06, Singapore 739256

Revision history: Pub. No. MAN0017528

Revision	Date	Description
C.0	1 February 2023	Removed the following skus from the Ion S5™ Installation Kit contents table. • A28195 • A27897 • A29022
B.0	22 February 2022	Update the quantity of the Ion S5™ Cartridge Tool in the Ion S5™ Installation Kit.
A.0	5 January 2018	New instrument user guide that includes instructions for Ion GeneStudio™ S5 Series instrument operation and maintenance.

The information in this guide is subject to change without notice.

Contents

About this guide

CAUTION! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the "Safety" appendix in this document.

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Purpose of the guide

The *Ion GeneStudio™ S5 Instrument User Guide* (Pub. No. MAN0017528) provides reference information for using and maintaining the Ion GeneStudio™ S5 (Cat. No. A38194), Ion GeneStudio™ S5 Plus (Cat. No. A38195), and Ion GeneStudio™ S5 Prime (Cat. No. A38196) Sequencers.

Note: In this user guide, Ion GeneStudio™ S5 Sequencer or System refers generically to the three Ion GeneStudio™ S5 Sequencers or Systems, unless otherwise specified.

The Ion S5™ and Ion S5™ XL Instruments continue to be supported by Thermo Fisher Scientific. See the *Ion S5™ and Ion S5™ XL Instrument User Guide* (Pub. No. MAN0010811) for reference information for the Ion S5™ and Ion S5™ XL Instruments.

1 Product information

Contents

The Ion GeneStudio™ S5 (Cat. No. A38194), Ion GeneStudio™ S5 Plus (Cat. No. A38195), and Ion GeneStudio™ S5 Prime (Cat. No. A38196) Systems contain the following boxes and components.

Ion GeneStudio™ S5 System (Cat. No. A38194)

Components	Part No.
Ion GeneStudio™ S5 Sequencer	A37904
Ion S5™ Installation Kit	A27215

Ion GeneStudio™ S5 Plus System (Cat. No. A38195)

Components	Part No.
Ion GeneStudio™ S5 Plus Sequencer	A37259
Ion S5™ Installation Kit	A27215

Ion GeneStudio™ S5 Prime System (Cat. No. A38196)

Components	Part No.
Ion GeneStudio™ S5 Prime Sequencer	A37260
Ion Torrent™ Server	A36305
Ion S5™ Installation Kit	A27215

Ion S5™ Installation Kit (Part No. A27215; Not available for separate purchase.)

Contents	Part No.	Quantity	Storage
Ion 540™ Chip Kit	A27765	4 pack	15°C to 30°C
Ion S5™ Sequencing Solutions	A27767	1	
Ion S5™ Sequencing Reagents	A27768	1	–30°C to –10°C
Ion S5™ Cartridge Tool	A28308	1	15°C to 30°C

Required materials and equipment not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

✓	Item[1]	Source
	Ion Chip™ Minifuge (120 V or 230 V; required for use only with the Ion OneTouch™ 2 System.)	4479672 (120V) *or* 4479673 (230V)
	Minifuge Proton Rotor and Bucket Kit (required for use only with the Ion OneTouch™ 2 System.)	4482578
	(*Optional*) Uninterruptible Power Supply (UPS) [2]	MLS
	Thermal cycler with a heated lid	MLS
	Microcentrifuge[3]	MLS
	1.5-mL or 1.7-mL microcentrifuge tubes	MLS
	0.2-mL MAXYMum Recovery™ Thin Wall PCR Tubes, Flat Cap (do not use polystyrene tubes)	Axygen, PCR-02-L-C
	Pipettes (P2, P10, P20, P200, P1000) and appropriate low-retention filtered tips	MLS
	Isopropanol (100%)	MLS
	Nuclease-free water molecular biology grade	MLS
	Standard laboratory vacuum line or vacuum pump	MLS
	Liquid trap	MLS
	Tygon™ tubing [4]	MLS
	Vortex mixer	MLS

[1] IMPORTANT! Thermo Fisher Scientific has verified the procedures in this user guide using these specific materials. Substitution can adversely affect system performance.

[2] For laboratories that experience frequent power outages or line voltage fluctuations, we recommend that you use an uninterruptible power supply that is compatible with 2500 W output or higher.

[3] Must fit standard 1.5- and 0.2-mL microcentrifuge tubes and generate 15,500 × *g*.

[4] As needed to connect laboratory vacuum to liquid trap and liquid trap to P200 pipette tip.

Instrument installation by trained personnel only

IMPORTANT! The Ion GeneStudio™ S5 System is installed by trained service personnel and must not be relocated without assistance from trained service personnel. See “Customer and technical support” on page 62.

Nucleic acid contamination

IMPORTANT! A primary source of contamination is DNA fragments from previously processed samples. Do not introduce amplified DNA into the library preparation laboratory or work area.

Instrument vibration and clearances

IMPORTANT! Significant vibration during sequencing may add noise and reduce the quality of the measurements. The Ion GeneStudio™ S5 System must be installed on a bench that is free from vibrations or in contact with equipment that can cause vibrations to the bench (freezers, pumps, and other similar equipment).

IMPORTANT! Place the instrument at least 40 in. (1 meter) away from major sources of electronic noise such as refrigerators or microwaves.

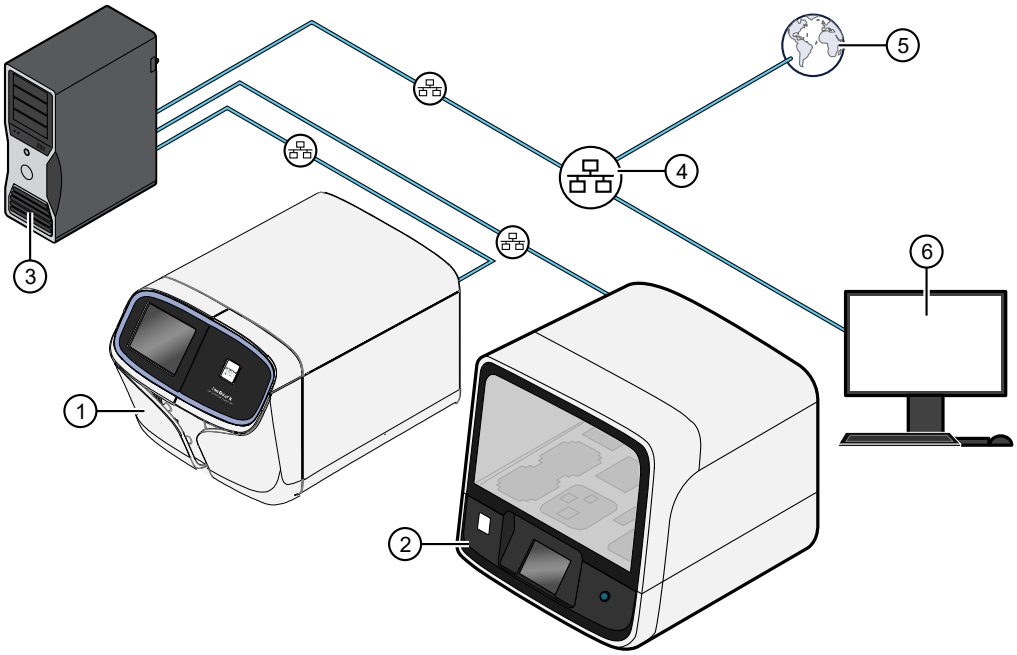
2 System components

Ion GeneStudio™ S5 System component configuration and layout

The Ion GeneStudio™ S5 Sequencer uses a virtual server, and the Ion GeneStudio™ S5 Plus Sequencer is equipped with an internal Ion Torrent™ Server. These instruments are not connected to an external Ion Torrent™ Server. The Ion GeneStudio™ S5 Prime System includes the Ion GeneStudio™ S5 Prime Sequencer and an external Ion Torrent™ Server.

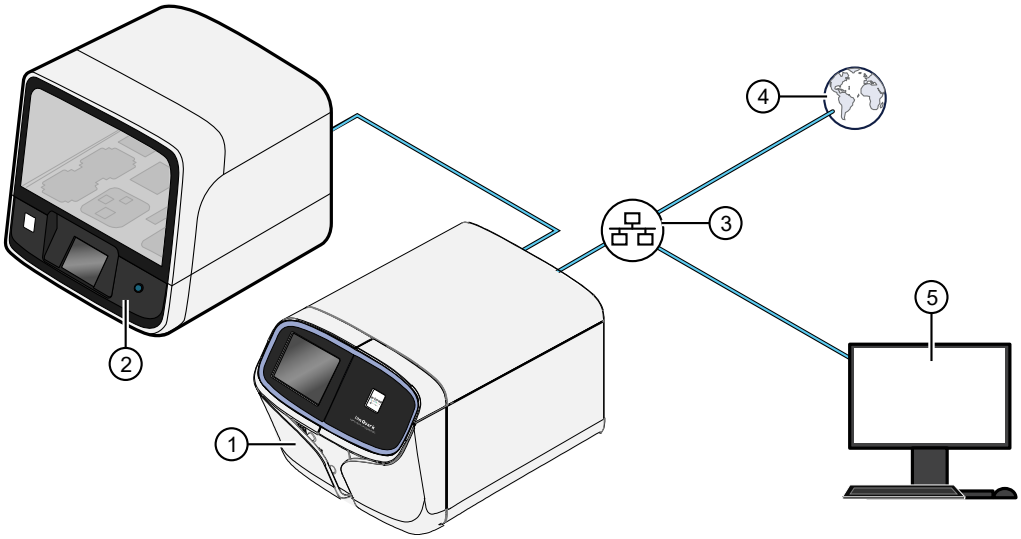
We support the layout in which the external Ion Torrent™ Server is connected directly to the Ion GeneStudio™ S5 Prime Sequencer, instead of through a local area network from a remote location such as a server room. Data are most robustly transferred from the Ion GeneStudio™ S5 Prime Sequencer to the Ion Torrent™ Server when they are connected directly by a standard Category 6 Ethernet cable that is provided with the installation materials.

IMPORTANT! The Ion GeneStudio™ S5 Prime Sequencer must be connected to the Ion Torrent™ Server by a standard Category 6 Ethernet cable. We do not troubleshoot data transfer problems that are associated with an indirect connection between the Ion GeneStudio™ S5 Prime Sequencer and the Ion Torrent™ Server.



Ion GeneStudio™ S5 Prime System configuration

1. Ion GeneStudio™ S5 Prime Sequencer
2. Ion template preparation instrument (Ion Chef™ Instrument (shown) or Ion OneTouch™ 2 System)
3. Ion Torrent™ Server
4. Local area network
5. Internet
6. Client computer



Ion GeneStudio™ S5 System, or Ion GeneStudio™ S5 Plus System configuration

1. Ion GeneStudio™ S5 Sequencer, or Ion GeneStudio™ S5 Plus Sequencer
2. Ion template preparation instrument (Ion Chef™ Instrument (shown) or Ion OneTouch™ 2 System)
3. Local area network
4. Internet
5. Client computer

Internet connectivity

The Ion GeneStudio™ S5 Sequencer, the Ion GeneStudio™ S5 Plus Sequencer, or the Ion Torrent™ Server (in an Ion GeneStudio™ S5 Prime System) should be connected to a network with internet access. Connecting to the Internet allows you to update your software and access remote system support easily. Software updates through the network/internet are free. If you do not connect your instrument or server to a network, software updates must be manually installed via USB.

IMPORTANT! Thermo Fisher Scientific does not support the USB method for updating software.

Any problems (for example, file corruption, incomplete updates) resulting from update of the sequencer or server using a USB device that require correction of the faulty update is not covered by your Ion GeneStudio™ S5, Ion GeneStudio™ S5 Plus, or Ion GeneStudio™ S5 Prime Sequencer warranty or any service contract you may have bought. You are required to schedule an on-site Time and Materials visit by a field service engineer to correct the problem at your own expense.

In providing outbound access to the Internet from the server, you enable the support team to provide inbound support. The Ion GeneStudio™ S5 Sequencer, the Ion GeneStudio™ S5 Plus Sequencer, and the Ion Torrent™ Server run a remote monitor agent that can provide service personnel with critical system information, such as installed software versions and instrument alarms. With your permission, the agent also allows service personnel to access remotely the Ion GeneStudio™ S5 Sequencer, the Ion GeneStudio™ S5 Plus Sequencer, or the Ion Torrent™ Server, which is required for system support. Without remote access, service personnel cannot access, view, and troubleshoot problems regarding machine performance.

To enable full support, the Ion Torrent™ Server must have outbound internet access (ports 22, 80, and 443) and be behind an appropriately configured firewall. Although not recommended, you can enable access to the Torrent Browser (the web server running on the Ion Torrent™ Server from the Internet). If you provide such access, you must restrict access to the server using HTTP and AUTH firewall rules, or a combination of the two. Implementing and maintaining such restrictions is the responsibility of your server administrator and not of Thermo Fisher Scientific.

Note: For further support and troubleshooting for Ion Torrent™ Server network access requirements, see Software Administration in *Torrent Suite™ Software Help*.

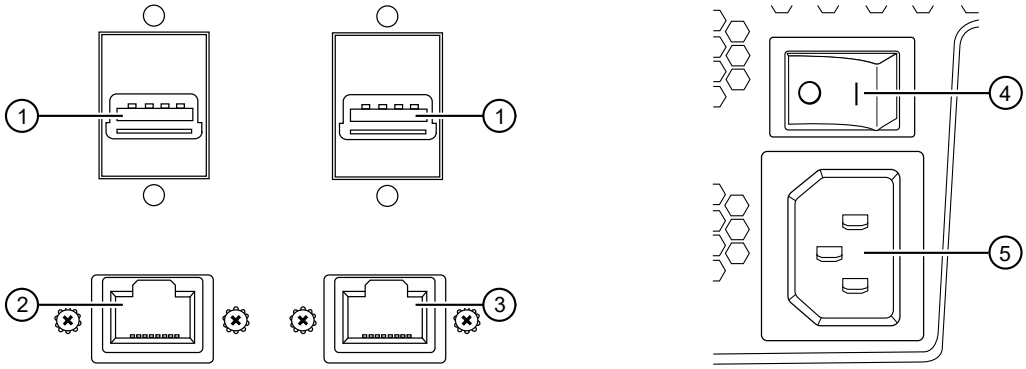
Ion GeneStudio™ S5 System component positions

1. Touchscreen
2. Power button
3. Ion S5™ Sequencing Reagents cartridge
4. Chip clamp
5. Ion S5™ Wash Solution bottle. Waste reservoir located behind the Ion S5™ Wash Solution bottle (shown on the right).
6. Ion S5™ Cleaning Solution bottle
7. Waste reservoir

Note:

- The system uses RFID technology to verify that the proper reagents are loaded in positions 3, 5, and 6. Reagents that exceed their expiration date or usage count generate an error message prompting the user to replace the reagent before performing the run.
- RFID regulatory information can be found on the main screen under **Options ▸ Regulatory info**.

Ion GeneStudio™ S5 Sequencer input and output connections



① USB ports – Connects a USB device to the instrument.

② Ethernet port – An RJ45 port that provides Ethernet (Gigabit) communication between the sequencer and a local area network.

③ Ethernet port – An RJ45 port that provides Ethernet (Gigabit) communication with the Ion Chef™ Instrument.

④ On/off switch – Power switch, where the states are on (|) or off (O).

⑤ Power port – 100-240VAC port that provides power to the instrument.

3 Instrument operation

Power the Ion GeneStudio™ S5 Sequencer on or off

Note: If the Ion GeneStudio™ S5 Sequencer is powered on, and the touchscreen is blank, touch the screen to "wake" the touchscreen.

Power on

If the touchscreen is unresponsive, check the power switch on the back of the instrument to ensure that the switch is in the on (|) position. If the power switch is in the off (0) position, proceed with step 1. If the power switch is already in the on position, proceed to step 2.

1. Turn the power switch on the back of the instrument to the on (|) position.
2. Press the power button on the left side of the instrument.
   The button illuminates. When the instrument touchscreen Main Menu appears, the instrument is ready for use.

Power off

It is not necessary to power off the instrument overnight or over the weekend. If the instrument will not be used for more than three days, power off the instrument as follows:

1. In the Main Menu, tap **Settings** ▸ **System Tools** ▸ **Shut Down**.
2. Select either **Shut Down** or **Reboot**.

   Note: If you select **Shut Down**, a confirmation message appears. Select **Yes** to power off the instrument.

Note: Do *not* press the power button during a run. Interrupting power to the instrument during a run can result in sequencing run failure and loss of sample.

Update the Ion GeneStudio™ S5 System software

Note: An internet connection is required for the Ion GeneStudio™ S5 System to receive alerts that software updates are available.

If an update to the Ion GeneStudio™ S5 Sequencer software is available, the **Notifications/Alarms** button illuminates red in the touchscreen Main Menu. Press the red **Alarms** button to see the detailed messages. If a message states ***New Software Available***, follow these steps to update the software:

1. In the Main Menu, press **Settings** ▸ **Check for Updates**.
2. Press **Update** to download and install the updates automatically.
3. Press **Done**.

The instrument automatically restarts when the software update is complete.

Ion GeneStudio™ S5 instrument run times

Approximate Ion GeneStudio™ S5 instrument run times for a given chip type, read length, and number of reads are shown in the following table. Run times indicate typical time from run start to BAM file delivery (1 run/initialization).

Chip type	Number of reads	Read length/output	Instrument run time		
			Ion GeneStudio™ S5 Sequencer	Ion GeneStudio™ S5 Plus Sequencer	Ion GeneStudio™ S5 Prime Sequencer
Ion 510™ Chip	2–3 M	200 bases 0.3–0.5 Gb	4.5 hours	3 hours	3 hours
		400 bases 0.6–1 Gb	10.5 hours	5 hours	5 hours
Ion 520™ Chip	4–6 M	200 bases 0.6–1 Gb	7.5 hours	3.5 hours	3 hours
		400 bases 1.2–2 Gb	12 hours	5.5 hours	5.5 hours
	3–4 M	600 bases 0.5–1.5 Gb	12 hours	5.5 hours	5.5 hours
Ion 530™ Chip	15–20 M	200 bases 3–4 Gb	10.5 hours	5 hours	4 hours
		400 bases 6–8 Gb	21.5 hours	8 hours	6.5 hours

(continued)

Chip type	Number of reads	Read length/output	Instrument run time		
			Ion GeneStudio™ S5 Sequencer	Ion GeneStudio™ S5 Plus Sequencer	Ion GeneStudio™ S5 Prime Sequencer
Ion 530™ Chip	9–12 M	600 bases 1.5–4.5 Gb	21 hours	8 hours	7 hours
Ion 540™ Chip	60–80 M	200 bases 10–15 Gb	19 hours	10 hours	6.5 hours
Ion 550™ Chip	100–130 M	200 bases 20–25 Gb	—	11.5 hours	8.5 hours

Maintain the sequencer

Materials required

- Lint-free wipes
- 70% isopropanol
- *(Optional)* 10% bleach solution

Clean or decontaminate the sequencer

In the event of a spill or leak on or inside the instrument, perform the following steps.

Note: Dispose of all waste in appropriate liquid or solid waste containers.

1. Remove the Ion S5™ Wash Solution bottle, then remove and empty the waste reservoir.
2. Remove the Ion S5™ Sequencing Reagents cartridge.
3. Inspect the waste and nucleotide reagent bays for liquid.
4. Using absorbent paper soak up as much liquid as possible, then wash the affected area with 10% bleach solution.
5. Wipe the affected surfaces with 70% isopropanol, then allow to air-dry.

Perform the sequencer cleaning manually

The Ion GeneStudio™ S5 Sequencers require cleaning before initialization. Cleaning is normally performed automatically at the completion of the previous sequencing run. To enable two sequencing runs on a single initialization, you must deselect the "Enable post-run clean" checkbox for the first sequencing run. The post-run cleaning is then performed normally after the second sequencing run. However, if the "Enable post-run clean" checkbox is deselected for the second run, the cleaning is not performed after either run. If the second sequencing run is not performed, or the cleaning is not performed automatically after the second run, the instrument will not allow the subsequent initialization to proceed until a manual cleaning has been performed.

If an Ion GeneStudio™ S5 Sequencer is initialized and

- a sequencing run is not started within 24 hours, *or*
- a run is not started or completed due to a power failure or an abort

do not perform a manual cleaning. An instrument reset run is required before reinitialization. See “Perform an instrument reset run with an initialized sequencer that is loaded with an unused Reagents cartridge” on page 20 for more information.

When a cleaning is necessary, use the following procedure:

1. On the home screen, press **Settings** ▸ **Clean Instrument**.
   The instrument door unlocks allowing access to the consumables.

2. Remove the Ion S5™ Wash Solution bottle to access the waste reservoir, then remove and empty the waste reservoir.

3. Reinstall the empty waste reservoir and a *used* Ion S5™ Wash Solution bottle.

4. Ensure the Ion S5™ Sequencing Reagents cartridge and Ion S5™ Wash Solution bottle are properly installed.

   IMPORTANT! Perform the cleaning with a used reagent cartridge and wash solution bottle installed. The cleaning procedure pumps cleaning solution into the wash solution bottle and reagent cartridge making them unsuitable for sequencing.

5. Place a used sequencing chip in the chip clamp, then push the chip clamp in all the way to engage.

6. Close the instrument door, then press **Next**.
   Cleaning takes ~35 minutes to complete. On completion, the instrument door automatically unlocks and the chip and cartridge clamps disengage.

Perform an instrument reset run with an initialized sequencer that is loaded with an unused Reagents cartridge

Cleaning is normally automatically performed at completion of a sequencing run. If an Ion GeneStudio™ S5 System is initialized, an instrument reset run is required to ensure proper cleaning before reinitialization in one of the following situations.

- A sequencing run is not started within 24 hours after initialization.
- A sequencing run is not completed due to a power failure or an abort, and <200 flows occurred before the stoppage.

Do NOT perform a manual cleaning with an unused, initialized Ion S5™ Sequencing Reagents cartridge.

Note:

- If a power failure or abort occurs during the second of two runs started after a single initialization, a manual cleaning is sufficient (see page 18).
- If the number of flows that occurred before a power failure or abort is unknown, perform an instrument reset run.

To perform an instrument reset run, use the following procedure before reinitialization.

1. In the instrument touchscreen main menu, tap **Run**.
   The instrument door unlocks and the chip clamp disengages.
2. Ensure that a used sequencing chip is in the chip clamp, then push the chip clamp in all the way to engage.
3. Close the instrument door, then tap **Next**.
4. When prompted, select **Planned Run (none)**. Ensure that the **Enable post-run clean** checkbox is selected, then tap **Review**.
5. In the **Select Run** screen, tap **Edit**, then in the **Detail** screen set the number of flows to **200**. Ensure that the **Post-Run/Clean** checkbox is selected, then tap **Close**.
6. Tap **Start run**, then tap **Accept** to confirm that Post-Run Clean is enabled, and to start the run.

When the instrument reset run completes, the instrument automatically performs the cleaning procedure. After cleaning, the touchscreen returns to the main menu.

A Touchscreen reference

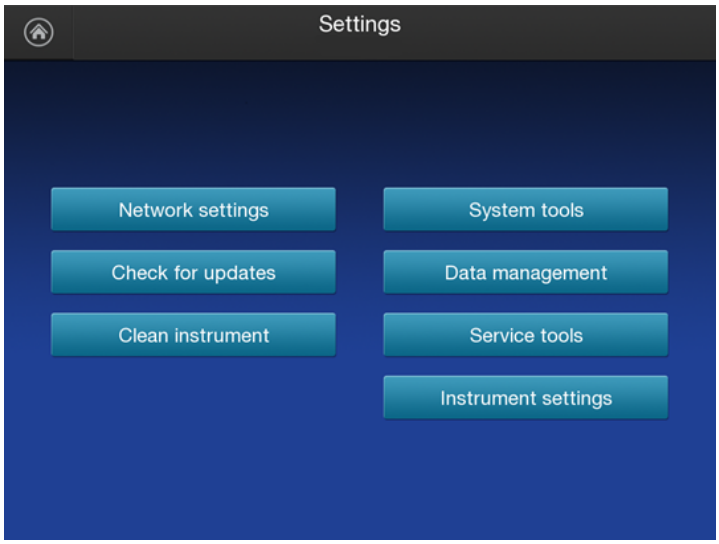
Clean, Initialize, and Run

In the **Home** screen, the **Clean**, **Initialize**, and **Run** programs lead you through the necessary steps to prepare the instrument for sequencing and to start a sequencing run. Press the main dial to start a program. Simple, easy to follow instructions are provided on the touchscreen.

- Cleaning must be performed before each initialization to ensure that the reagents from the previous run are cleared from the fluid lines. The **Clean** program is normally performed automatically at the completion of the previous sequencing run. Perform a **Clean** if for any reason the sequencing run was not properly completed. Follow the instructions that are provided on the touchscreen.
- The **Initialize** program must be performed before each run to load and prepare the run reagents. The **Initialize** program walks you through:
  - Emptying the waste reservoir
  - Loading the reagent cartridge, wash solution, and cleaning solution
- The **Run** program walks you through steps leading up to and through sequencing, including:
  - Placing a loaded chip on the instrument
  - Selecting a Planned Run created in the Torrent Suite™ Software
  - Performing sequencing

Settings

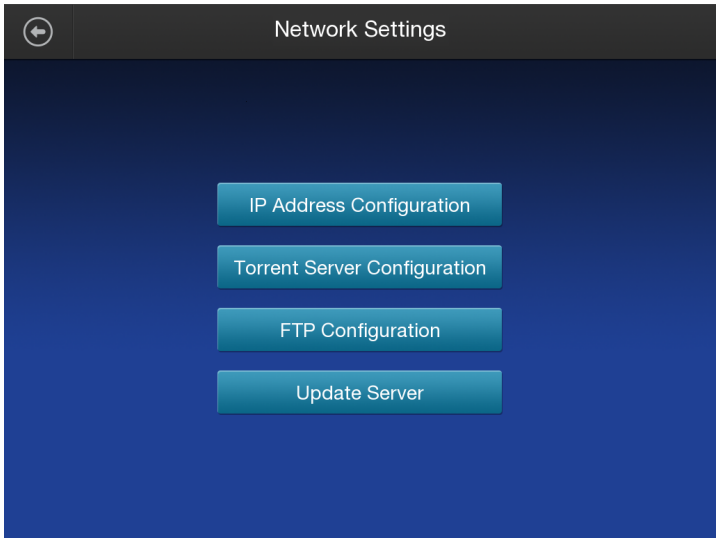
In the **Settings** menu, users can view and/or change instrument settings, manage data and network configurations, and update the installed software.

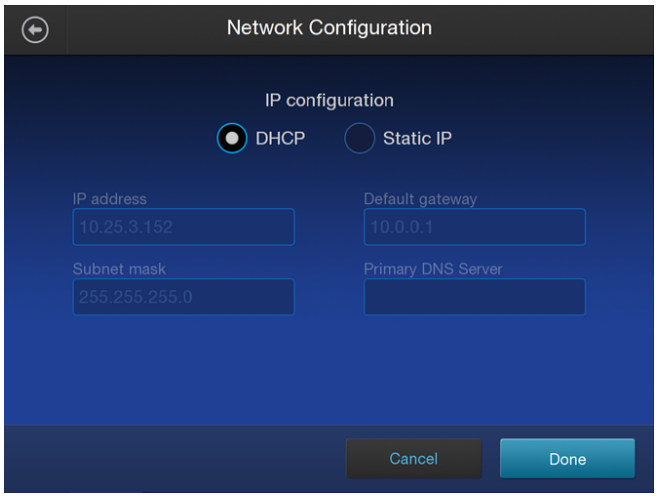
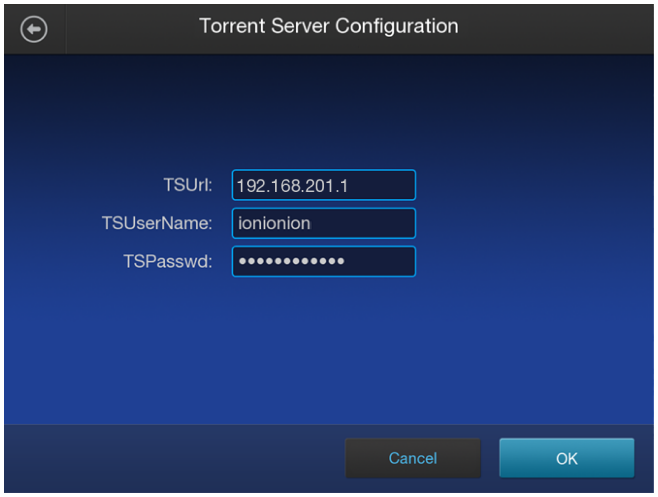


Note: The **Service tools** option is for use by trained service personnel only.

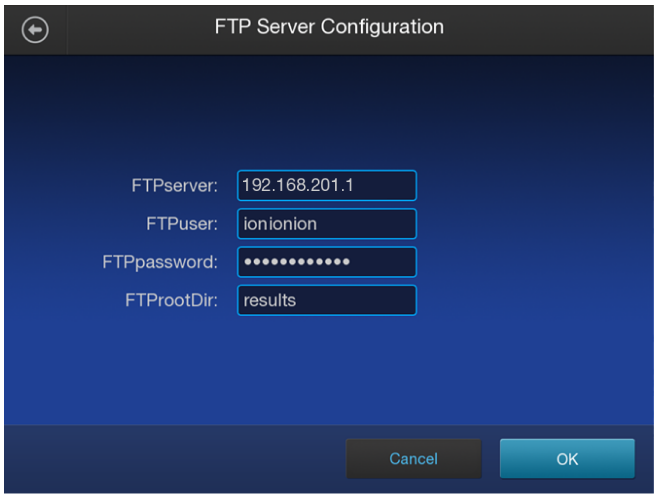
Network Settings

The **Network Settings** menu allows you to configure IP address, Ion Torrent™ Server, and FTP settings.

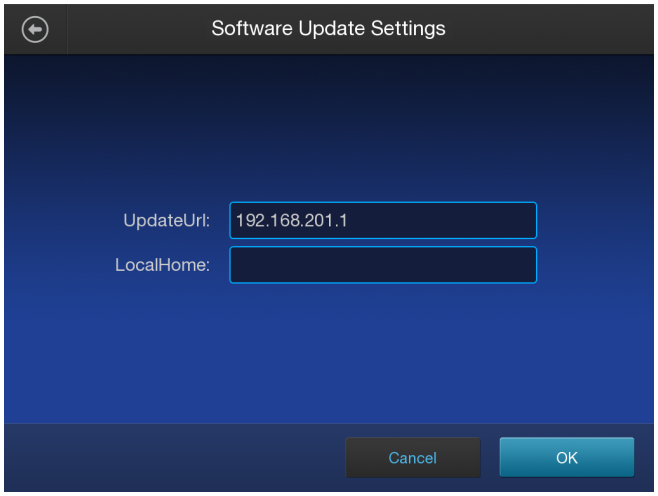


Item	Description	When/How to use
IP Address Configuration	Allows users to set or change the IP configuration (DHCP or Static IP). 	1. Select either the **DHCP** or **Static IP** radio button. 2. Touch the screen in the field you want to edit to activate the field. A virtual keyboard appears. 3. Enter the new information, then press **Done**.
Ion Torrent™ Server Configuration	Allows users to change the Ion Torrent™ Server IP address and user information.  Enter one of the following IP addresses in the **TSUrl** field for your instrument: • Ion GeneStudio™ S5/Ion GeneStudio™ S5 Plus Sequencer: 192.168.122.249 • Ion GeneStudio™ S5 Prime Sequencer: 192.168.201.1	When a change to the Ion Torrent™ Server IP address or user information is required.

(continued)

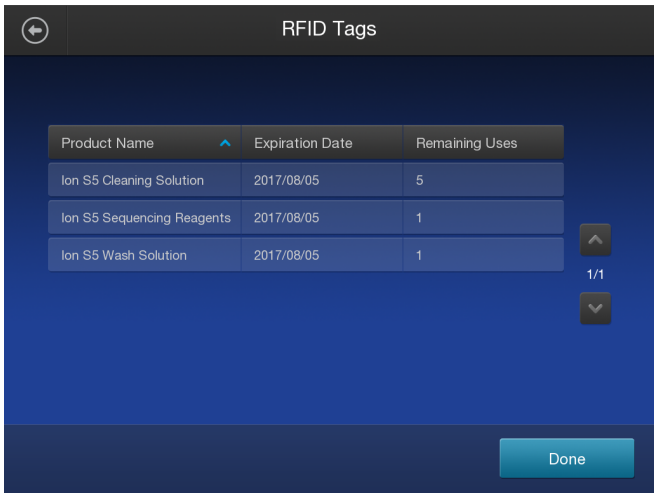
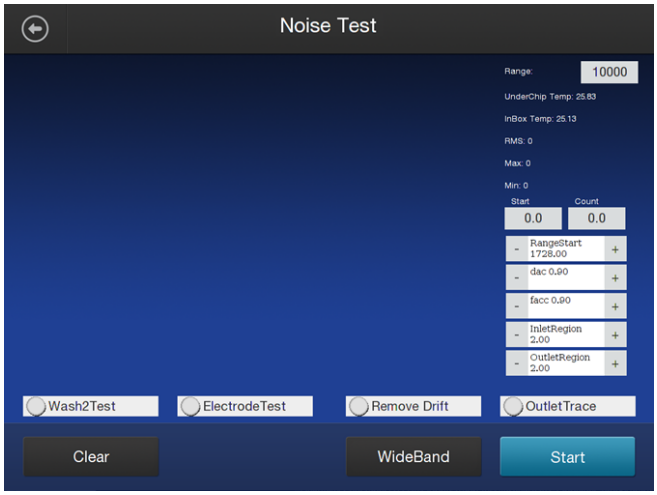
Item	Description	When/How to use
FTP Server Configuration	Allows users to change the FTP Server IP address and user information.  Enter the following IP address in the **FTPserver field** for your instrument: • Ion GeneStudio™ S5/Ion GeneStudio™ S5 Plus Sequencer: 192.168.122.249 • Ion GeneStudio™ S5 Prime Sequencer: 192.168.201.1	When a change to the FTP Server IP address or user information is required.

(continued)

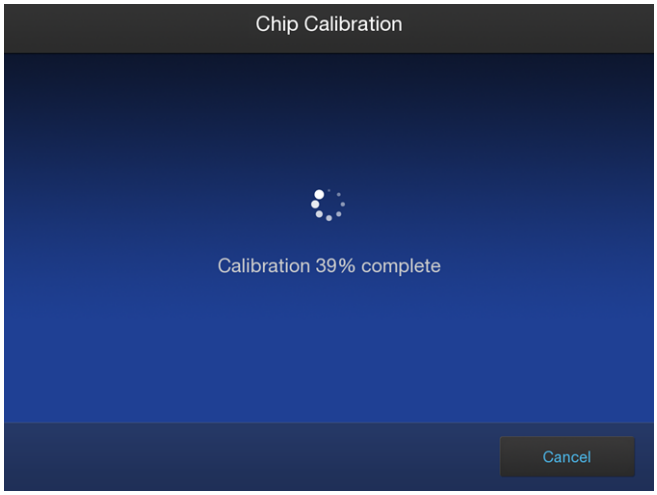
Item	Description	When/How to use
Update Server	Allows users to update the software update server.  Enter the following IP address in the **UpdateUrl** field for your instrument: • Ion GeneStudio™ S5/Ion GeneStudio™ S5 Plus Sequencer: 192.168.122.249 • Ion GeneStudio™ S5 Prime Sequencer: 192.168.201.1	When a change to the URL for the software update server is required. The values on this screen are set at installation. Do not change the settings except at the direction of Thermo Fisher Scientific service staff.

System Tools

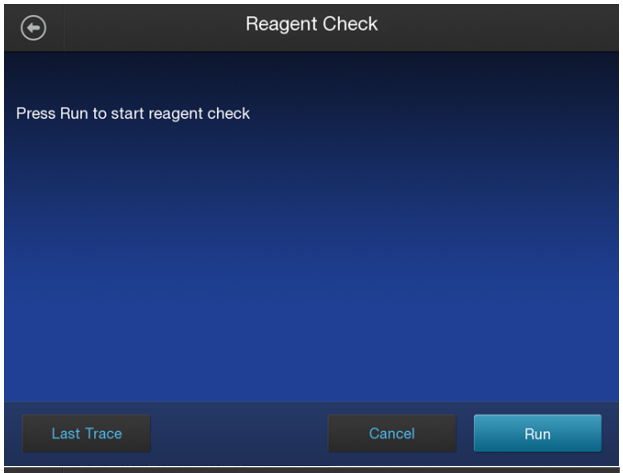
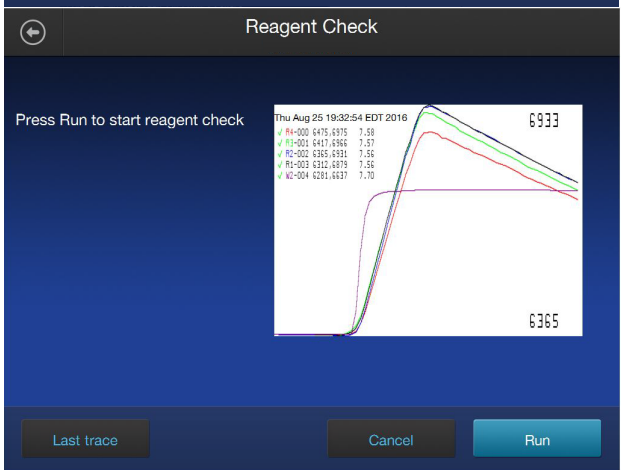
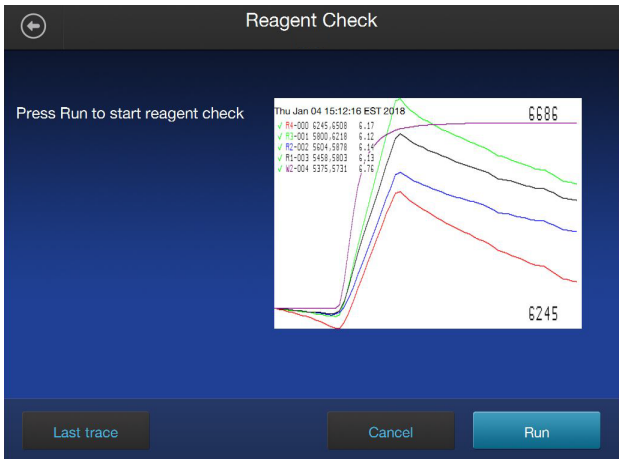
The **System Tools** menu enables you to upload instrument diagnostics, manage data, and shut down or reboot the instrument.

Item	Description	When/How to use
RFID	Lists the product, product expiration date, and remaining uses of the product. 	To determine if reagents that were left on an instrument during a period of instrument shutdown have expired. 1. Press the screen within the row of the product to view an **RFID Tag Detail** page. 2. Press **Done** to return.
Noise Test	Provides real-time measurement of electrical noise readings on the chip. 	For troubleshooting if directed to do so by Technical Support.

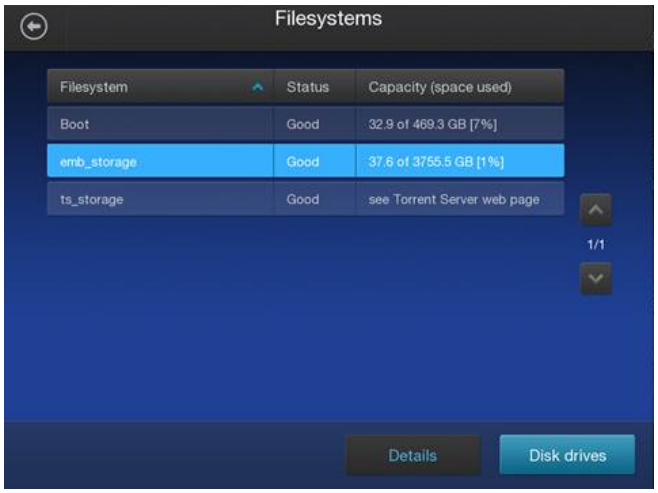
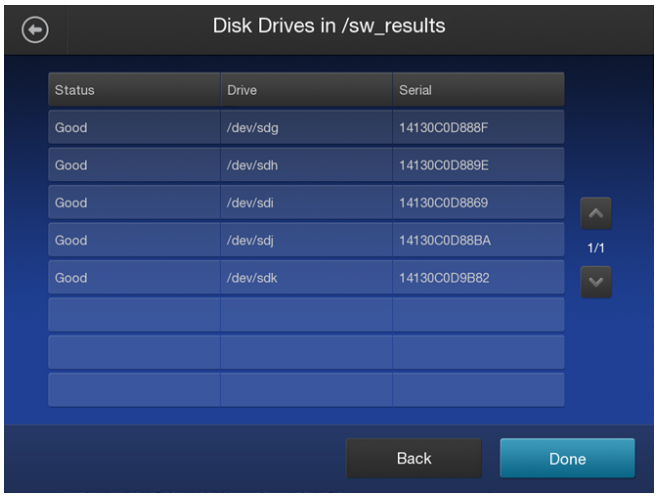
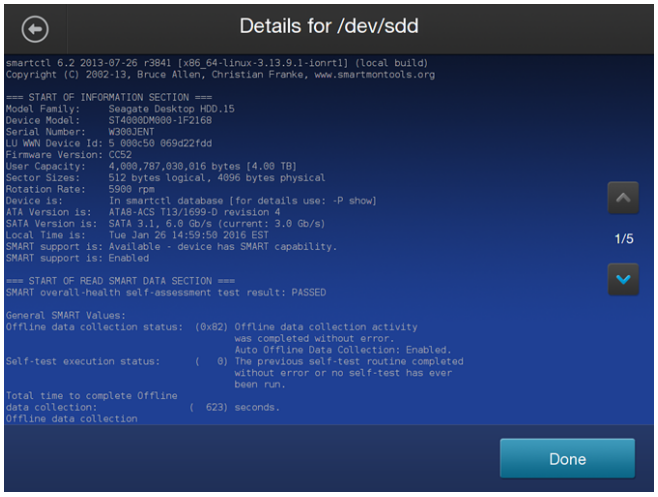
(continued)

Item	Description	When/How to use
Chip Calibration	Checks the status of a chip. 	1. Insert a chip into the chip clamp. 2. Press **Chip Calibration** to start the chip calibration. 3. In the dropdown lists, select the **Image** to display and the type of **Calibration** to perform. 4. Press **Cancel** to quit the chip calibration.

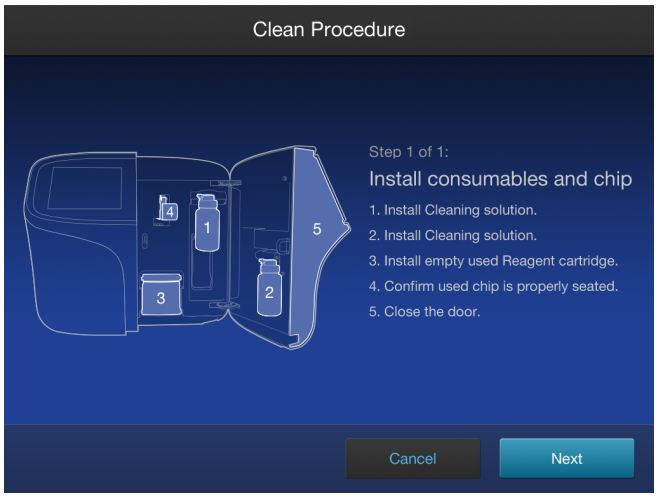
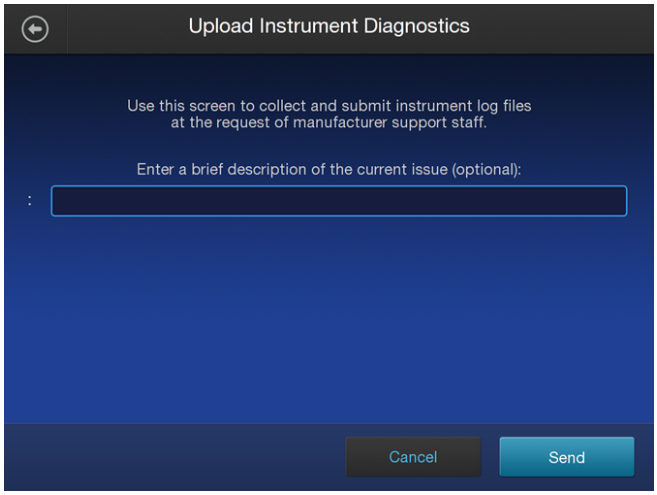
(continued)

Item	Description	When/How to use
Reagent Check	Reagent Check, performed by the instrument as part of initialization, confirms that initialization passes by testing reagent flow and signal detection.   Example Reagent Check for an Ion 540™ Chip  Example Reagent Check for an Ion 550™ Chip	If directed to do so by Technical Support as part of a troubleshooting procedure. • Press **Run** to start the reagent check. • Press **Last Trace** to review the most recent reagent check result trace. **Note:** Reagent Check confirms that the chip calibration passes, and will only fail if the chip is defective, or Ion S5™ Wash Solution is not flowing.

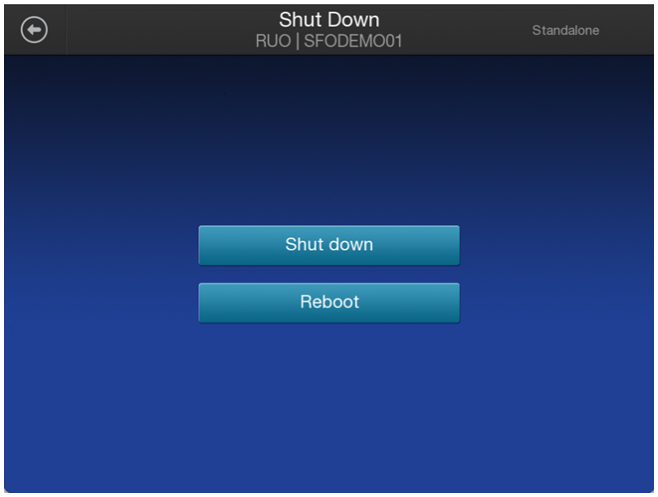
(continued)

Item	Description	When/How to use
Filesystems and disks	Provides real-time drive activity status and disk space used.   	Use if directed to do so as part of a troubleshooting procedure: 1. In the System Tools menu, press **Filesystems and disks**. The **Filesystems** screen opens. 2. Press the screen within the row of the **Filesystem** you want to view, then press **Details**. The **Disk Drives in <Filesystem>** screen opens. 3. Press the screen within the row of the **Drive** to view details of the disk drive. The **Details for <Drive>** screen opens. 4. Press **Done** to return to the previous screen.

(continued)

Item	Description	When/How to use
Pulse clean	Provides step by step instruction to perform a pulse clean of the instrument if a solid-state buffer leak occurs. 	Use if directed to do so by Technical Support as part of a troubleshooting procedure: **1.** Install consumables and a used sequencing chip. **Note:** Install Cleaning solution in both the Wash solution (1) and Cleaning solution (2) locations. Use an empty Reagent cartridge. **2.** Close the instrument door, then press **Next**. The pulse cleaning procedure starts automatically. See "Solid-state buffer leak" on page 47 for more information.
Upload Diagnostics	Allows upload of instrument diagnostics files for review by support personnel. 	For troubleshooting if directed to do so by Technical Support. **Note:** The system must be connected to an active network port. If no network is present, the file is pushed to the Torrent Browser. You can then download the file and email it to Technical Support. To retrieve the file using the Torrent Browser, navigate to **Help** ▸ **Instrument Diagnostics**, then follow the on-screen instructions.

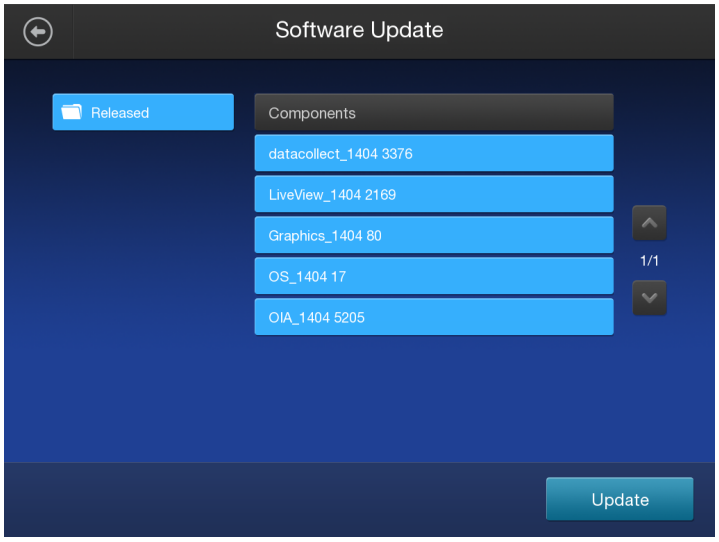
(continued)

Item	Description	When/How to use
Shut Down	Access to "Shut Down" and "Reboot" commands.  **Note:** It is not necessary/recommended to shut down the instrument overnight or over the weekend. If needed to shut down the instrument, see “Power off” on page 15.	If directed to do so by Technical Support as part of a troubleshooting procedure.

Check for software updates

When software updates are available, users receive a notification through the home screen.

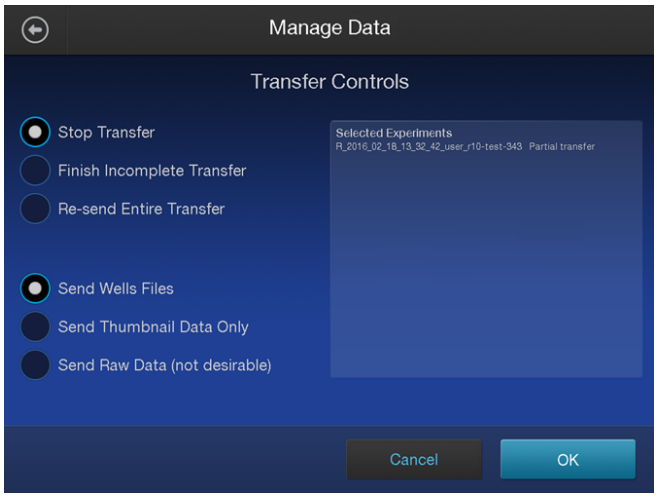
1. In the **Settings** menu, tap **Check for Updates**.
   The **Software Update** screen lists the available updates.



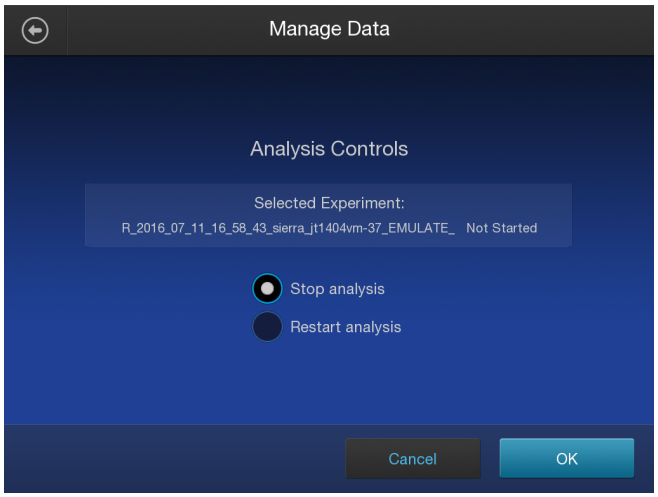
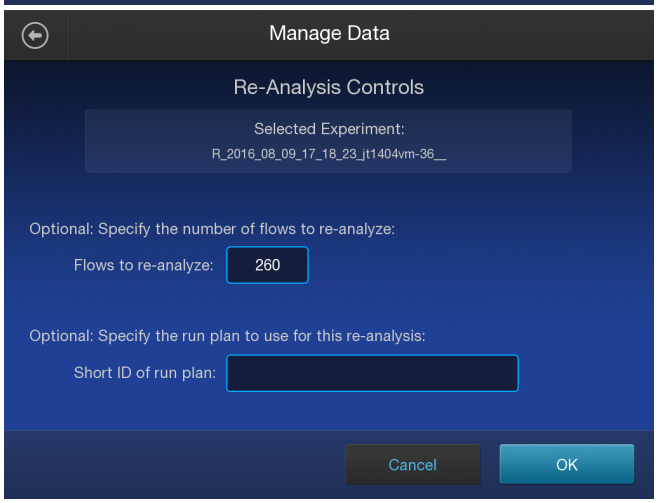
2. Tap **Update** to download and install the updates automatically.

Data management

The **Data management** function allows users to delete run data manually, analyze the data, or transfer the data to the Ion Torrent™ Server. Under normal conditions, run data are automatically transferred to the server, then deleted from the instrument hard drive.

Item	Description	When/How to use
Delete data	Manually delete run data from the instrument.	If the instrument hard drive becomes full, see “Manually delete run data” on page 34.
Transfer data		Transfer run data from the instrument hard drive to the Ion Torrent™ Server. 1. Tap the screen in the **Experiment Name** row of the individual experiment to be transferred. 2. Tap **Transfer**. 3. Select the radio button of the action to be performed. 4. Select the radio button of the files to be transferred. 5. Tap **OK**.

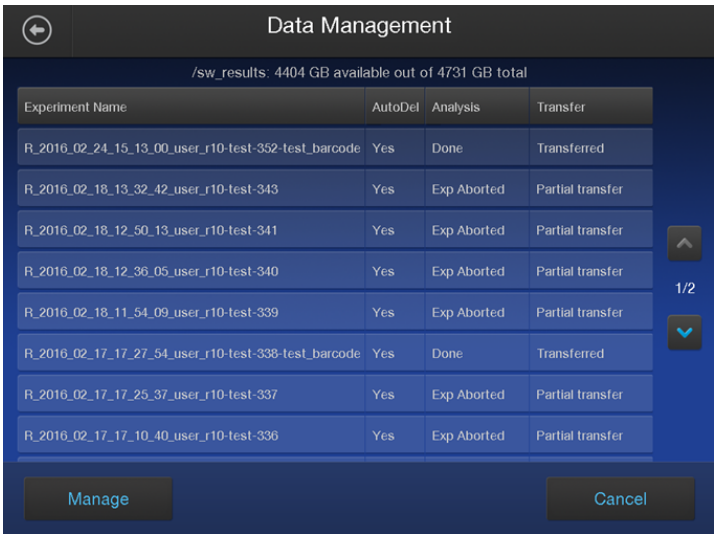
(continued)

Item	Description	When/How to use
Analysis Controls	Allows the user to stop or reanalyze the data of individual runs.  	1. Tap the screen in the **Experiment Name** row of the individual experiment to be analyzed, then press **Analysis**. 2. Select the **Stop Analysis** or **Restart Analysis** radio button, then tap **OK**. 3. If performing a re-analysis, follow the on-screen instructions, then tap **OK**.

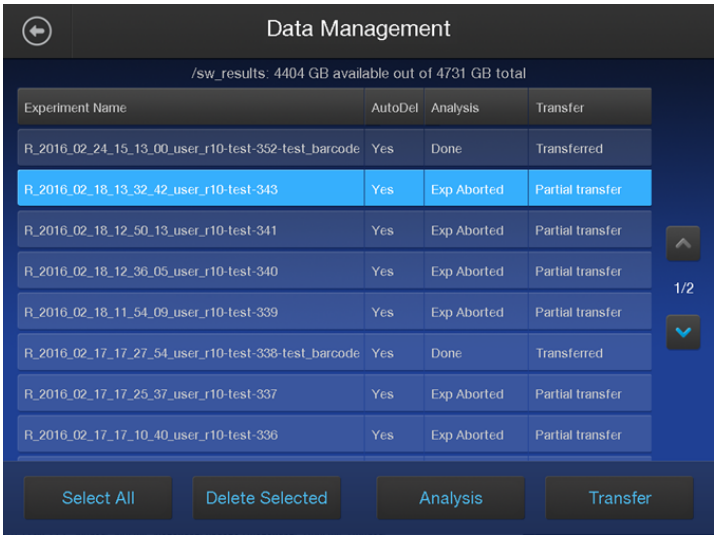
Manually delete run data

To troubleshoot data management problems the **Data Management** function allows users to delete run data manually, or transfer the data to the server. Under normal conditions, run data are automatically transferred to the Ion Torrent™ Server, then deleted from the instrument hard drive.

1. In the **Settings** menu, tap **Data management** to access the **Data Management** screen, then tap **Manage**.



2. Tap **Select All** to select all the available experiments, or tap the screen in the **Experiment Name** row of the individual experiment to be managed.



3. Press **Delete Selected**.

Perform the instrument Clean operation

The **Clean** program is normally performed automatically at the completion of the previous sequencing run. Perform a **Clean** if the sequencing run:

- was aborted or had a power failure during the second of two runs started after a single initialization.
- is not completed and >200 flows occurred before the stoppage.
- the post-sequencing run cleaning was not completed.

IMPORTANT! Do NOT perform a manual cleaning with an unused, initialized Ion S5™ Sequencing Reagents cartridge. Perform an instrument reset run instead. See “Perform an instrument reset run with an initialized sequencer that is loaded with an unused Reagents cartridge” on page 20 for more information.

---

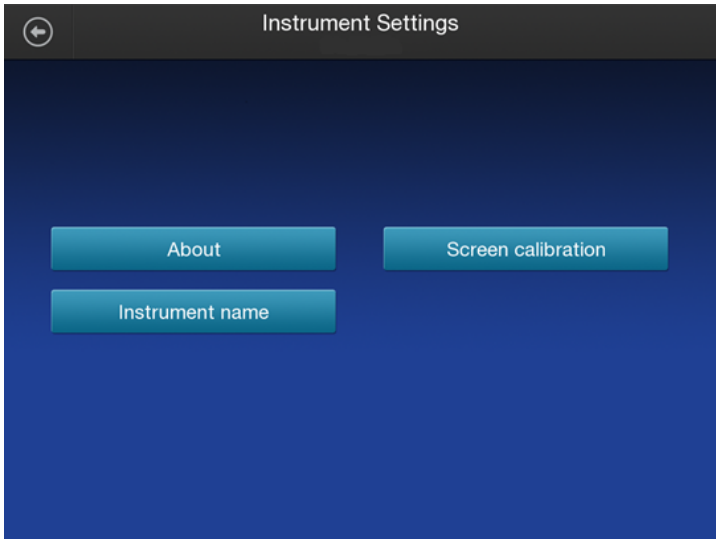
1. In the **Settings** menu, tap **Clean Instrument**.
   The Clean procedure begins automatically.

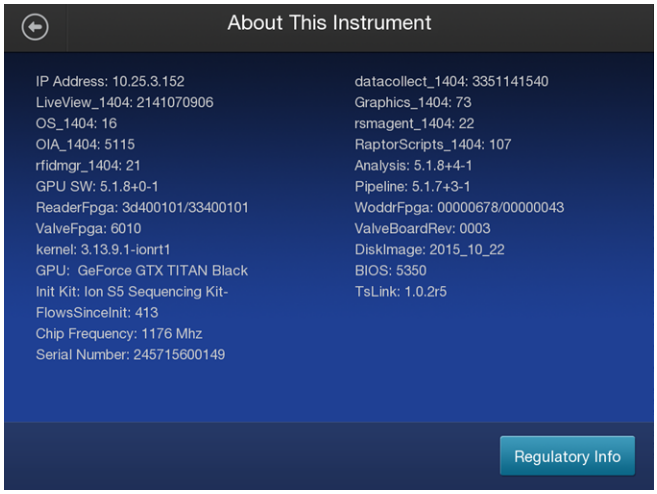
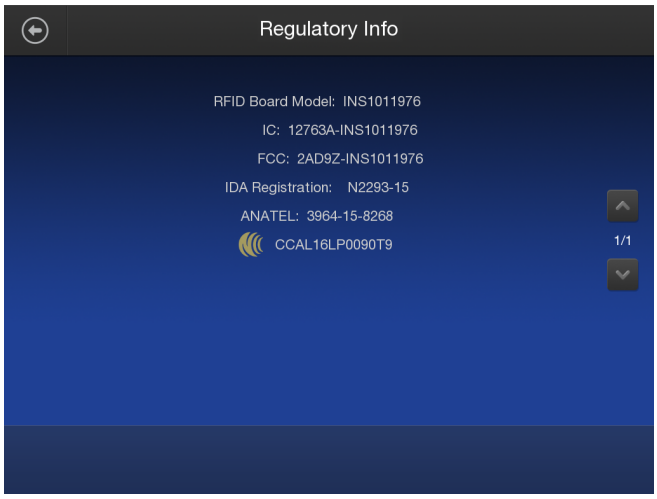
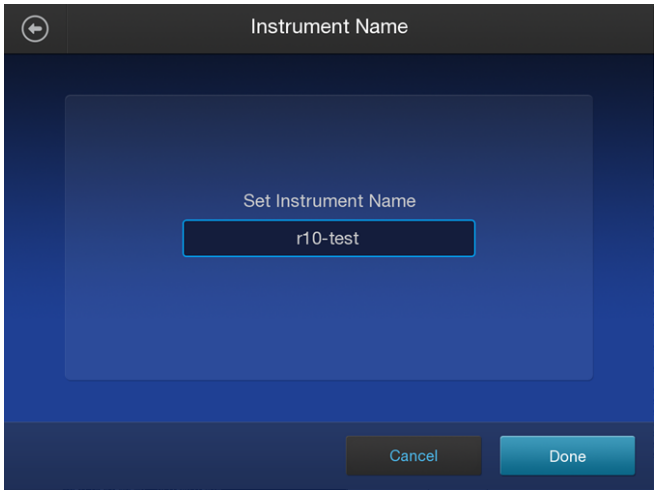
2. Follow the on-screen instructions (see page 18 for more information), then tap **Next**.

The user interface returns to the Home screen when the cleaning is complete.

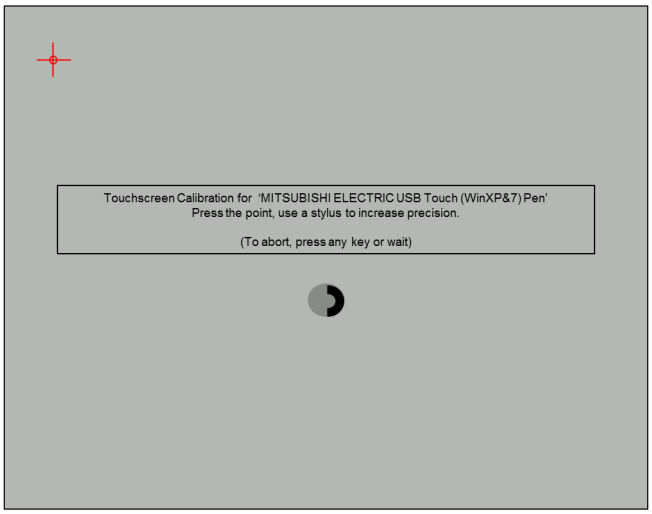
Instrument settings

The **Instrument Settings** menu provides information about the instrument and allows you to set the instrument name and calibrate the touchscreen.

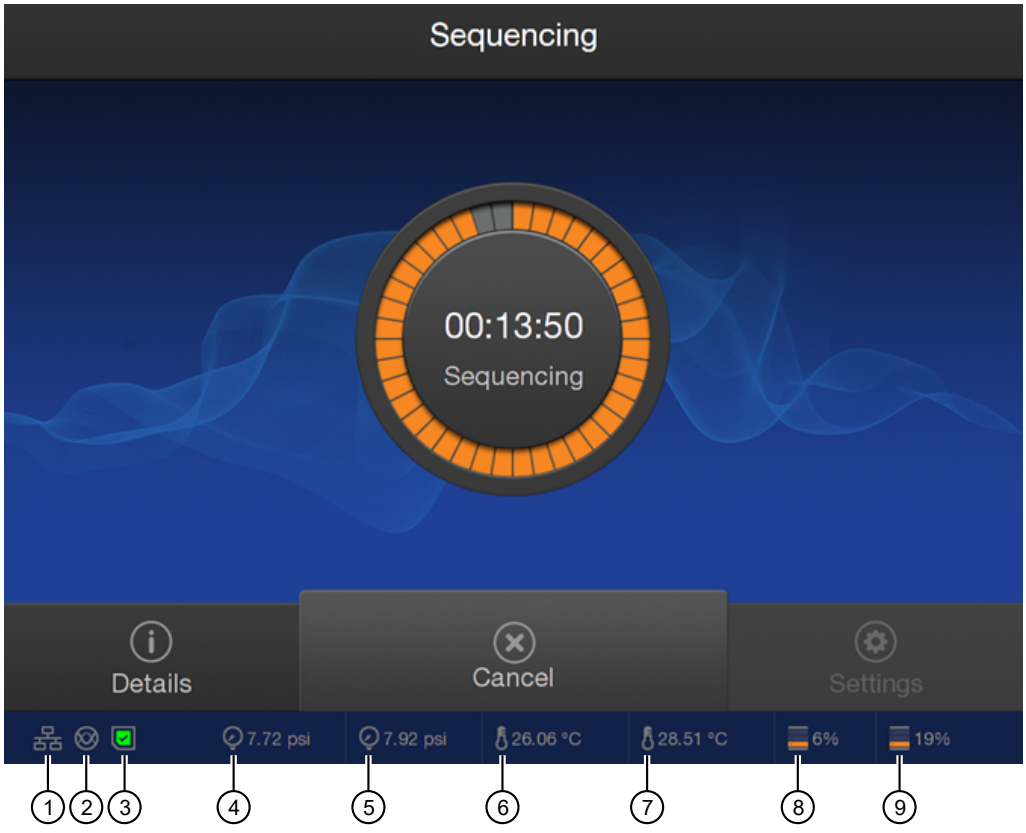


Item	Description	When/How to use
About	Provides instrument details. 	To view instrument reference information or access regulatory information.
Regulatory Info	Lists instrument-specific regulatory information. 	To view instrument regulatory information.
Instrument Name		To change the instrument name.

(continued)

Item	Description	When/How to use
Screen Calibration		For troubleshooting if directed to do so by Technical Support. Touch the red cross with your finger or a stylus each time it appears. In total, you will touch the screen 4 times, one time in each corner.

Touchscreen icons



Number	Icon	Description
1		Network connectivity – connected
		Network connectivity – not connected
2		Instrument idle
		Sequencing in progress
		Instrument ready
		Error
3		Chip status – Absent
		Chip status – Standby
		Chip status – Connecting
		Chip status – Ready
		Chip status – Imaging
		Chip status – Error
4		Manifold air pressure – Good
		Manifold air pressure – Bad. If the icon reads "0" and there is an alarm, contact Technical Support.
5		Regulator air pressure – Good
		Regulator air pressure – Bad. If the icon reads "0" and there is an alarm, contact Technical Support.
6		Manifold temperature – Good
		Manifold temperature – Bad. Check for related alarms. If the alarm persists contact Technical Support.
7		Chip block temperature – Good
		Chip block temperature – Bad. If the icon reads "0" and there is an alarm, contact Technical Support.
8		Instrument File System Space, the percent of file space used is indicated.[1] The instrument checks for sufficient disk space before each run and notifies the user if there is not enough.
9		Torrent Server File System Space, the percent of file space used is indicated. **Note:** If the indicator turns red, archive data from the server to free up disk space. Refer to the Torrent Suite™ Software help for more information on archiving data.

[1] Indicator turns yellow when disk space is ≥67% full, indicator turns red when ≥90% full.

Alarms, Notifications, and Events

If the Alarms/Notifications indicator appears, press the indicator to open the **Notifications** screen, then press **Alarms** or **Events** see detailed messages.

1 Alarms and Notifications indicator.

Alarm	Description	Recommended Action
Fatal alarms		
Fluidics Calibration Needed.	—	Contact Technical Support.
Chip Cooler can not reach desired temperature.	—	Contact Technical Support.
Drive sdX failed smartctl health check.	Where X is the letter of the drive that failed. Indicates that a drive is failing.	Contact Technical Support.
Failed to mount the results filesystem.	One or more drives are missing from the RAID that makes up sw_results.	Contact Technical Support.
System sensor check failed ipmi -sensors.	A component on the motherboard is failing or has failed.	Contact Technical Support.
System sensor check failed, CC.CC V (LL.LL/HH.HH)	Where CC.CC = current voltage, LL.LL = the low voltage threshold, HH.HH = the high voltage threshold. Indicates a voltage drop on the motherboard.	Contact Technical Support.

(continued)

Alarm	Description	Recommended Action
CPU temperature check failed, Physical Id X.	Where X is the processor number. Indicates that the processor is over heating.	Contact Technical Support.
Front/Rear FanX speed below threshold YYYY.	Where X is the fan number and YYYY is the low threshold speed for the system fan.	Contact Technical Support.
FPGA failed to connect in emulate mode.	Internal error.	Contact Technical Support.
Valve Link Failure.		
OIA (On Instrument Analysis) is not running.		
FPGA temperature is above the threshold of 70°C.	One or more of the system fans has failed.	Contact Technical Support.
Boot drive exceeded 75%.	The boot drive is unexpectedly full.	Contact Technical Support.
RAID degraded one disk has failed.	One of the Ion Torrent™ Server drives has failed. System is still operational but must be serviced soon.	Contact Technical Support.
RAID failure detected, more than one disk failed.	More than one of the Ion Torrent™ Server drives has failed. System is no longer operational.	
/sw_results directory not mounted.	One or more of the drives used during data collection has failed.	
No GPU detected.	GPU has failed.	Contact Technical Support.
No GPU Driver detected.		
Air Compressor failure.	Air compressor is either leaking or has stopped working.	Contact Technical Support.
Non fatal alarms		
Instrument must be cleaned before starting another run.	Maximum number of runs since last clean has been exceeded.	Perform an instrument cleaning. See “Perform the sequencer cleaning manually” on page 18.
Ambient temperature is above the threshold of 35°C.	Verify the temperature in the lab.	Contact Technical Support if the temperature of the lab is within normal operating temperature (20°C to 30°C).
RAID degraded one disk has failed.	One of the Ion Torrent™ Server drives has failed. System is still operational but must be serviced soon.	Contact Technical Support.

(continued)

Alarm	Description	Recommended Action
No Connectivity to Torrent Server.	Check that the Ethernet cable connecting the instrument to the server is properly connected, see page 14 for the Ethernet port location.	Reconfigure the Ion Torrent™ Server as needed, see "Network Settings" on page 22.
No Connectivity to FTP server.		Reconfigure the FTP server as needed, see "Network Settings" on page 22.
Network Manager not connected.	Check that the Ethernet cable connecting the instrument to the local area network is properly connected, see page 14 for the Ethernet port location.	If the problem persists, contact Technical Support.
Newer Software Available	Notification that software updates are available.	Update your software. See "Check for software updates" on page 31.

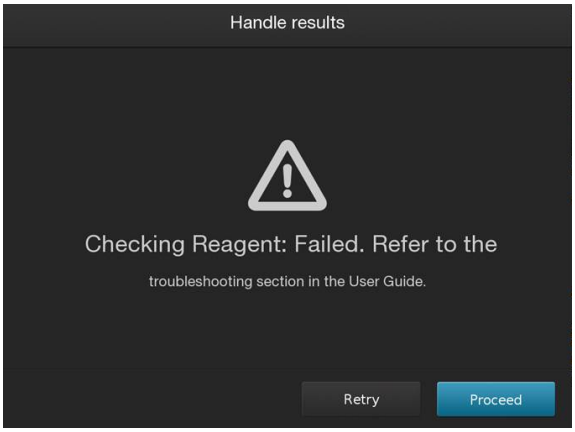
B

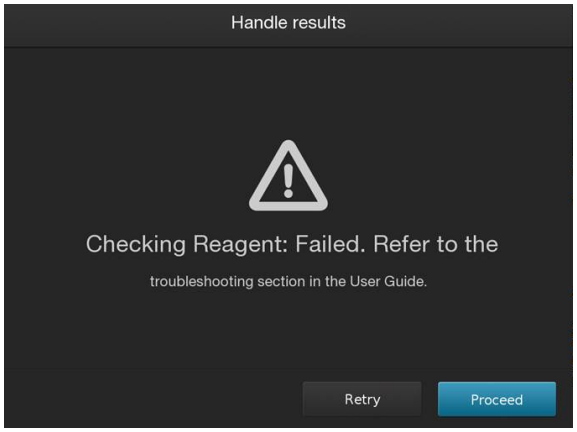
Troubleshooting

Instrument alarms and events

Observation	Possible cause	Recommended action
Red "Alarms" and/or "Events" message in Main Menu 	• Available software updates were detected. • Connectivity issues were detected. • Instrument did not detect required files or hardware.	Tap the red **Alarms** icon to see detailed messages. • If a message states "Newer Software Available": **IMPORTANT!** After updates are installed, the instrument must be restarted. a. In the main menu, tap **Settings** ▸ **Check for Updates**. b. Select the **Released Updates** checkbox, then tap **Update**. c. When installation is complete, follow the onscreen prompts to restart the instrument. **Note:** In some cases, the instrument restarts automatically after software installation. • If a message states "No Connectivity to Torrent Server", "No Connectivity to ftp server", or "Network Manager not connected", disconnect and re-connect the ethernet cable, confirm that the router is operational, and verify that the network is up and running. • For any other messages: a. Power off the instrument: On the home screen, tap **Settings** ▸ **System Tools** ▸ **Shut Down** ▸ **Shut Down**. b. Wait 30 seconds, then press the button on the side of the instrument to power on the instrument. • If the red "Alarms" and/or "Events" message still appears in the main menu, contact Technical Support.

Initialization fails

Observation	Possible cause	Recommended action
Chip Check fails	• The chip clamp was not closed. • The chip was not properly seated. • The chip was damaged.	1. Open the chip clamp, remove the chip, and look for signs of water outside the flow cell. 2. If the chip appears damaged, replace it with a new one. 3. Close the clamp, then repeat the Chip Check. 4. If the chip passes, click **Next**. If the chip fails, replace it with a new chip, then press **Chip Check**. 5. If Chip Check continues to fail, there could be a problem with the chip socket. Contact Technical Support.
Reagent Check fails 	A chip failure was detected.	1. Replace the used sequencing chip used during initialization with a different used chip. 2. Press **Retry**. 3. If the initialization completes without failure, touch **Home**, then continue with your sequencing run. 4. If the Reagent Check continues to fail, contact Technical Support.

Observation	Possible cause	Recommended action
Reagent Check fails  *(continued)*	A wash failure was detected.	1. Perform a manual cleaning of the sequencer, see page 18. 2. Replace the Reagents cartridge, Wash Solution bottle, and Cleaning Solution bottle, then repeat initialization of the sequencer. 3. If the initialization completes without failure, press **Home**, then continue with your sequencing run. 4. If the Reagent Check continues to fail, contact Technical Support.
		Ensure that you install the Ion S5™ Wash Solution bottle with the instrument door fully open. If you install the bottle impeded by a partially closed door, the port can be obstructed, and a faulty installation and wash failure can result.
Bottle leak check fails	• Bottle seal was not tight. • Bottle was damaged or defective.	1. Finger-tighten the bottles. 2. If the bottle continues to leak, replace the bottle. 3. If leak check continues to fail, contact Technical Support.

Troubleshooting using control libraries

Observation	Possible cause	Recommended action
Ion Sphere™ Test Fragments are not present in the Test Fragment Report section of the run report, and library sequencing is poor	• Chip loading was poor. • Control Ion Sphere™ Particles were not added to the sample	1. Confirm that the Control Ion Sphere™ Particles (included in the Ion S5™ Installation Kit) were added. 2. If controls were added, contact Technical Support.
Control Ion Sphere™ Particles are present in the run report, but AQ20 throughput is poor	• The quality of the library was poor. • The quality of the template preparation was poor.	Verify the quality of the library and template preparations using quality assessment procedures recommended in the appropriate library and template preparation user guides.
		1. Use the Human CEPH Genomic DNA Control or Human CEPH Control 200 Library, included in the Ion S5™ Controls Kit (Cat. No. A27760), to prepare template-positive ISPs with the Ion OneTouch™ 2 Instrument, or the Ion Chef™ Instrument. 2. Use the ISPs in an Ion GeneStudio™ S5 run. 3. If AQ20 throughput is still below specification, verify the quality of unenriched and enriched ISPs to identify a problem in template preparation. 4. If ISP quality is good, but AQ20 throughput is below specification, contact Technical Support.

Troubleshooting using Control Ion Sphere™ Particles

To prepare Control Ion Sphere™ Particles, provided in the Ion S5™ Installation Kit, for an installation or troubleshooting sequencing run:

1. Create a Planned Run.
2. Clean and initialize the Ion GeneStudio™ S5 Sequencer.
3. Prepare the Control Ion Sphere™ Particles for sequencing:
   a. Vortex the Control Ion Sphere™ Particles for 5 seconds, then centrifuge for 2 seconds before taking aliquots.
   b. Add 66 µL of Control Ion Sphere™ Particles to an empty 0.2-mL PCR tube (non-polystyrene).

c. Add 150 µL of Ion S5™ Annealing Buffer to the tube.

4. Anneal the sequencing primer to the enriched ISPs, then follow the remaining procedures in your appropriate user guide to load a chip and start the sequencing run.

Solid-state buffer leak

Observation	Possible cause	Recommended action
Two or more of the following observed • Excessive read trimming or the number of short reads is significantly higher than expected. • Percentage of low quality reads is significantly higher than expected. • Poor sequencing performance or no Test Fragments.	A solid-state buffer leak occurred.	Contact Technical Support. Perform the pulse cleaning protocol if directed to do so by Technical Support or your Field Service Engineer (FSE).

Pulse clean the sequencer

You should only pulse clean your Ion GeneStudio™ S5 Sequencer if directed by Technical Support, or your Field Service Engineer (FSE), to recover instrument performance from a diagnosed solid-state buffer leak.

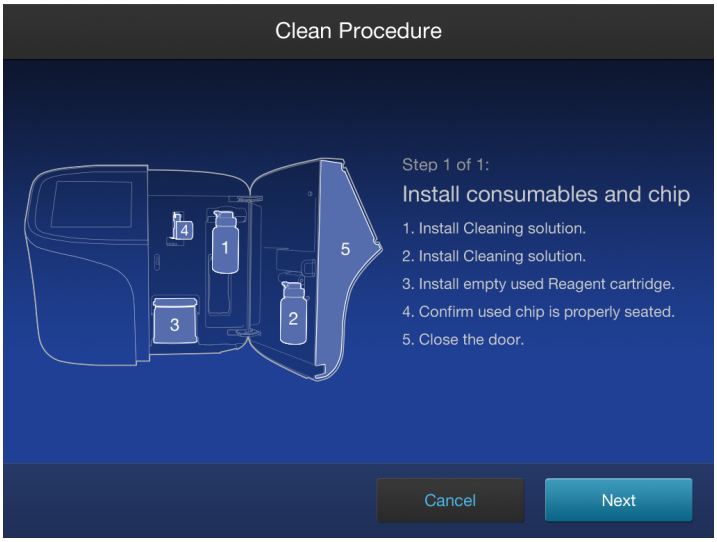
Note: You must contact Technical Support, or your FSE, to diagnose whether a solid-state buffer leak has occurred and obtain the required materials to perform the pulse cleaning.

1. Ensure that the sequencer has completed a normal post-run cleaning. In the main menu:

Display text	Description
Run	Instrument requires cleaning before a pulse cleaning can be performed, see “Perform the sequencer cleaning manually” on page 18.
Clean	Instrument requires cleaning before a pulse cleaning can be performed, see “Perform the sequencer cleaning manually” on page 18.
Initialize	Instrument is ready to be pulse cleaned. Proceed to step 2.

2. Press **(Settings) ▸ System Tools ▸ Pulse clean**.

3. Follow the onscreen prompts to prepare the instrument.
    - Install new bottles of Ion S5™ Cleaning Solution in both the Wash and Cleaning solution positions.
    - Replace the Ion S5™ Sequencing Reagents cartridge with an empty used cartridge.
    - Install a used sequencing chip.



4. Close the instrument door, then touch **Next**.
   The pulse cleaning procedure begins automatically. The user interface returns to the main menu when the cleaning is complete and the sequencer is ready to be initialized.

C

Supplemental procedures

Set up and test the Ion Chip™ Minifuge

Note: The Ion Chip™ Minifuge is only required when using the Ion OneTouch™ 2 System for template preparation. Ion Chef™ Instrument users do not require an Ion Chip™ Minifuge.

The Ion Chip™ Minifuge (Cat. No. 4479672 or 4479673) is used to load sequencing chips manually for use on Ion PGM™, Ion Proton™, and Ion GeneStudio™ S5 System sequencing platforms. To accommodate the larger chip size, Ion Proton™ and Ion GeneStudio™ S5 System sequencer users must:

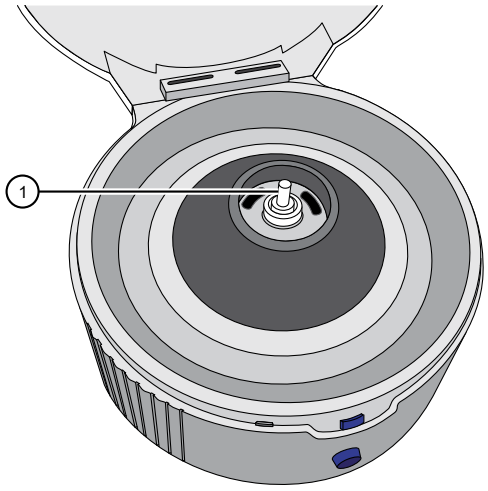
- install the Ion S5™/Ion Proton™ Rotor and Buckets (Cat. No. 4482578).
- test the minifuge to confirm that no liquid is lost during centrifugation.

before using the minifuge to load chips for the first time.

Note: The following protocols may also be used to convert the Ion Chip™ Minifuge back for use with Ion PGM™ sequencing chips.

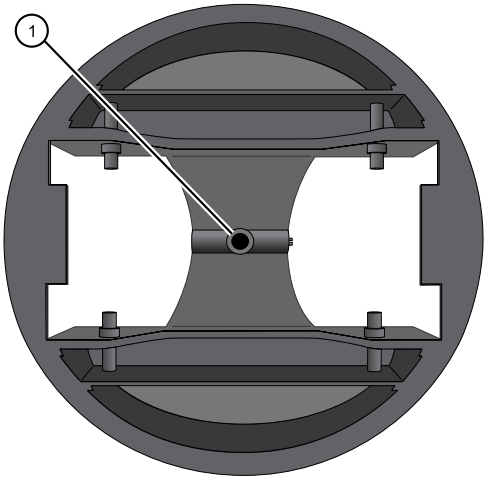
Install the Ion S5™ /Ion Proton™ Rotor and Buckets

1. Grasp the existing rotor and pull straight up to remove the rotor from the motor shaft.



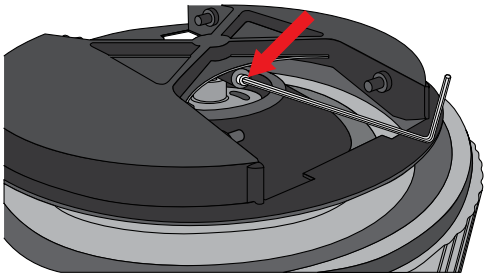
① Motor shaft

2. Press the Ion S5™/Ion Proton™ Rotor down onto the motor shaft to install.



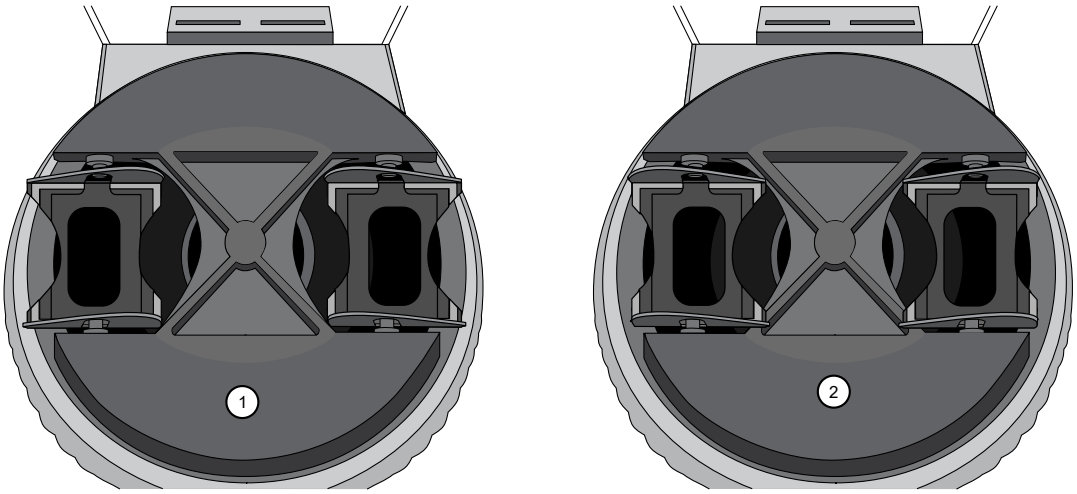
① Insert motor shaft here

3. Tighten the set screw (arrow) with a 1.5-mm hex wrench.



Note: A newer version of the rotor lacks a set screw. In this case, simply press the rotor firmly onto the motor shaft to install.

4. Install the two buckets. Position the buckets with the larger semi-circular cut-outs facing out, and ensure that the buckets hang freely.



① Correct orientation
② Incorrect orientation

Test the minifuge

1. Prepare two previously-used chips:
   a. Inject 100 µL of isopropanol two times into the loading port of each chip. After each injection, remove the expelled liquid from the opposite port.

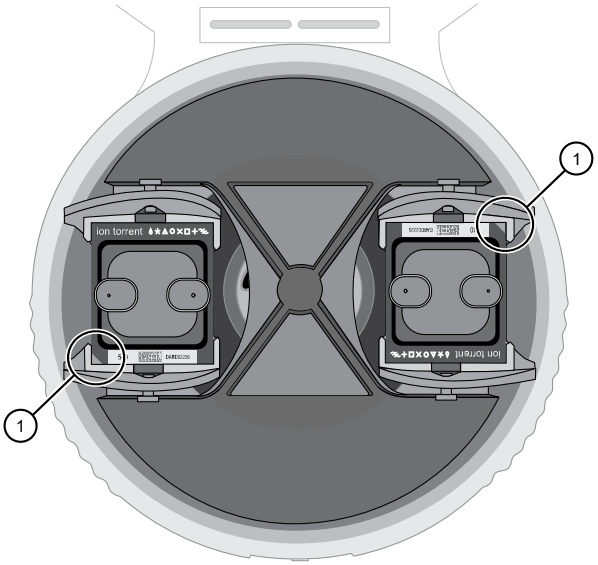
   Note: Use 50 µL volume of isopropanol if testing Ion PGM™ sequencing chips.

   b. Aspirate the remaining isopropanol from the flow cells for 5–10 seconds. Confirm that the chips are dry.

   Note: To aspirate the isopropanol, attach a P200 pipette tip to a vacuum line, then place the pipette tip in the chip loading port.

2. Place the two chips prepared in step 1 in the centrifuge buckets, with the chip notch pointing out. Add 55 µL of nuclease-free water to each chip loading well (do not inject into the chip loading port).

Note: Use 35 µL volume of nuclease-free water if testing Ion PGM™ sequencing chips.



① Chip notch

3. Centrifuge for 5–10 seconds, then examine each chip.

   The flow cell in each chip should be completely filled with liquid with no air bubbles. A small volume of liquid will remain in the loading well; this is normal.

Result	Action
The chips are NOT completely filled...	Contact Technical Support.
The chips ARE completely filled...	Centrifuge the chips for an additional 10 minutes, then check the chips again for air bubbles, especially near the inlet and outlet ports.
The chips have air bubbles after the additional 10 minute centrifugation...	Contact Technical Support.
The chips remain completely filled...	The centrifuge is ready to use for chip loading.

D Instrument warranty

For new Ion Torrent™ instruments, Life Technologies warrants to and only to buyer for twelve (12) months from the date of shipping, that the Ion Torrent™ software and Ion Torrent™ instruments are free from defects in material and workmanship and conform to Life Technologies' published specifications in all material respects. Where a valid and timely claim in respect of breach of Ion Torrent™ warranty is submitted to Life Technologies, Life Technologies may, at its discretion, replace, repair or modify the Ion Torrent™ instrument. Any agreed replacement shall be at 1:1, like-kind basis, at no cost to the buyer. For Ion Torrent™ chips or reagents reasonably determined by Life Technologies to be defective, independent of user error, shall be replaced by Life Technologies on a 1:1, like-kind basis at no cost to buyer, provided that such defective Ion Torrent™ chips or reagents were used by buyer prior to their expiration date, or if there is no expiration date, the Ion Torrent™ chips or reagents were used within six (6) months of receipt, and the defect was promptly reported with appropriate detail to Life Technologies' technical support.

NO OTHER WARRANTIES SHALL BE APPLICABLE TO ION TORRENT PRODUCTS (WHETHER OR NOT ANY FURTHER WARRANTY DOCUMENTATION MAY BE INCLUDED IN THE SHIPMENT), WITH THE EXCEPTION OF THIRD PARTY WARRANTIES WITH RESPECT TO THIRD PARTY PRODUCT. ANY THIRD PARTY PRODUCTS ARE NOT COVERED BY THIS SECTION AND ANY WARRANTIES FOR THIRD PARTY PRODUCTS ARE PROVIDED BY THE ORIGINAL MANUFACTURER OF THE THIRD PARTY PRODUCT. Warranties are made only to buyer purchasing the Ion Torrent™ Product directly from Life Technologies, are not transferable and do not extend to the benefit of any other person or entity, unless otherwise expressly stated in writing by Life Technologies. ANY PRODUCT NOT COVERED BY AN EXPRESS WRITTEN WARRANTY IS SOLD AND PROVIDED "AS IS," WITHOUT WARRANTY OF ANY KIND, STATUTORY, EXPRESS OR IMPLIED. Any description of Ion Torrent™ Product recited in Life Technologies' quotation is for the sole purpose of identifying Ion Torrent™ Product, and any such description is not part of any contract between Life Technologies and buyer and does not constitute a warranty that Ion Torrent™ Product shall conform to that description. Any sample or model used in connection with Life Technologies' quotation is for illustrative purposes only, and is not part of any contract between Life Technologies and buyer and does not constitute a warranty that Ion Torrent™ Product will conform to the sample or model. No affirmation of fact or promise made by Life Technologies, whether or not in Life Technologies' quotation, shall constitute a warranty that Ion Torrent™ Product will conform to the affirmation or promise. Unless otherwise specified in writing in documentation shipped with Ion Torrent™ Product or otherwise agreed by Life Technologies in writing. Life Technologies does not provide service or support for custom products or other products made to buyer's specifications. THE WARRANTIES IDENTIFIED IN THIS CLAUSE ARE LIFE TECHNOLOGIES' SOLE AND EXCLUSIVE WARRANTIES WITH RESPECT TO Ion Torrent™ PRODUCT AND ARE IN LIEU OF ALL OTHER WARRANTIES, STATUTORY, EXPRESS OR IMPLIED, ALL OF WHICH OTHER WARRANTIES ARE EXPRESSLY DISCLAIMED, INCLUDING WITHOUT LIMITATION ANY IMPLIED WARRANTY OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, NON-INFRINGEMENT, OR REGARDING RESULTS OBTAINED THROUGH THE USE OF ANY PRODUCT (INCLUDING, WITHOUT LIMITATION, ANY CLAIM OF INACCURATE, INVALID OR INCOMPLETE RESULTS), WHETHER ARISING FROM A STATUTE OR OTHERWISE IN LAW OR FROM A COURSE OF PERFORMANCE, DEALING OR USAGE OF TRADE.

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

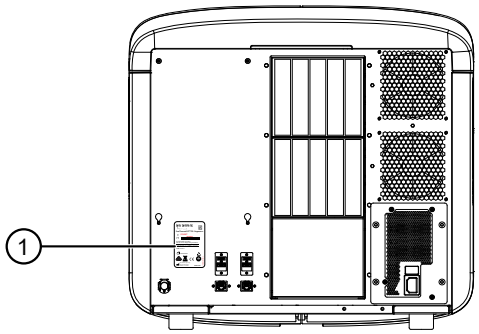
Safety alerts on this instrument

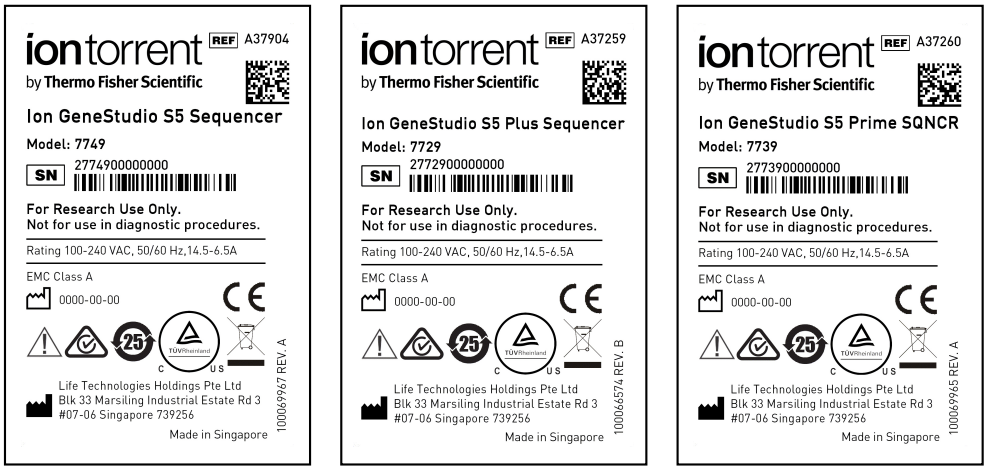
Additional text may be used with one of the symbols described above when more specific information is needed to avoid exposure to a hazard. See the following table for safety alerts found on the instrument.

English	Français
CAUTION! Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.	**MISE EN GARDE ! Produits chimiques dangereux.** Lire les fiches signalétiques (FS) avant de manipuler les produits.
CAUTION! Hazardous waste. Refer to SDS(s) and local regulations for handling and disposal.	**MISE EN GARDE ! Déchets dangereux.** Lire les fiches signalétiques (FS) et la réglementation locale associées à la manipulation et à l'élimination des déchets.

Location of safety labels on this instrument

Ion GeneStudio™ S5 Series sequencers have warnings at the location shown:





Ion GeneStudio™ S5 Sequencer labels

Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

Cleaning and decontamination

CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment.

MISE EN GARDE ! Nettoyage et décontamination. Utiliser uniquement les méthodes de nettoyage et de décontamination indiquées dans la documentation du fabricant destinée aux utilisateurs. L'opérateur (ou toute autre personne responsable) est tenu d'assurer le respect des exigences suivantes:

- Ne pas utiliser d'agents de nettoyage ou de décontamination susceptibles de réagir avec certaines parties de l'appareil ou avec les matières qu'il contient et de constituer, de ce fait, un DANGER.
- L'instrument doit être correctement décontaminé a) si des substances dangereuses sont renversées sur ou à l'intérieur de l'équipement, et/ou b) avant de le faire réviser sur site ou de l'envoyer à des fins de réparation, de maintenance, de revente, d'élimination ou à l'expiration d'une période de prêt (des informations sur les formes de décontamination peuvent être demandées auprès du Service clientèle).
- Avant d'utiliser une méthode de nettoyage ou de décontamination (autre que celles recommandées par le fabricant), les utilisateurs doivent vérifier auprès de celui-ci qu'elle ne risque pas d'endommager l'appareil.

Electrical safety

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

AVERTISSEMENT ! Veiller à utiliser une alimentation électrique appropriée. Pour garantir le fonctionnement de l'instrument en toute sécurité :

- Brancher le système sur une prise électrique correctement mise à la terre et de puissance adéquate.
- S'assurer que la tension électrique est convenable.
- Ne jamais utiliser l'instrument alors que le dispositif de mise à la terre est déconnecté. La continuité de la mise à la terre est impérative pour le fonctionnement de l'instrument en toute sécurité.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.

AVERTISSEMENT ! Cordons d'alimentation électrique. Utiliser des cordons d'alimentation adaptés et approuvés pour raccorder l'instrument au circuit électrique du site.

WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

AVERTISSEMENT ! Déconnecter l'alimentation. Pour déconnecter entièrement l'alimentation, détacher ou débrancher le cordon d'alimentation. Placer l'instrument de manière à ce que le cordon d'alimentation soit accessible.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety

Reference	Description
EU Directive 2014/35/EU	European Union “Low Voltage Directive”
IEC 61010-1 UL 61010-1 CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-010	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials*

EMC

Reference	Description
Directive 2014/30/EU	European Union “EMC Directive”
EN 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
AS/NZS CISPR 22 2009+A1 2010	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-003, Issue 5	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*
FCC Part 15 Subpart B (47 CFR)	*U.S. Standard Radio Frequency Devices*

Environmental design

Waste and hazardous substances

Reference	Description
Directive 2012/19/EU	European Union “WEEE Directive” – Waste electrical and electronic equipment
Directive 2011/65/EU	European Union “RoHS Directive” – Restriction of hazardous substances in electrical and electronic equipment

Radio compliance

Reference	Description
Directive 2014/53/EU	European Radio Equipment Directive (RED)
RFID	***FCC Notice (for U.S. Customers):*** This device complies with Part 15 of the FCC Rules. Operation is subject to the following conditions: **1.** This device many not cause harmful interference, and **2.** This device must accept any interference received, Including interference that may cause undesired operation Changes and Modifications not expressly approved by **Thermo Fisher Scientific** can void your authority to operate this equipment under Federal Communications Commissions rules. Canada: ***This device complies with Industry Canada licence-exempt RSS standard(s).*** Operation is subject to the following two conditions: (1) this device may not cause interference, and (2) this device must accept any interference, including interference that may cause undesired operation of the device. Le présent appareil est conforme aux CNR d'Industrie Canada applicables aux appareils radio exempts de licence. L'exploitation est autorisée aux deux conditions suivantes : (1) l'appareil ne doit pas produire de brouillage, et (2) l'utilisateur de l'appareil doit accepter tout brouillage radioélectrique subi, même si le brouillage est susceptible d'en compromettre le fonctionnement.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! **PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES.** Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l’utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d’utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l’inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu’avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l’absence de fuite ou d’écoulement des produits chimiques. En cas de fuite ou d’écoulement d’un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Documentation and support

Related documentation

Title	Pub. No.
Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef User Guide	MAN0016854
Ion 540™ Kit – Chef User Guide	MAN0010851
Ion 550™ Kit – Chef User Guide	MAN0017275
Ion 520™ & Ion 530™ ExT Kit – Chef User Guide	MAN0015805
Ion 520™ & Ion 530™ Kit – OT2 User Guide	MAN0010844
Ion 540™ Kit – OT2 User Guide	MAN0010850
Ion GeneStudio™ S5 System Site Preparation Guide	MAN0017529

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Ion GeneStudio™ S5 System

Publication Number MAN0017529 Revision C.0

This guide contains the information that is needed to prepare your site for installation of an Ion GeneStudio™ S5 System, Ion GeneStudio™ S5 Plus System, or Ion GeneStudio™ S5 Prime System. An external Ion Torrent™ Server is required for use with the Ion GeneStudio™ S5 Prime Sequencer and is included in the Ion GeneStudio™ S5 Prime System.

Note: In this guide, Ion GeneStudio™ S5 Sequencer or System refers generically to the series of three Ion GeneStudio™ S5 Sequencers or Systems, unless otherwise specified.

The Ion S5™ System and the Ion S5™ XL System are no longer available for purchase and are replaced by the Ion GeneStudio™ S5 System and Ion GeneStudio™ S5 Prime System, respectively. Both of the Ion S5™ and Ion S5™ XL Sequencers continue to be supported by Thermo Fisher Scientific. See the *Ion S5™ and Ion S5™ XL System Site Preparation Guide* (Pub. No. MAN0010810) for site preparation information for these systems.

ThermoFisher SCIENTIFIC

Site preparation workflow

A Thermo Fisher Scientific service representative will contact you to schedule the installation. When the installation date is scheduled, perform the following tasks.

Site preparation workflow

Review this site preparation guide

Complete the site preparation checklist (page 3)

Complete, date, and initial all items in the checklist before the scheduled installation date.

Receive and inspect the shipment (page 25)

- Verify items ordered were shipped.
- Inspect the shipping containers and report any damage.
- Unpack the installation kit and store as directed.

Move the crated shipment to the installation site (page 26)

- Prepare the installation site.
- Move the crated instrument to the site.

Installation timeline and training

After your sequencer is uncrated, installation and testing of the Ion GeneStudio™ S5, Ion GeneStudio™ S5 Plus, or Ion GeneStudio™ S5 Prime Sequencer and Torrent Server (required for use with the Ion GeneStudio™ S5 Prime Sequencer) takes ~8 hrs.

The Ion Chef™ System or Ion OneTouch™ 2 System is purchased and installed separately. Installation requires an additional 4–6 hours.

During and/or after installation, the Thermo Fisher Scientific service representative reviews data and provides basic operator training. For additional training and reference information, see the user documents that are provided with the Ion GeneStudio™ S5 Sequencers.

Site preparation checklist

IMPORTANT! Complete, date, and initial all items in the following checklist before the scheduled installation date. If the site preparation checklist is not complete when the Thermo Fisher Scientific service representative arrives, the scheduled installation can be postponed.

✓	Date	Initials	Site preparation requirement	See page
☐			Customer responsibilities have been reviewed and personnel have been assigned.	4 –5
☐			The installation site is identified and meets requirements:	
			☐ Space and clearance	5
			☐ Environmental	16
			☐ Electrical	19
			☐ Network	22
			☐ Safety	24
☐			All materials needed for installation and operation are available.	24
☐			The instrument was received and inspected:	25
			☐ All items on the shipping list are the same items ordered at the time of purchase.	
			☐ Any damage to shipping containers was reported to the shipping company that delivered the instrument.	
			☐ Any damage or mishandling was recorded on the shipping documents.	
			☐ The reagents box was unpacked and stored as specified.	
☐			The installation site is cleared and ready for instrument installation.	26
☐			The crated instrument and other shipping containers are moved to the installation site.	

Customer responsibilities

Personnel	Responsibilities
Site preparation/ installation coordinator	• Reviews the site preparation guide for safety information and instrument requirements. • Coordinates personnel and tasks. • Chooses the site. • Reviews checklists with applicable personnel, then with the service representative to verify that the site is properly prepared. • Receives and inspects the Ion GeneStudio™ S5 Sequencer. • Stores the reagents box according to the specifications indicated in the product inserts. • Schedules the installation and informs personnel of the installation day. • Ensures that the site is clear of unnecessary material on the installation day. • Is available to assist the service representative throughout installation.
Laboratory safety representative	• Reviews the site preparation guide for safety information. • Ensures that the required safety practices and equipment are in place. • Is in the vicinity and available to the service representative at all times while the service representative is at the customer's facility.
Laboratory personnel/ primary users	• Review safety information. • Ensures that all customer-provided materials for installation are present at the site. • Ensures that primary users (responsible for training other users) are available during the installation, so that they can be trained on the instrument.
Facilities personnel	• Ensures that the installation requirements are met for: – Space at the installation site – Building clearances – Temperature and humidity – Waste collection – Electrical supply – Computer – Safety and installation materials • If possible, moves the crated Ion GeneStudio™ S5 Sequencer to the site before the installation date. • Is available to assist service representative and laboratory personnel throughout installation. • If applicable, ensures that at least two people are available to help the service representative move and position the instrument.

(continued)

Personnel	Responsibilities
Network or IT specialist (if the instrument will be connected to a network)	• Ensures that active, tested local area network (LAN) connections are in place before the scheduled installation date. • Ensures that network hardware is compatible with an RJ45-type connector. • If necessary, supplies additional cables. • Is available during installation to connect the Ion GeneStudio™ S5 Sequencer to the network. • If applicable, provides and installs a network or dedicated printer. **IMPORTANT!** Do not attempt to connect the Ion GeneStudio™ S5 Sequencer components to the network before the service representative arrives.

Site requirements

Dimensions and weights

To prepare for installation, provide space for receipt and configuration of the components listed in this section. This section provides dimensions and weights for the packages you will receive, and it describes the dimensions of the components after installation and configuration.

Crates and packages

WARNING! PHYSICAL INJURY HAZARD. Do not attempt to lift or move the crated instrument without professional assistance. The crated instrument is heavy. Any incorrect lifting or moving of the crated instrument can cause serious injury.

Ensure the building clearances allow for the passage of the instrument crates and packages.

Crate	Height	Length (depth)	Width	Weight
Ion GeneStudio™ S5 Sequencer	25.5 in (64.8 cm)	39 in (99 cm)	28 in (71.1 cm)	200 lbs (90.7 kg)
Ion GeneStudio™ S5 Plus Sequencer	25.5 in (64.8 cm)	39 in (99 cm)	28 in (71.1 cm)	200 lbs (90.7 kg)
Ion GeneStudio™ S5 Prime Sequencer	25.5 in (64.8 cm)	39 in (99 cm)	28 in (71.1 cm)	200 lbs (90.7 kg)
Ion Torrent™ Server[1]	28.3 in (71.9 cm)	27.8 in (70.6 cm)	13.5 in (34.3 cm)	116.9 lbs (53.0 kg)
Ion Chef™ Instrument	28 in (71.1 cm)	34 in (86.4 cm)	34 in (86.4 cm)	295 lbs (134.0 kg)
Ion OneTouch™ 2 Instrument	18 in (45.7 cm)	18 in (45.7 cm)	21 in (53.3 cm)	44 lbs (20.0 kg)
Ion OneTouch™ ES Instrument	14.5 in (36.8 cm)	14.5 in (36.8 cm)	17.5 in (44.5 cm)	~13 lbs (5.9 kg)

[1] Required for use with the Ion GeneStudio™ S5 Prime Sequencer.

Components

Ensure that the installation site bench space is level and can accommodate the dimensions and support the weights.

Component	Height		Length (depth)	Width		Weight
	Open	Closed		Open	Closed	
Ion GeneStudio™ S5 Sequencer	—	20 in (50.9 cm)	31.75 in (80.6 cm)	34 in (86.4 cm)	21.4 in (54.2 cm)	140 lb (63.5 kg)
Ion GeneStudio™ S5 Plus Sequencer	—	20 in (50.9 cm)	31.75 in (80.6 cm)	34 in (86.4 cm)	21.4 in (54.2 cm)	140 lb (63.5 kg)
Ion GeneStudio™ S5 Prime Sequencer	—	20 in (50.9 cm)	31.75 in (80.6 cm)	34 in (86.4 cm)	21.4 in (54.2 cm)	140 lb (63.5 kg)
Ion Torrent™ Server[1]	—	17.5 in (44.4 cm)	27.5 in (69.8 cm)	—	12.1 in (30.8 cm)	101.4 lbs (46.0 kg)
Ion Chef™ Instrument	33 in (83.9 cm)	22.1 in (56.1 cm)	27.6 in (70.0 cm)	—	28.1 in (71.4 cm)	150 lbs (68.2 kg)
Ion OneTouch™ 2 Instrument	—	12 in (30.5 cm)	16 in (40.6 cm)	—	14 in (35.6 cm)	37.5 lbs (17.0 kg)
Ion OneTouch™ ES Instrument	—	9.5 in (24.1 cm)	12.5 in (31.8 cm)	—	11 in (27.9 cm)	12 lbs (5.4 kg)

[1] Required for use with the Ion GeneStudio™ S5 Prime Sequencer.

WARNING! PHYSICAL INJURY HAZARD.

Do not attempt to lift or move the instrument without professional assistance. The instrument is heavy. Any incorrect lifting or moving of the instrument can cause serious injury.

Instrument clearances

During instrument setup and maintenance, it is necessary to access the back and sides of all of the instruments. If the back of an instrument component faces a wall, it is necessary to have enough space to allow rotation of the instrument on the bench for access.

IMPORTANT! For safety, the power outlet used for powering the instrument components must be accessible at all times.

Component	Top	Front	Left/Right	Back
Ion GeneStudio™ S5 Sequencer[1]	12.0 in (30.5 cm)	12.0 in (30.5 cm)[2]	4.0 in/12 in[3] (10.0 cm/30.5 cm)	12.0 in (30.5 cm)
Ion GeneStudio™ S5 Plus Sequencer[1]	12.0 in (30.5 cm)	12.0 in (30.5 cm)[2]	4.0 in/12 in (10.0 cm/30.5 cm)[3]	12.0 in (30.5 cm)
Ion GeneStudio™ S5 Prime Sequencer[1]	12.0 in (30.5 cm)	12.0 in (30.5 cm)[2]	4.0 in/12 in (10.0 cm/30.5 cm)[3]	12.0 in (30.5 cm)
Ion Torrent™ Server	2.0 in (5.0 cm)	12.0 in (30.5 cm)	2.0 in (5.0 cm)	24.0 in (61.0 cm)
Ion Chef™ Instrument	14 in (35.6 cm)	6.7 in (17.0 cm)	4.0 in (10.0 cm)	4.0 in (10.0 cm)
Ion OneTouch™ 2 Instrument	12.0 in (30.5 cm)	12.0 in (30.5 cm)	4.0 in (10.0 cm)	4.0 in (10.0 cm)
Ion OneTouch™ ES Instrument	12.0 in (30.5 cm)	12.0 in (30.5 cm)	12.0 in (30.5 cm)	4.0 in (10.0 cm)

[1] Clearance requirements for all Ion GeneStudio™ S5 Sequencers are identical.

[2] The instrument requires 36.0 in (90.0 cm) aisle in front of bench for operator access.

[3] To allow sufficient clearance for the instrument door to open.

Placement of the instrument and server

If any of the instruments will be placed on a mobile bench, verify that the bench is level and meets the requirements in “Crates and packages” on page 5.

IMPORTANT! The Ion GeneStudio™ S5 Sequencer must be installed on a level surface.

Note: Thermo Fisher Scientific is not responsible for any damage caused by using a laboratory bench that does not meet the minimum weight capacity requirements mentioned above.

Laboratory layout of the Ion GeneStudio™ S5 Sequencers and Ion Torrent™ Server

The Ion GeneStudio™ S5 Sequencer contains a virtual Ion Torrent™ Server, and the Ion GeneStudio™ S5 Plus Sequencer is equipped with an internal Ion Torrent™ Server. These instruments are not connected to an external Ion Torrent™ Server and only require connection to a local area network (see Figure 1). For the Ion GeneStudio™ S5 Prime Sequencer, we support the layout in which the Ion Torrent™ Server is directly connected to the sequencer (see Figure 2), instead of through the local area network from a remote location such as a server room. Data are most robustly transferred from the Ion GeneStudio™ S5 Prime Sequencer to the Ion Torrent™ Server when they are directly connected by a category 6 Ethernet cable that is provided with the installation materials.

IMPORTANT! The Ion GeneStudio™ S5 Prime Sequencer must be connected to the Ion Torrent™ Server by a category 6 Ethernet cable. We do not troubleshoot data transfer problems that are associated with an indirect connection between the Ion GeneStudio™ S5 Prime Sequencer and the Ion Torrent™ Server.

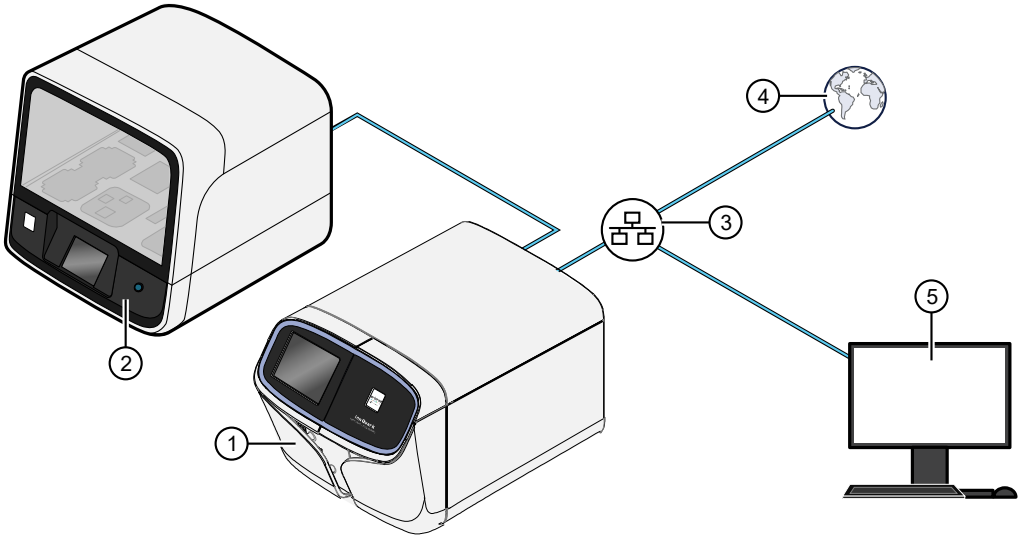


Figure 1 Ion GeneStudio™ S5 Sequencer and Ion GeneStudio™ S5 Plus Sequencer configuration

1. Ion GeneStudio™ S5 Sequencer or Ion GeneStudio™ S5 Plus Sequencer
2. Ion template preparation instrument (Ion Chef™ Instrument (shown) or Ion OneTouch™ 2 System)
3. Local area network
4. Internet
5. Client computer

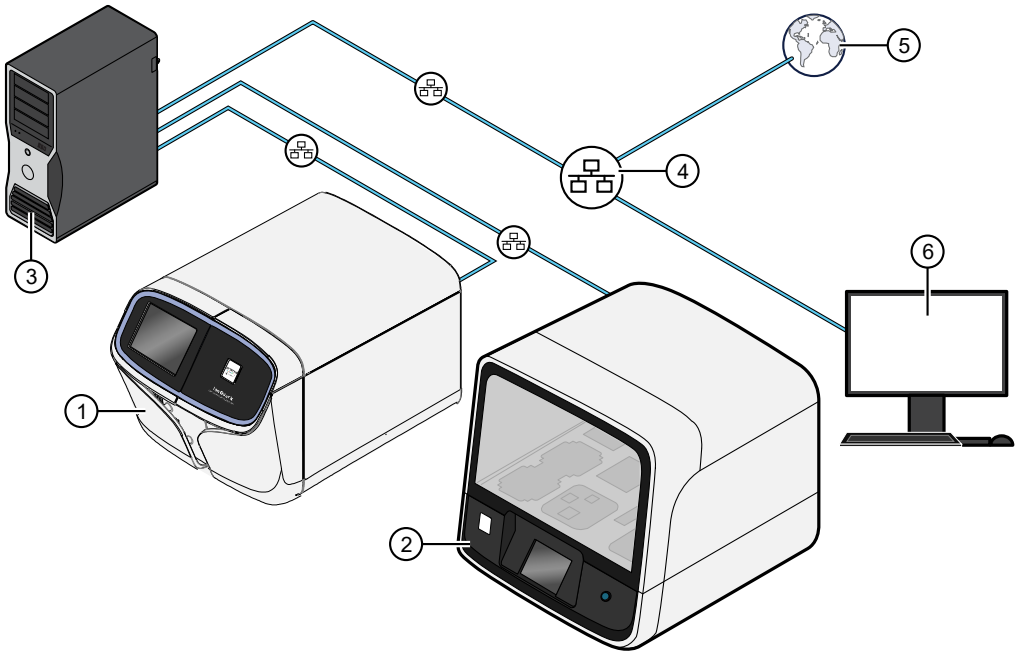


Figure 2 Ion GeneStudio™ S5 Prime Sequencer configuration

1. Ion GeneStudio™ S5 Prime Sequencer
2. Ion template preparation instrument (Ion Chef™ Instrument (shown) or Ion OneTouch™ 2 System)
3. Ion Torrent™ Server
4. Local area network
5. Internet
6. Client computer

Note: In either configuration, the Ion Chef™ Instrument must be connected to the Ion Torrent™ Server either directly using a category 6 Ethernet cable, or indirectly through a local area network that has been configured to allow HTTP-443, SSH-22, and FTP-20/21 traffic. Unlike the Ion GeneStudio™ S5 Prime Sequencer, the Ion Chef™ Instrument does not require a robust network connection to the Ion Torrent™ Server and can accommodate standard network connection speeds.

Internet connectivity

The Ion GeneStudio™ S5 Sequencer, the Ion GeneStudio™ S5 Plus Sequencer, or the Ion Torrent™ Server (in an Ion GeneStudio™ S5 Prime System) should be connected to a network with internet access. Connecting to the Internet allows you to update your software and access remote system support easily. Software updates through the network/internet are free. If you do not connect your instrument or server to a network, software updates must be manually installed via USB.

IMPORTANT! Thermo Fisher Scientific does not support the USB method for updating software.

Any problems (for example, file corruption, incomplete updates) resulting from update of the sequencer or server using a USB device that require correction of the faulty update is not covered by your Ion GeneStudio™ S5, Ion GeneStudio™ S5 Plus, or Ion GeneStudio™ S5 Prime Sequencer warranty or any service contract you may have bought. You are required to schedule an on-site Time and Materials visit by a field service engineer to correct the problem at your own expense.

In providing outbound access to the Internet from the server, you enable the support team to provide inbound support. The Ion GeneStudio™ S5 Sequencer, the Ion GeneStudio™ S5 Plus Sequencer, and the Ion Torrent™ Server run a remote monitor agent that can provide service personnel with critical system information, such as installed software versions and instrument alarms. With your permission, the agent also allows service personnel to access remotely the Ion GeneStudio™ S5 Sequencer, the Ion GeneStudio™ S5 Plus Sequencer, or the Ion Torrent™ Server, which is required for system support. Without remote access, service personnel cannot access, view, and troubleshoot problems regarding machine performance.

To enable full support, the Ion Torrent™ Server must have outbound internet access (ports 22, 80, and 443) and be behind an appropriately configured firewall. Although not recommended, you can enable access to the Torrent Browser (the web server running on the Ion Torrent™ Server from the Internet). If you provide such access, you must restrict access to the server using HTTP and AUTH firewall rules, or a combination of the two. Implementing and maintaining such restrictions is the responsibility of your server administrator and not of Thermo Fisher Scientific.

Planning the installation

In preparation for the Ion GeneStudio™ S5 Sequencer installation, you must plan the layout of your laboratory to accommodate library and template preparation activities, in addition to those related to chip preparation and sequencing. This section describes the stations involved in the sequencer workflow and the basic laboratory layouts.

Layout of instrument components and equipment

When designing your PCR laboratory layout, follow good laboratory practices to ensure reliable and contamination-free PCR results. Pay particular attention to the need to separate the areas for pre- and post-PCR activities. Isolating the amplicon source, separating pre-PCR from post-PCR activities, and dedicating laboratory supplies and/or equipment to each space can significantly reduce the potential for contamination.

You can deploy the Ion GeneStudio™ S5 Sequencer in both one- and two-room laboratory configurations. The two-room layout is highly recommended due to the protection that it affords against contamination; however, the sub-optimal one-room layout will produce acceptable results if proper precautions are observed.

If you choose to deploy the Ion GeneStudio™ S5 Sequencer in a one-room layout:

- Establish clearly-labeled, separate sets of pipettes for library preparation, emulsion PCR setup, and emulsion-breaking stations (if using the Ion OneTouch™ 2 System).
- Always move from "clean" to "dirty" (for example, from pre- to post-PCR). We do not recommend moving from "dirty" to "clean" (for example, do not handle post-amplification samples and then make libraries).

Note: The positions of the stations in the pre-and post- PCR rooms are not important.

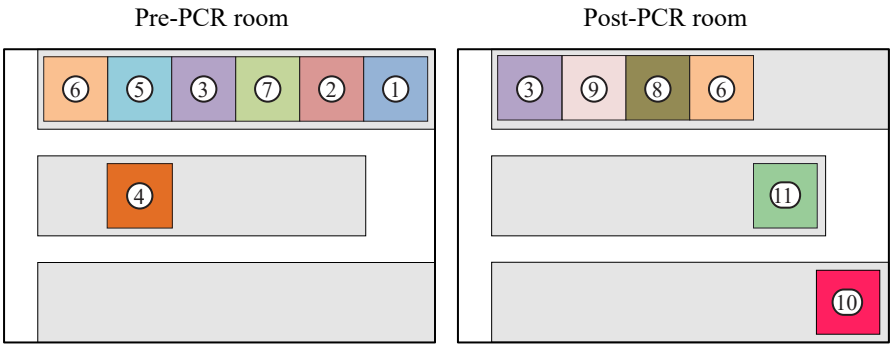


Figure 3 Two-room layout

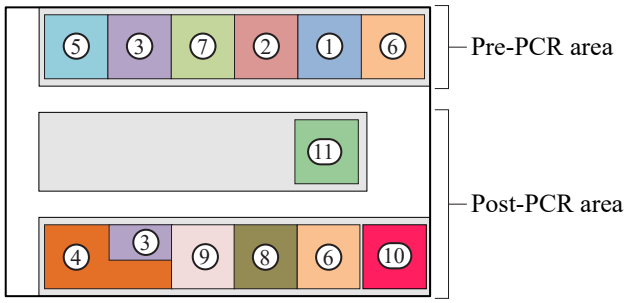


Figure 4 One-room layout

1. Agilent™ 2100 Bioanalyzer™ instrument
2. (*Optional*) Diagenode™ Bioruptor™ Pico sonication device system
3. Centrifuge
4. Amplification mixture setup hood
5. Library area setup hood
6. Pipettes
7. (*Optional*) Pippin Prep™ instrument
8. Qubit™ Fluorometer
9. Thermal cycler
10. Ion Chef™ or Ion OneTouch™ 2 Instrument
11. Ion GeneStudio™ S5 Sequencer, Ion GeneStudio™ S5 Plus Sequencer, *or* Ion GeneStudio™ S5 Prime Sequencer and Ion Torrent™ Server

Laboratory workstations

The following table describes workstations that are associated with the generic laboratory layout for the Ion GeneStudio™ S5 Sequencer. The stations are categorized in terms of their involvement pre- and post-PCR activities. Note that some stations (pipette and centrifuge) are present in both environments, but the equipment is not necessarily shared between the pre- and post-PCR stations.

#	Station	Location	Description/requirements
1	Agilent™ 2100 Bioanalyzer™ Instrument	Pre-PCR area	Use the Agilent™ 2100 Bioanalyzer™ Instrument to perform library construction quality control, providing yield and size characterization. When planning the instrument placement, consider isolating the Agilent™ 2100 Bioanalyzer™ Instrument from the sequencer, preferably in a room dedicated for library preparation, to minimize contamination. **Note:** If needed, you can substitute a qPCR instrument for the Agilent™ 2100 Bioanalyzer™ Instrument. In general, qPCR does not yield size distribution but does provide more accurate counts.
2	*(Optional)* Bioruptor™ System	Pre-PCR area	If required, the Bioruptor™ Instrument performs acoustic shearing/fragmentation of DNA samples for library preparation. You can perform DNA shearing through the use of a Covaris™ Focused-Ultrasonicator or the Ion Express Library Kit, both of which are acceptable substitutes for the Bioruptor™ Instrument. Mechanical shearing is recommended (using Bioruptor™ or Covaris™ instruments), but enzymatic shearing is acceptable and can be less expensive. When planning Bioruptor™ or Covaris™ instrument placement: • Consider placing the instrument in an isolated room to minimize the noise hazard. • Consider isolating the instrument from the sequencer, preferably in a dedicated room for library preparation, to minimize contamination.
3	Centrifuges	Pre- and post-PCR areas	Use centrifuges during library preparation and chip loading, so access is required in both the pre- and post-PCR areas. When planning centrifuge placement: • Consider dedicating separate centrifuges for the pre- and post-PCR operations to minimize contamination. • If only one centrifuge is available, place it in a central location, which is accessible from both areas. • To limit vibrational interference, do not place centrifuges on the same bench as the sequencer instrument. • Most importantly, place the centrifuges in convenient locations.

(continued)

#	Station	Location	Description/requirements
4	Amplification mixture setup hood (if using the Ion OneTouch™ 2 System)	Pre-PCR area	Whenever possible, perform amplification mixture preparation in a dedicated hood. When selecting a location for amplification mixture preparation: • The amplification mixture setup station requires a set of dedicated pipettes. • If only one hood is available in the pre-PCR area, dedicate the hood for amplification mixture preparation and perform library preparation elsewhere. • If a hood is unavailable in the pre-PCR area, select a bench that is sterilized regularly and preferably isolated from the neighboring stations. **IMPORTANT!** Separation of the amplification mixture preparation area from the other stations is critical to preventing contamination. **IMPORTANT!** If possible, physically separate the library and amplification mixture setup areas.
5	Library area setup hood	Pre-PCR area	Whenever possible, perform library preparation in a dedicated hood. The use of a dedicated hood allows for UV treatment of the library preparation area and minimizes contamination from post-PCR material containing adaptors. When selecting a location for library preparation: • In a one-room laboratory layout, we highly recommend the use of a dedicated hood for library preparation, but not required. • The library area setup station requires a set of dedicated pipettes. • If a dedicated hood is unavailable, select a bench that is preferably isolated from the neighboring stations.
6	Pipettes	Pre- and post-PCR areas	Both the pre- and post-PCR areas require a complete set of dedicated pipettes. If needed, you can share the pipettes between neighboring stations, except for the amplification mixture and library setup stations, both of which require dedicated sets of pipettes.
7	*(Optional)* Pippin Prep™ Instrument	Pre-PCR area	If required, use the Pippin Prep™ Instrument to perform automated size selection before template preparation. Use of the Pippin Prep™ Instrument is necessary only if you require size selection for your sample type. **Note:** You can also do size selection through the use of the E-Gel™ Imager Gel Documentation System, an acceptable substitute for the Pippin Prep™ Instrument.
8	Qubit™ System	Post-PCR area	You can use the Qubit™ 2.0, Qubit™ 3.0, or Qubit™ 4 Fluorometer to perform library quantification or quality control following PCR or just before library preparation. When selecting a location for the instrument, place the Qubit™ System close to the Ion OneTouch™ 2 System.

(continued)

#	Station	Location	Description/requirements
9	Primer-Annealing Thermal Cycler	Post-PCR area	Use a 96-well thermal cycler to perform the primer-annealing step during template preparation. When selecting a location for the thermal cycler, ensure that the location meets all clearance and environmental requirements for the instrument. **Note:** If you are using Ion AmpliSeq™ Primers, a pre-PCR thermal cycler is required in the pre-PCR room. **Note:** Primer-annealing thermal cycler is not required if using the Ion Chef™ System for template preparation.
10	Ion Chef™ or Ion OneTouch™ 2 System	Post-PCR area	When planning the placement of the Ion Chef™ Instrument or Ion OneTouch™ 2 System, ensure that the location meets all clearance and environmental requirements described in this document. **Note:** If possible, install the Ion GeneStudio™ S5 Sequencer and Ion Chef™ Instrument to a dedicated power outlet.
11	Ion GeneStudio™ S5 Sequencer, Ion GeneStudio™ S5 Plus Sequencer, *or* Ion GeneStudio™ S5 Prime Sequencer and Ion Torrent™ Server	Post-PCR area	When planning the placement of the Ion GeneStudio™ S5 Prime Sequencer and Ion Torrent™ Server, ensure that the location meets all clearance and environmental requirements described in this document. **IMPORTANT!** The Ion GeneStudio™ S5 Sequencers are sensitive to electrical noise, vibration, and temperature changes. **Note:** Because the Ion Torrent™ Server requires a direct connection to the Ion GeneStudio™ S5 Prime Sequencer via a standard Category 6 Ethernet cable, the server is typically installed to the bench directly beneath the instrument.

Environmental requirements

Ensure that the installation room is maintained under correct environmental conditions. Avoid placing the sequencer or server next to heaters, cooling ducts, or in direct sunlight. Place the sequencer at least 1 meter away from major sources of electronic noise such as refrigerators or microwaves. Fluctuations between day and night temperatures can cause system instability.

Component	Acceptable range
Altitude	Located between sea level and 6500 ft (2000 m) above sea level
Humidity	**Ion GeneStudio™ S5, Ion GeneStudio™ S5 Plus, or Ion GeneStudio™ S5 Prime Sequencer** : 40-60%, non-condensing **Ion Torrent™ Server**: 20-80%, non-condensing **Ion Chef™ Instrument**: 40-60%, non-condensing **Ion OneTouch™ 2 Instrument/Ion OneTouch™ ES Instrument**: Relative humidity 80% for temperatures up to 31°C, decreasing linearly to 50% relative humidity at 40°C
Operating Temperature	**Ion GeneStudio™ S5, Ion GeneStudio™ S5 Plus, or Ion GeneStudio™ S5 Prime Sequencer**: 20°C to 30°C (68°F to 86°F) **Note:** The room temperature must not fluctuate more than 2°C over a 2-hour period. **Ion Torrent™ Server**: 10°C to 35°C (50°F to 95°F) **Ion Chef™ Instrument**: 20°C to 25°C (68°F to 77°F) **Ion OneTouch™ 2 Instrument/Ion OneTouch™ ES Instrument**: 20°C to 25°C (68°F to 77°F)
Vibration	Install the instrument(s) on benches that have no contact with equipment that causes vibration (freezers, pumps, and similar equipment). Significant vibration during sequencing can add noise and reduce the quality of the sequencing measurements. **Note:** Where possible we recommend placing the Ion Chef™ Instrument on a separate bench from the Ion GeneStudio™ S5, Ion GeneStudio™ S5 Plus, or Ion GeneStudio™ S5 Prime Sequencer as the Ion Chef™ Instrument can itself cause vibration.
Pollution	The Ion GeneStudio™ S5 Sequencers, the Ion Chef™ Instrument, Ion OneTouch™ 2 Instrument, and the Ion OneTouch™ ES Instrument are for use in Office or Laboratory controlled environments.
Overvoltage category	The Ion GeneStudio™ S5 Sequencers, Ion Chef™ Instrument, Ion OneTouch™ 2 Instrument, and the Ion OneTouch™ ES Instrument have installation (overvoltage) categories of II (2), and are classified as portable equipment.
Other conditions	For indoor use only. Install the Ion GeneStudio™ S5 Sequencers on a level surface. The installation location must be away from any vents that could expel particulate material on the system components.

Thermal specifications for the instrument and server

During operation, the thermal outputs based on the typical current draw of the components are:

Component	Typical draw (W)	Thermal output (BTU/h)
Ion GeneStudio™ S5 Sequencer	520[1]	1774
Ion GeneStudio™ S5 Plus Sequencer	700[1]	2388
Ion GeneStudio™ S5 Prime Sequencer	700[1]	2388
Ion Torrent™ Server[2]	1100	3753
Ion Chef™ Instrument	990	3378
Ion OneTouch™ 2 Instrument	550	1877
Ion OneTouch™ ES Instrument	410	1399

[1] Maximum draw: 1350W

[2] Minimum Efficiency: 65% (Energy Star Qualified); 85% Efficient Power Supply.

Ventilation requirements

Allow at least 4 inches (10 cm) of clearance around the Ion GeneStudio™ S5 System for ventilation. Do not block air inlets or outlets to allow proper ventilation.

Cleaning or decontamination

See the user documentation for your Ion GeneStudio™ S5 Sequencer for information on how to clean or decontaminate the instrument.

Wear appropriate protection, including gloves, laboratory goggles, and coat whenever you work with the fluids that are used on this instrument, or parts that may come into contact with these fluids.

Use only the cleaning agents as described in the user documentation for your Ion GeneStudio™ S5 Sequencer. Use of cleaning agents that are not described in user documentation can impair the instrument. Contact Technical Support if you have questions.

Wipe off any liquid on or near the instrument using a lint-free tissue.

Disposing of waste

WARNING! CHEMICAL HAZARD. Refer to Safety Data Sheets (SDSs) and local regulations for handling and disposing of plastic consumables. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of plastic consumables.

WARNING! DANGER CHIMIQUE. Consulter les fiches de données de sécurité (FDS) et les réglementations locales en matière de manipulation et d'élimination des consommables en plastique. Se conformer à la réglementation locale relative à l'élimination des déchets usuels pour réduire l'impact environnemental des consommables en plastique.

WARNING! CHEMICAL HAZARD. Before handling chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! DANGER CHIMIQUE. Avant de manipuler des produits chimiques, se référer à la fiche de données de sécurité (FDS) fournie par le fabricant et respecter toutes les précautions d'usage.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! DANGER CHIMIQUE. Tous les produits chimiques contenus dans l'instrument, notamment le liquide dans les lignes, sont potentiellement dangereux. Toujours déterminer les produits chimiques utilisés dans l'instrument avant le remplacement de réactifs ou de composants de l'instrument. Porter des gants, des vêtements de protection et des protections oculaires appropriés lors de toute intervention sur l'instrument.

WARNING! CHEMICAL HAZARD. Waste produced by instruments can be hazardous and can cause injury or illness.

WARNING! DANGER CHIMIQUE Les déchets produits par les instruments peuvent être dangereux et entraîner des blessures ou des maladies.

Electrical requirements

WARNING! For safety, the power outlet used for powering the instrument must be accessible at all times. See "Instrument clearances" on page 7 for information about the space needed between the wall and the instrument. In case of emergency, you must be able to immediately disconnect the main power supply to all the equipment. Allow adequate space between the wall and the equipment so that the power cords can be disconnected in case of emergency.

WARNING! Par souci de sécurité, la prise de courant alimentant l'instrument doit être accessible à tout moment. En cas d'urgence, il doit être possible de débrancher immédiatement l'alimentation principale de l'ensemble des équipements. Laisser suffisamment d'espace entre le mur et les équipements afin de pouvoir débrancher les câbles d'alimentation sans encombre, en cas d'urgence.

- Electric receptacle required: 2-prong with ground pin
- Mains AC line voltage tolerances must be up to ± 10% percent of nominal voltage

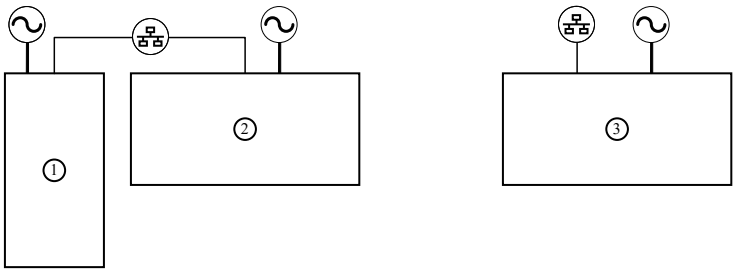
Component	Input voltage (VAC)	Frequency (Hz)	Rated Current (A)[1]
Ion GeneStudio™ S5 Sequencer	100-240	50/60	14.0
Ion GeneStudio™ S5 Plus Sequencer	100-240	50/60	14.0
Ion GeneStudio™ S5 Prime Sequencer	100-240	50/60	14.0
Ion Torrent™ Server[2]	90-265	50/60	12
Ion Chef™ Instrument	100-240	50/60	12
Ion OneTouch™ 2 Instrument	100-240	50/60	5.5
Ion OneTouch™ ES Instrument	99-132/198-264	50/60	0.5

[1] Based on rated current at minimum input voltage.

[2] Minimum Efficiency: 65% (Energy Star Qualified); 85% Efficient Power Supply.

Electrical requirements for the Ion GeneStudio™ S5 System and Ion Torrent™ Server

Use an approved UL Listed detachable power supply cord to connect the sequencer to the wall. Route power cords away from the workspace to avoid accidental disconnection.



① Ion Torrent™ Server
② Ion GeneStudio™ S5 Sequencer
③ Ion GeneStudio™ S5/Ion GeneStudio™ S5 Plus Sequencer

Power cords are provided with the instrument. If not suitable for installation in your region, ensure that any power cord you do use is:

- Maximum 10 ft (3 m) in length
- Grounding type
- Compatible with the power supply receptacles used to connect to main power
- Suitable for the rating of the instrument and mains power supply
- Compliant with local safety requirements (for example, UL Listed for North America, JIS approved for Japan, HAR or agency certified for Europe)

Electrical requirements for the Ion OneTouch™ Instrument

- Use the external power supply provided with the instrument.
- Facility to supply via building code compliant 15A circuit.
- Use an approved UL Listed detachable power supply cord, as supplied, to connect the system to the wall.

Electrical protective devices

We recommend several protective devices to protect the system in environments with large voltage and power fluctuations.

Device	Description
Power line regulator	We recommend the use of a 1.5-kVA power line regulator in areas where the supplied power fluctuates in excess of ± 10% of the normal voltage. Power fluctuations can adversely affect the function of the instrument and computer. **Note:** A power line regulator monitors the input current and adjusts the power supplied to the instrument or computer. It does not protect against a power surge or failure.
Uninterruptible power supply (UPS)	We recommend the use of a 1.5-kVA uninterruptible power supply (UPS), especially in areas prone to power failure. Power failures and other events that abruptly terminate the function of the instrument and computer can corrupt data and possibly damage the system. ⚠ **WARNING! PHYSICAL INJURY HAZARD.** Do not attempt to lift the UPS unit without assistance of at least two people. Improper lifting can cause painful and permanent back injury. Refer to the UPS manufacturer user guide for more information. ⚠ **WARNING! RISQUE DE BLESSURES CORPORELLES.** Ne pas essayer de soulever l'onduleur sans l'aide d'au moins deux personnes. En soulevant incorrectement l'appareil, l'opérateur risque de se blesser au dos de façon permanente. Voir le guide de l'utilisateur du fabricant de l'onduleur pour plus d'informations. **IMPORTANT!** UPSs provide power for a limited time. They are meant to delay the effects of a power outage, not to serve as replacement power sources. In the event of a power loss, power off the instrument and computer unless you expect to regain power within the battery life of the UPS.
Surge protector	We recommend the use of a 10-kVA surge protector (line conditioner) in areas with frequent electrical storms or near devices that are electrically noisy, such as refrigerators, air conditioners, or centrifuges. Short-duration, high-voltage power fluctuations can abruptly terminate the function of, and thereby damage the components of, the computer and the instrument. **Note:** A dedicated line and ground between the instrument, computer, and the building's main electrical service can also prevent problems caused by power fluctuations.

Network requirements

The Ion Torrent™ Server is factory-configured for the TCP/IP protocol. The product includes a fast Ethernet adapter (10/100 Mbps) with an RJ45-type connector and one 3-m (9.8-ft) crossover Ethernet cable that connects the computer and the instrument.

For the instrument to be connected to a LAN, an active, tested LAN connection must be in place before the scheduled installation date.

You must supply a standard Category 6 Ethernet cable of the required length to connect the computer to your LAN.

Network configuration

The following requirements must be met before installation of the Ion Torrent™ Server. Discuss any discrepancies in the checklist with your field service representative prior to the visit.

- An information technologies resource must be available to assist with the network connection on the date that the Ion Torrent™ Server will be installed.
- A static (or dynamic) IP address must be reserved for the Ion GeneStudio™ S5 Plus Sequencer or Ion Torrent™ Server.

  Note: If necessary, field service can provide the Ion GeneStudio™ S5 Plus Sequencer or Ion Torrent™ Server's MAC address prior to the installation.

- The room where the Ion Torrent™ Server will be deployed must contain at least one active network jack.
- The site DNS Server must be configured for the Ion Torrent™ Server so that users can access the server URL from their workstations.
- The Ion GeneStudio™ S5 Plus Sequencer and/or the Ion Torrent™ Server must be protected behind an appropriately configured network firewall and have outbound internet access as described below.

  Note: While not recommended, you can enable access to the Ion Torrent™ Server from the internet (via the web server running on the Ion Torrent™ Server). If you provide such access, you must restrict access to the server using HTTP and AUTH firewall rules, or a combination of the two. Implementing and maintaining such restrictions is the responsibility of the server administrator and not of Thermo Fisher Scientific.

- The Ion GeneStudio™ S5 Plus Sequencer and Ion Torrent™ Server require outbound Internet access through HTTP/port-80 to **http://ionupdates.com** and **http://us.archive.ubuntu.com** to retrieve software updates.

  Note: The Ion GeneStudio™ S5 Sequencer and Ion Torrent™ Server require an efficient Internet connection to download software updates. If they are isolated from the Internet, you must download and install updates manually to the server.

  IMPORTANT! Manually installing server updates is not supported by Thermo Fisher Scientific. If you choose to download the updates to a USB and manually update the Ion Torrent™ Server you do so at your own risk. Any issues updating the server in this manner requiring Thermo Fisher Scientific intervention are not covered by the warranty or any service contract you may have purchased. You will need to schedule an on-site visit by your field service engineer at your own expense.

- The Ion GeneStudio™ S5 Sequencer and Ion Torrent™ Server require outbound Internet access through HTTPS/port-443 and SSH/port-22 to allow us to provide remote support via the Axeda Remote System Monitoring (RSM) Agent. The server includes the Axeda Agent to assist you in maintaining your Ion GeneStudio™ S5 System and to provide timely technical support (for more information, see **http://www.axeda.com/community/customers/applied-biosystems**).

  At minimum, whitelist the following outbound addresses for the server:

  - drm.appliedbiosystems.com on HTTPS (443)
  - rssh.iontorrent.net on SSH (22)

  IMPORTANT! Without access through HTTPS/port-443 and SSH/port-22, we cannot support your site in a timely fashion and we may forego remote support for your site altogether at our discretion.

  Note: The Axeda Agent is already in use with the Ion PGM™ and Ion Proton™ Sequencers to perform instrument diagnostics, preventive maintenance, failure prediction, and proactive notification. The agent does not collect any sequencing reports, results, or data. For more information on the Axeda Agent, see: **http://lifetech-it.hosted.jivesoftware.com/message/1546#1546**

- If the Ion GeneStudio™ S5 System will be installed without a connection to a local area network, then you must provide a router to access data generated by the Ion GeneStudio™ S5 Sequencer. Connection and configuration of the router is not the responsibility of the field service engineer.
- If the Ion Torrent™ Server will be more than 10 feet from the Ion GeneStudio™ S5 Prime Sequencer, you must provide a standard Category 6 Ethernet Cable of sufficient length at the time of installation.

  Note: The supported configuration of the Ion GeneStudio™ S5 Prime Sequencer is a direct connection between the instrument and the server.

- Use a shielded Ethernet cable when connecting the Ion Torrent™ Server to the Ion OneTouch™.

Safety requirements

Safety practices

A safety representative from your facility must ensure that:

- Personnel establish and follow all applicable safety practices and policies to protect laboratory personnel from potential hazards.
- All applicable safety devices and equipment are available at all times.

Required safety equipment

Your laboratory has specific safety practices and policies designed to protect laboratory personnel from potential hazards that are present. Follow all applicable safety-related procedures at all times.

The following safety equipment and protection from hazards must be available at the installation site:

- Protection from any sources of hazardous chemicals, radiation (for example, lasers, radioisotopes, radioactive wastes, and contaminated equipment), and potentially infectious biological material that may be present in the area where the service representative will work.
- Eyewash
- Safety shower
- Eye and hand protection
- Adequate ventilation, including vent line/fume hood, if applicable
- Biohazard waste container, if applicable
- First-aid equipment
- Spill cleanup equipment
- Applicable Safety Data Sheets (SDSs)

Materials for installation and operation

Installation checklist

See the *Ion GeneStudio™ S5 Systems Pre-Installation Checklist* (Pub No. MAN0013777) for the materials required for instrument installation. The checklist specifies the materials that must be present onsite before installation and subsequent training can take place.

Installation kit

The Ion S5™ Installation Kit (Cat. No. A27215) is available to first-time owners of an Ion GeneStudio™ S5 System and is shipped with the instrument. The kit contains the core reagents and controls that are used during the installation, training, and operation of the instruments.

Ion S5™ Installation Kit (Part No. A27215; Not available for separate purchase.)

Contents	Part No.	Quantity	Storage
Ion 540™ Chip Kit	A27765	4 pack	15°C to 30°C
Ion S5™ Sequencing Solutions	A27767	1	
Ion S5™ Sequencing Reagents	A27768	1	–30°C to –10°C
Ion S5™ Cartridge Tool	A28308	1	15°C to 30°C

Operation materials

Additional supplies and consumables are necessary for routine operation. Contact a sales representative to order these additional supplies. Use only supplies as specified by Thermo Fisher Scientific.

Materials for template and library preparation

For more information about Ion Torrent™ sequencing applications and materials and equipment required for library and template preparation, see the documentation at **thermofisher.com**.

Receive and inspect the shipment

1. Verify that the items shown on the shipping list are the items that were ordered at the time of purchase.
2. Carefully inspect the shipping containers. Take photographs of any damage, then report the damage to the shipping company and to your service representative. Record any damage or mishandling on the shipping documents.
3. Immediately unpack the reagents or installation kit box (boxed separately from the instrument components). Store the reagents at the temperatures specified on the product packaging or labels. Storage temperatures are also listed in .

IMPORTANT! Other than reagents or plates that require storage at specific conditions, do not unpack the instrument shipping containers at this time. To protect yourself from liability for damage that occurred during shipping, inspect the shipping containers and report damage as described above.

IMPORTANT! Do not unpack the instrument shipping containers at this time. To protect yourself from liability for damage that occurred during shipping, inspect the shipping containers and report damage as described above.

Move the crated instrument to the installation site

1. Clear the installation site of all unnecessary materials.
2. If possible, move the crated instrument and other shipping containers to the installation site. Do not uncrate.

CAUTION! PHYSICAL INJURY HAZARD. Do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more people.

IMPORTANT! Do not tip the crated instrument on end. Tipping may damage the instrument hardware and electronics.

Note: After installation, retain the crate and instrument packaging in case you need to relocate the instrument.

Related documentation and support

Related documentation

Document	Pub. No.	Description
Ion GeneStudio™ S5 Instrument User Guide	MAN0017528	Describes the Ion GeneStudio™ S5 System hardware and software, and provides information on preparing, maintaining, and troubleshooting the system.
Ion GeneStudio™ S5 Systems Pre-Installation Checklist	MAN0013777	Describes the tasks that you should complete before installation, and lists the materials needed for a successful Ion GeneStudio™ S5 System installation and training.

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Life Technologies Holdings Pte Ltd | Block 33 | Marsiling Industrial Estate Road 3 | #07-06, Singapore 739256

Revision history: Pub. No. MAN0017529

Revision	Date	Description
C.0	1 February 2023	Removed the following skus from the Ion S5™ Installation Kit contents table. • A28195 • A27897 • A29022
	21 February 2022	Update the quantity of the Ion S5™ Cartridge Tool in the Ion S5™ Installation Kit. Update site preparation workflow to current format.
A.0	5 January 2018	New site preparation guide for Ion GeneStudio™ S5 Series sequencers.

The information in this guide is subject to change without notice.

iontorrent

Ion Chef™ Instrument

USER GUIDE

Maintenance, calibration, and troubleshooting

Catalog Number 4484177

Publication Number MAN0018668

Revision A.0

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

Life Technologies Holdings Pte Ltd | Block 33 | Marsiling Industrial Estate Road 3 | #07-06, Singapore 739256
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.

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Revision history: Pub. No. MAN0018668

Revision	Date	Description
A.0	21 June 2019	New user guide

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Contents

About this guide

CAUTION! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the "Safety" appendix in this document.

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

This guide is a reference for routine maintenance and calibration of the Ion Chef™ Instrument that is enabled for the user to perform. The guide is also a general reference for troubleshooting and ordering compatible reagent kits and sequencing chips, and provides instrument layout and safety information.

1 Product information

Product description

The Ion Chef™ System (Cat. No. 4484177) provides automated, high-throughput template preparation and chip loading for use with an Ion PGM™ Sequencer, Ion Proton™ Sequencer, Ion S5™ Sequencer, Ion S5™ XL Sequencer, or Ion GeneStudio™ S5 Series Sequencer. The system includes a complete set of cartridge-based consumables and reagents that enable a user to load two chips in 13–18 hours, depending on the panel and sequencing chip, with less than 15 minutes of hands-on time. The Ion Chef™ System features network integration with Torrent Suite™ Software to enable sample and reagent traceability throughout the chip preparation workflow.

The Ion Chef™ System can also be used for automated Ion AmpliSeq™ library preparation from up to 8 DNA or RNA samples per run in approximately 7 hours, with 15 minutes of hands-on time.

Software compatibility

To access the deck calibration routines described in this guide, you need to update your instrument firmware with updates in Torrent Suite™ Software 5.12 or later. For more information, see “Install a firmware update“ on page 30.

Ion Chef™ reagent kits

The following table lists the reagent kits that are available from Thermo Fisher Scientific for use with the Ion Chef™ System. The template preparation kits include reagents and supplies for both template preparation and sequencing, excluding sequencing chips. For detailed information on use, see the kit user guides listed in "Related documentation" on page 53).

Kit	Cat. No.
Library preparation	
Ion AmpliSeq™ Kit for Chef DL8	A29024
Template preparation—Ion PGM™ System	
Ion PGM™ Hi-Q™ View Chef Kit	A29902
Ion PGM™ Hi-Q™ View Chef 400 Kit	A30798
Template preparation—Ion Proton™ System	
Ion PI™ Hi-Q™ Chef Kit	A27198
Template preparation—Ion GeneStudio™ S5 Systems	
Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef	A34461/A34019
Ion 520™ & Ion 530™ ExT Kit – Chef	A30670
Ion 540™ Kit – Chef	A30011
Ion 550™ Kit – Chef	A34541
Ion 550™ Single Chip Supplemental Kit	A36953

Compatible Ion Torrent™ sequencing chips

The following table lists the sequencing chip kits available from Thermo Fisher Scientific that are compatible with the Ion Chef™ System. Store all chips at 15°C–30°C.

Sequencing platform	Kit	Quantity	Cat. No.
Ion PGM™ System	Ion 314™ Chip Kit v2 BC	8 chips	4488144
	Ion 316™ Chip Kit v2 BC	4 chips	4488145
		8 chips	4488149
	Ion 318™ Chip Kit v2 BC	4 chips	4488146
		8 chips	4488150
Ion Proton™ System	Ion PI™ Chip Kit v3	8 chips	A26771
Ion GeneStudio™ S5 Systems Ion S5™/Ion S5™ XL Systems	Ion 510™ Chip Kit	8 chips	A34292
	Ion 520™ Chip Kit	8 chips	A27762
	Ion 530™ Chip Kit	8 chips	A27764
	Ion 540™ Chip Kit	8 chips	A27766
	Ion 550™ Chip Kit	4 chips	A34537
		8 chips	A34538

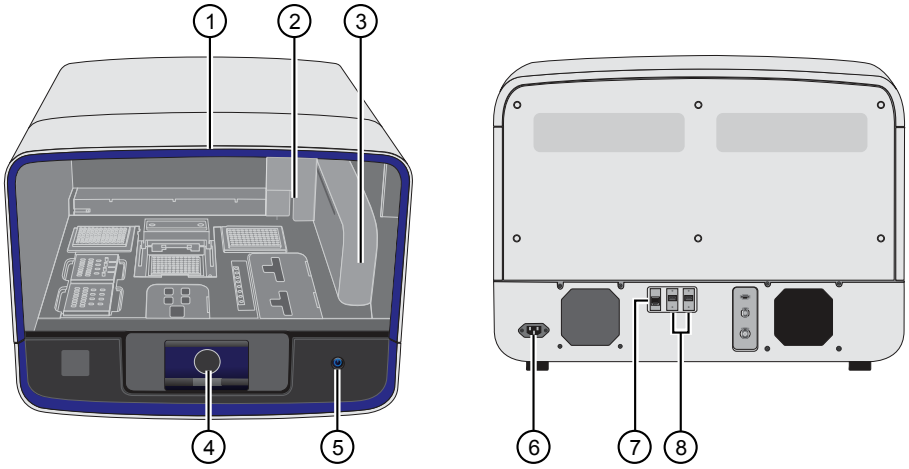
Ion Chef™ Calibration Supplies Kit

The Ion Chef™ Calibration Supplies Kit (Cat. No. A34301) has enough components to perform ten XYΘ calibrations and ten Z calibrations (reuse of the Ion Chef™ Tip Cartridge required).

Call out #	Description	Part No.	Quantity	Storage
1	Ion Chef™ PCR Plate	4483352	10	15°C–30°C
2	Black tip, 1 mL	INS1011428	96	
3	Ion Chef™ Recovery Tube	INS1011324	12	
4	Pierce tool	INS1010975	10	
5	Ion Chef™ Tip Cartridge and Tub	INS1011431 INS1008924	2 each	

Ion Chef™ Instrument components

The following figure illustrates the major external and internal features of the Ion Chef™ Instrument.



① **Door** – Provides access to the interior of the instrument. The door is locked in the closed position during operation.

② **Micropipettor** – A mechanical positive-displacement pipettor that performs all fluid transfers during sample and chip preparation.

③ **Robotic arm** – Enables fluid transfer by the Micropipettor. The arm also contains an optical sensor that reads the barcodes of instrument reagents and consumables.

④ **Touchscreen** – Provides access to all instrument functions for operation, maintenance, and troubleshooting.

⑤ **Power button** – Power switch for the Ion Chef™ Instrument, where the states are on (illuminated) and off.

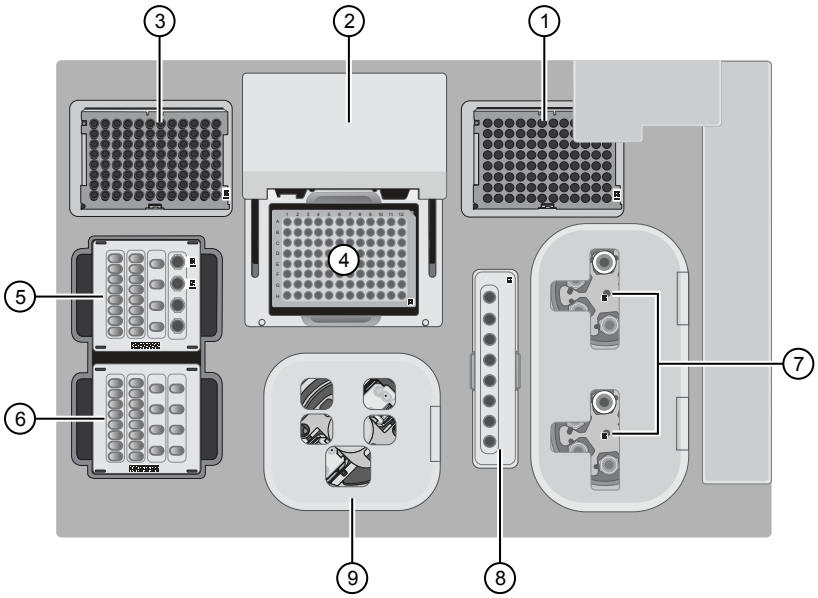
⑥ **Power port** – A 100–240 VAC port that provides power to the Ion Chef™ Instrument.

⑦ **Ethernet port** – An RJ45 port that provides Ethernet (100 Mbit) communication with the Ion Chef™ Instrument.

⑧ **USB port** – Provides USB communication with the Ion Chef™ Instrument. Used to update the instrument firmware and to transfer data during service or maintenance.

Interior hardware and consumables

The following figure illustrates the interior of the Ion Chef™ Instrument and describes the stations involved in the preparation of sequencing chips.



① **Waste pipette tip rack** – The position of the rack containing waste (used) pipette tips.

② **Automated heated cover** – Transfers the plate cover to the PCR reaction plate within the sample block. During thermal cycling, the heated cover applies compression to seal the reaction plate and heats the cover to prevent condensation.

③ **New pipette tips** – The position of the rack containing unused pipette tips.

④ **Thermal cycler sample block** – Performs thermal cycling of the sequencing reactions on a 96-well PCR reaction plate.

⑤ **Reagents station** – The position on the instrument deck of the diluted libraries, NaOH, and the Ion Chef™ Reagents cartridge.

⑥ **Solutions station** – The position on the instrument deck of the Ion Chef™ Solutions cartridge, which is maintained at room temperature.

⑦ **Recovery centrifuges** – Twin stations that perform centrifugation of the Ion Sphere™ Particles (ISPs) during the recovery phase of template preparation.

⑧ **Enrichment station** – The position of the rack containing consumables for the enrichment of the template-positive ISPs.

⑨ **Chip-loading centrifuge** – Performs centrifugation of sequencing chips that have been mounted to chip-loading adapters and loaded with template-positive ISPs.

2 Maintain and calibrate the Ion Chef™ Instrument

About the Ion Chef™ System touchscreen interface

The Ion Chef™ System features a simple interface for loading chips, cleaning the instrument, and performing system maintenance and configuration tasks.

① **Set up run** button – Set up the Ion Chef™ template preparation and chip-loading routine. Choose **Step by Step** to have the instrument lead you stepwise through installation of reagents and consumables, or choose **Quick Start** to proceed if you have already installed the consumables.

② **Open Door** button.

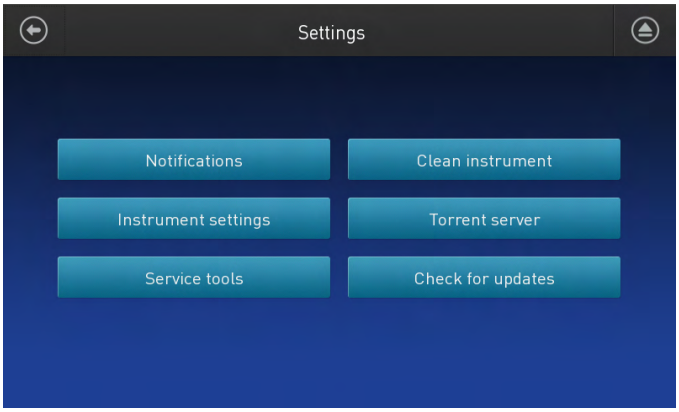
③ **Notifications** button – View notifications about instrument status during and between runs.

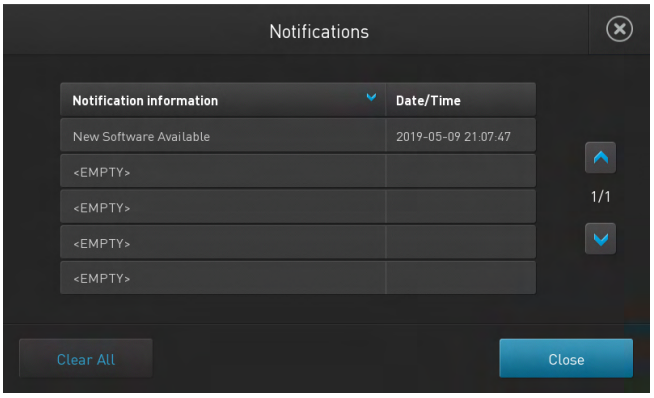
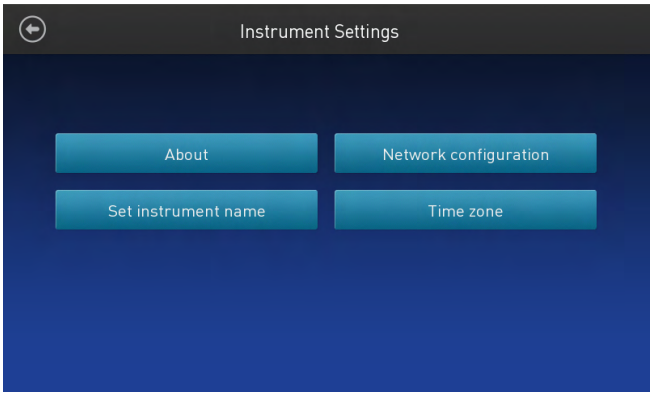
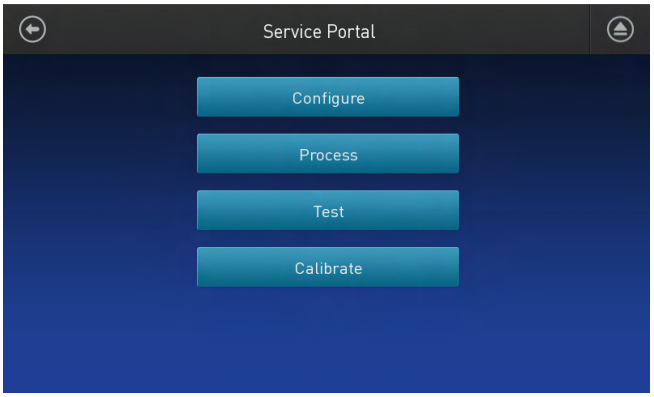
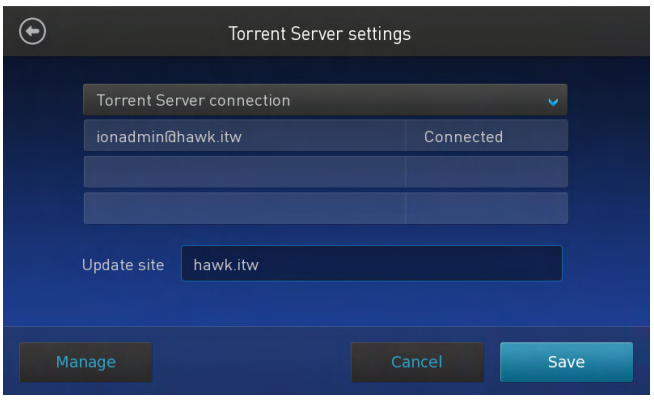
④ **Quick Start** button – Proceed directly to the Quick Start instrument setup mode. User verifies the loading of a new pipette tip cartridge and an empty pipette tip rack to hold waste tips generated during the run, before proceeding to Deck Scan.

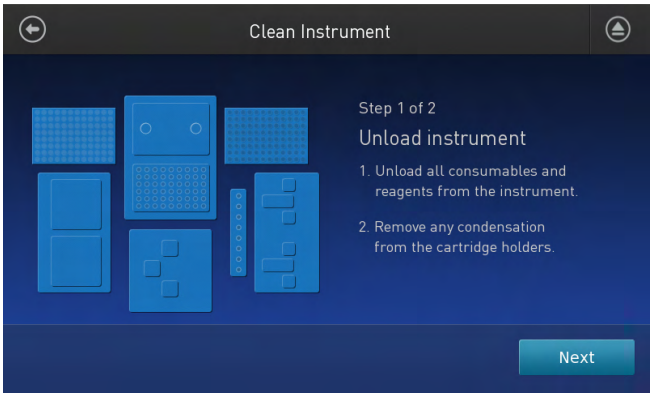
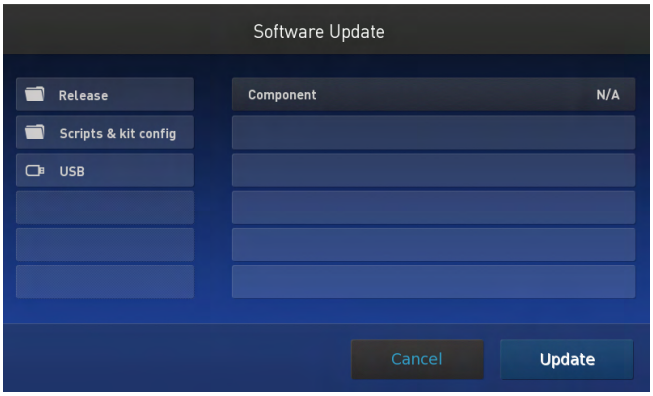
⑤ **Settings** button – Advance to the **Settings** screen with instrument configuration and maintenance options.

Settings screen

From the Ion Chef™ **Settings** screen you can access instrument settings and configuration screens, and maintenance and calibration procedures.



Item	Settings screen	When/How to use
Notifications		View notifications about instrument status during and between runs. **Note:** The **Notifications** screen is the same screen that opens after clicking **Notifications** in the lower left corner of the home screen.
Instrument settings		View current settings and network configuration, set instrument name, adjust time zone. For more information on setting a new instrument name, see "Change the instrument name" on page 16. For more information on configuring Ion Chef™ network connections, see the *Ion Chef™ Instrument Network Connection User Guide* (Pub. No. MAN0013444).
Service tools		Access screens for service-related maintenance and instrument diagnostics. Users can perform deck calibrations after updating instrument firmware in Torrent Suite™ Software 5.12. For more information on performing XYΘ and Z calibrations, see "Run the XYΘ calibration" on page 17 and "Run the Z calibration" on page 21.
Torrent server		Add and manage Ion Torrent™ Server connections. For more information on configuring Ion Chef™ and Ion Torrent™ Server connections, see the *Ion Chef™ Instrument Network Connection User Guide* (Pub. No. MAN0013444).

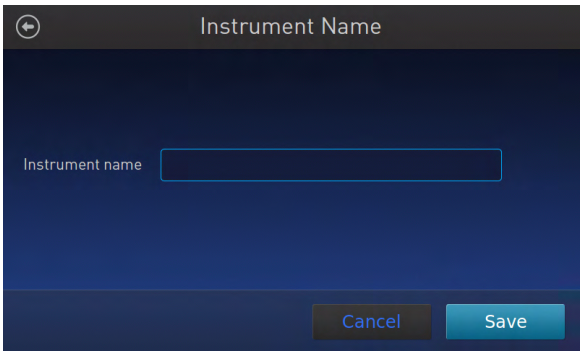
Item	Settings screen	When/How to use
Clean instrument		Proceed directly to the instrument cleaning routine. For more information on running the cleaning routine, see "Clean the Ion Chef™ System" on page 25.
Check for updates		Check availability of system software updates, and update system. For more information on updating instrument firmware, see "Install a firmware update" on page 30.

Change the instrument name

The following procedure describes how to change the name that is used to identify the Ion Chef™ Instrument both on the network and in the data that it generates.

1. In the Ion Chef™ Instrument home screen, tap **Settings**.
2. In the **Settings** screen, tap **Instrument settings**.
3. In the **Instrument Settings** screen, tap **Set Instrument Name**. Tap in the **Instrument name** field, enter a new instrument name using the keypad that appears, then tap **Save**.

   Note: Use only alphanumeric characters. Do not use special characters or spaces in the name.



4. In the next screen, tap **Save** again. Power off and on the Ion Chef™ Instrument to effect the name change.

Run the XYΘ calibration

The XYΘ calibration finds the precise location of the Z pump head (tip mandrel) relative to each consumable cartridge and a home position for the three centrifuges. To complete these tasks, the XYΘ calibration routine performs a series of X and Y position measurements, and it performs visual checks using the camera and the black dots.

Run XYΘ and Z calibrations if you see low chip loading or sample to sample variability problems that are not related to library input. We recommend running the XYΘ and Z calibrations after moving the instrument.

Note: Users can only perform this deck calibration after updating the Ion Chef™ firmware using Torrent Suite™ Software 5.12 or later.

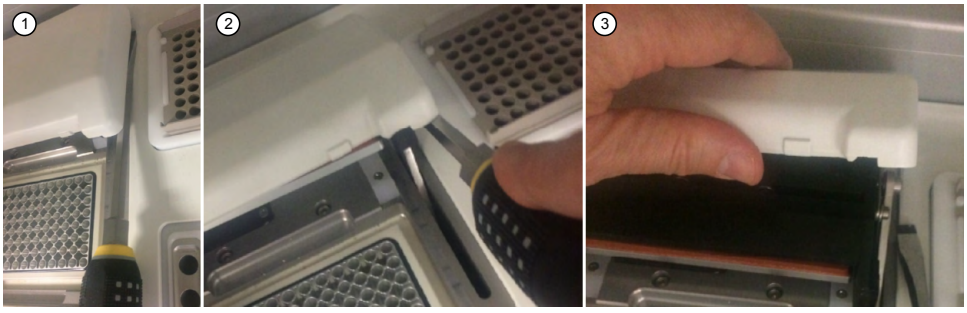
Required materials

Cat. No.	Description
A34301	Ion Chef™ Calibration Supplies Kit (see page 9) XYΘ calibration requires these components from the kit: • Ion Chef™ PCR Plate • Ion Chef™ Tip Cartridge (2) • Pierce tool

① Ion Chef™ PCR Plates
② 1-mL black tips
③ Ion Chef™ Recovery Tubes
④ Pierce tools
⑤ Ion Chef™ Tip Cartridge

Set up the instrument

1. Power on the instrument, then wait for it to come to idle.
2. Open the instrument door.
3. Remove any consumables that may be loaded in the instrument.
4. Remove the PCR lid cover.



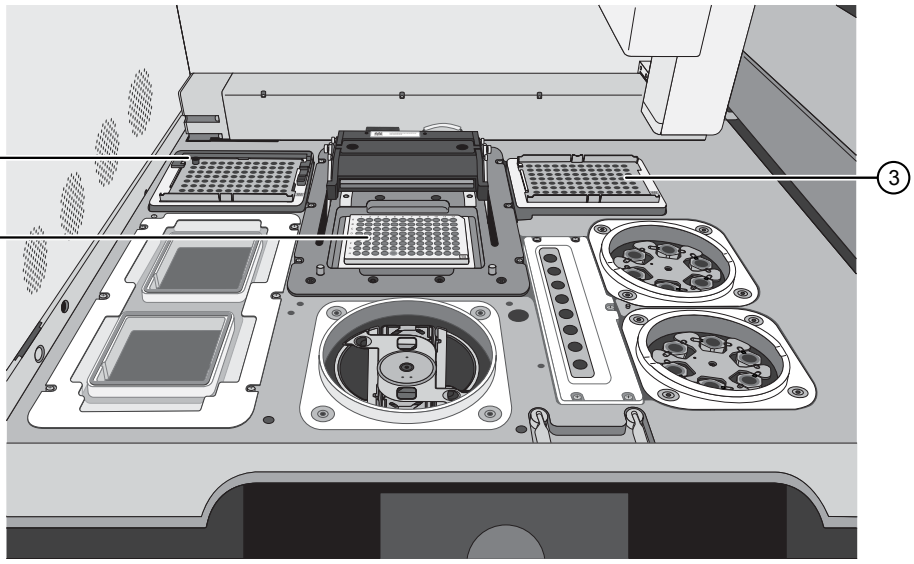
① Using a flat bladed screwdriver with a shaft that is at least 4 inches (100 mm) long, slide the blade under one side of the PCR plate cover.
② Lift up and pry off the PCR lid cover.
③ Remove the lid cover.

5. After removing the PCR lid cover, lift the deck plate at the front corners to detach the deck plate from deck magnets that hold it in place. Using two hands, carefully lift the deck plate above the deck and thermal cycler, then remove the deck plate from the instrument.

IMPORTANT! Be careful not to damage the PCR ribbon cable.

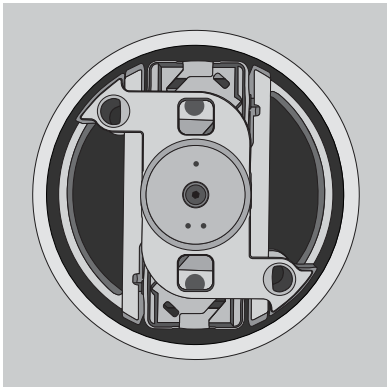
6. Install one new Pierce tool in the upper left position (A1) of an Ion Chef™ Tip Cartridge.
7. Load the deck.

Call out #	Deck position	Item
1	New pipette tips (left rear)	Ion Chef™ Tip Cartridge with Pierce tool in position A1
2	Thermal cycler sample block	Ion Chef™ PCR Plate
3	Waste pipette tip rack (right rear)	Empty Ion Chef™ Tip Cartridge



① Ion Chef™ Tip Cartridge with Pierce tool in position A1
② Ion Chef™ PCR Plate
③ Empty Ion Chef™ Tip Cartridge

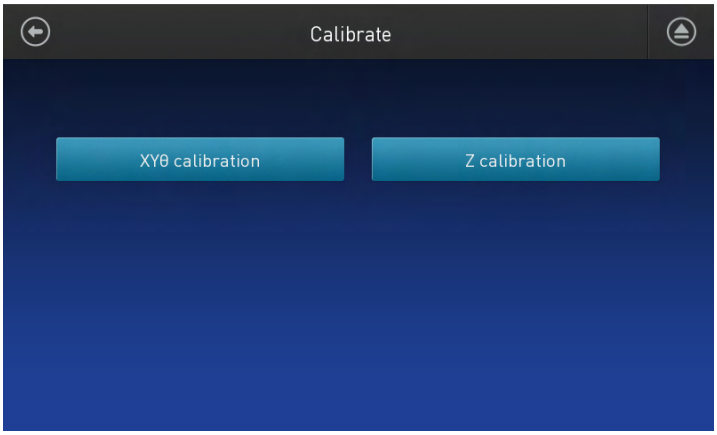
IMPORTANT! Ensure that the Chip-loading centrifuge is in the locked position with the bucket 2 position oriented toward the front of the deck.



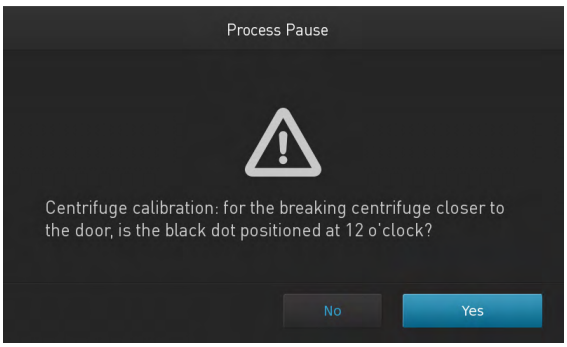
8. Close the instrument door.

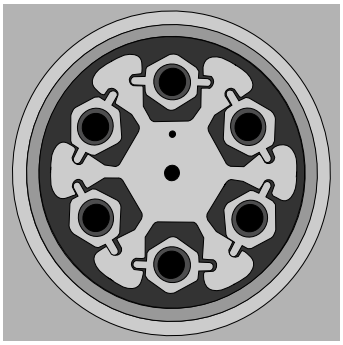
Run the XYΘ calibration

1. In the main menu, tap **Settings** ▸ **Service tools** ▸ **Calibrate**.
2. Tap **XYΘ Calibration**, then tap **Start** to start the calibration.



3. Follow the screen instructions to complete the calibration. The calibration takes approximately 20–25 minutes to complete.
4. Toward the end of the calibration run, a **Process Pause** screen appears. You are prompted to confirm the rotor position of each Recovery centrifuge (also called a breaking centrifuge).
   - If the black dot on the rotor in question is positioned at 12 o'clock, tap **Yes**. The calibration continues to the next stage.
   - If the black dot is *not* positioned at 12 o'clock, tap **No**. The calibration fails. Repeat the calibration from the beginning.





Rotor position with the black dot at the 12 o'clock position.

5. After the XYΘ calibration completes, tap **Done** to return to the main menu.

6. Remove, then discard the Pierce tool.

   Note: Reuse the tip cartridges and PCR plate in the Z calibration.

7. Reinstall the deck plate and PCR lid cover.

- If the calibration fails, follow the error message instructions to correct the problem, then run the calibration again.
- If the calibration fails a second time, contact Technical Support.

Proceed to "Run the Z calibration". Always run a Z calibration following an XYΘ calibration.

Run the Z calibration

The Z calibration routine uses eight 1-mL tips to conduct eight repeats on some calibration locations. Results are calculated based on these repeats.

Note: Run the Z calibration with the PCR lid cover and deck plate installed.

IMPORTANT! Always run a Z calibration after performing an XYΘ calibration.

Required materials

Cat. No.	Description
A34301	Ion Chef™ Calibration Supplies Kit (see page 9) Z calibration requires these components from the kit: • Ion Chef™ PCR Plate • Ion Chef™ Tip Cartridge (2) • 1-mL black tips (8) • Ion Chef™ Recovery Tubes (2)

① Ion Chef™ PCR Plates
② 1-mL black tips
③ Ion Chef™ Recovery Tubes
④ Pierce tools
⑤ Ion Chef™ Tip Cartridge

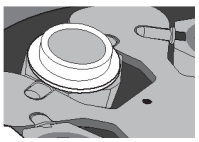
Set up the instrument

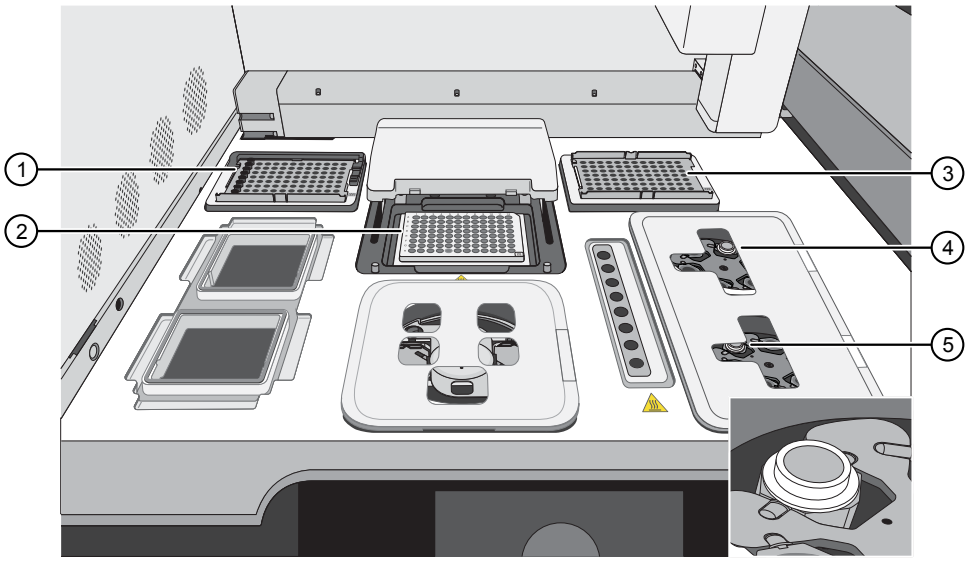
IMPORTANT! Before you run the Z calibration, note the following:

- Always use NEW, UNUSED 1-mL black tips.
- Ensure that no consumables are loaded in the instrument before setting up a Z calibration run. The Z calibration will fail and potentially cause major hardware damage if the calibration is performed with liquid in reagent strips.

1. Open the instrument door.
2. From the Ion Chef™ Calibration Supplies Kit, install at least 8 NEW 1-mL black tips in the left column (positions A1 to H1) of an Ion Chef™ Tip Cartridge.
3. Load the deck:

Call out #	Deck position	Item
1	New pipette tips (left rear)	Ion Chef™ Tip Cartridge with 8 NEW UNUSED 1-mL black tips loaded in the left column (A1 to H1)
2	Thermal cycler sample block	Ion Chef™ PCR Plate
3	Waste pipette tip rack (right rear)	Empty Ion Chef™ Tip Cartridge

Call out #	Deck position	Item
4	Recovery centrifuge (rear)	One Ion Chef™ Recovery Tube in the bucket position nearest to the black dot on the rotor
5	Recovery centrifuge (front)	

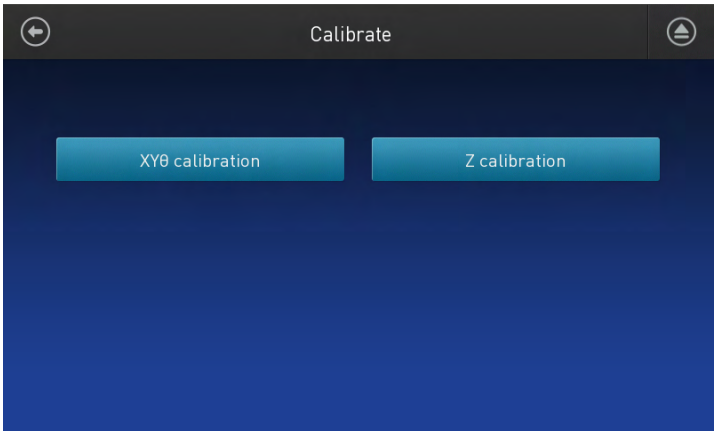


① Ion Chef™ Tip Cartridge with eight 1-mL black tips loaded in the left column (A1 to H1)
② Ion Chef™ PCR Plate
③ Empty Ion Chef™ Tip Cartridge
④ One Ion Chef™ Recovery Tube in the bucket position nearest to the black dot on the rotor (see figure inset)
⑤ One Ion Chef™ Recovery Tube in the bucket position nearest to the black dot on the rotor (see figure inset)

4. Close the instrument door.

Run the Z calibration

1. In the main menu, tap **Settings** ▸ **Service Tools** ▸ **Calibrate**.
2. Tap **Z calibration**, then tap **Start** to start the calibration.



3. Follow the screen instructions to complete the calibration.
4. After the Z calibration completes, tap **Done** to return to the main menu.
5. Unload the following consumables from the deck.
   - Ion Chef™ Recovery Tubes
   - Ion Chef™ PCR Plate
   - Ion Chef™ Tip Cartridges and tips.

   Dispose of the used PCR plate and tips, but save the tip cartridges and recovery tubes for reuse.

- If the calibration fails, follow the error message instructions to correct the problem, then run the calibration again.

  IMPORTANT! Do not reuse the 1-mL black tips after a failed calibration. Use only new tips.

- If the calibration fails a second time, contact Technical Support.

Clean the Ion Chef™ System

About the cleaning protocol

The Ion Chef™ System has an automated cleaning function that must be performed after every run. The cleaning routine is started from the instrument touchscreen and is designed to minimize potential contamination. During the routine, the instrument irradiates the deck with ultraviolet light for one minute after all consumables have been removed from the instrument.

Cleaning can be started by tapping **Clean instrument** in the **Settings** screen at any time.

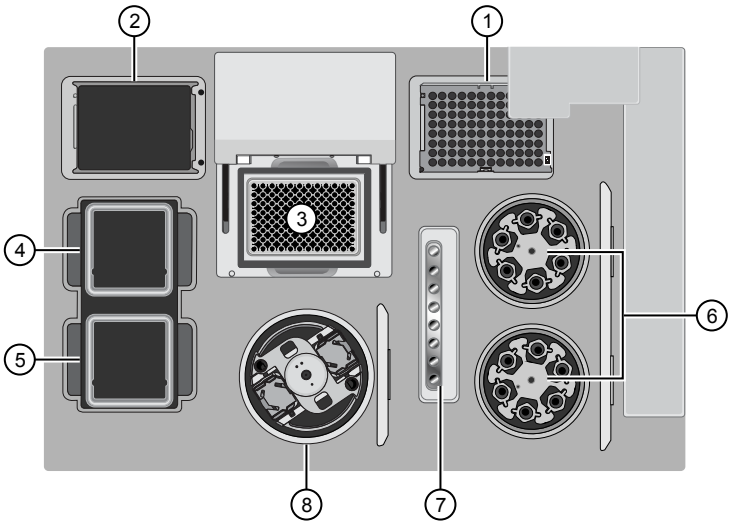
IMPORTANT! Although the cleaning routine provides some protection against contamination, it is not a substitute for good laboratory technique or precautions. When preparing DNA libraries or when preparing the Ion Chef™ Instrument, ensure that you always observe sterile laboratory procedures to ensure minimal contamination.

Materials required

- Gloves, powder-free nitrile
- Isopropanol, 70% solution
- Wipes, lint-free

Clean the Ion Chef™ Instrument

IMPORTANT! Clean the Ion Chef™ Instrument as described in the following pages after every run. To prevent contamination, do not operate the instrument unless it has been recently cleaned.



Ion Chef™ Instrument stations

1. Waste pipette tip position
2. Empty Tip Cartridge v2: move to waste pipette tip station
3. Thermal cycler sample block
4. Reagents station
5. Solutions station
6. Recovery centrifuges
7. Enrichment station
8. Chip-loading centrifuge

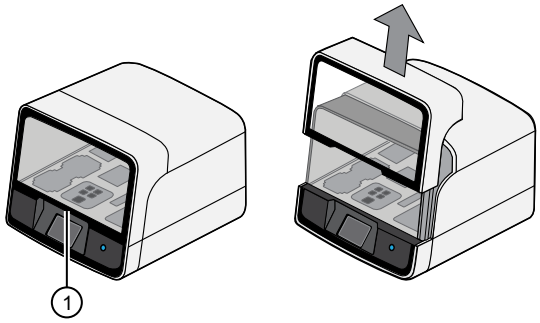
Remove and dispose of used consumables

Follow these steps if used consumables are loaded on the instrument deck. If consumables from the previous run have already been removed, proceed to "Inspect and clean the Recovery centrifuges and buckets" on page 27.

IMPORTANT!

- Do not discard the empty Tip Cartridge v2.
- Make sure to transfer the QC samples before you remove and discard the Reagents cartridge.

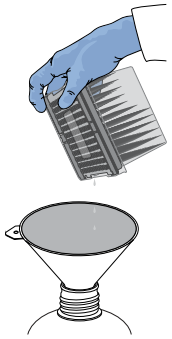
1. Tap ⏏ **(Open Door)** in the instrument touchscreen, then wait for the latch to open.
2. Lift the instrument door to the top of the travel until the latch mechanism engages.



① Hold here, then lift

3. Remove, then discard the PCR Plate from the thermal cycler sample block.
4. Remove, then discard the box of used pipette tips from the waste tip position.

IMPORTANT! Handle the disposable reservoir in the waste tip position with care. During the run, liquid waste collects in the reservoir. Dispose of the liquid waste by tipping the reservoir on one corner and pouring the waste into an appropriate waste container:



IMPORTANT! Do not reuse the waste pipette tip rack. Always move the empty Tip Cartridge v2 from the new tip position to the waste tip position.

5. Move the empty Tip Cartridge v2 to the waste tip position.

6. Remove, then discard the following cartridges.
   - Reagents cartridge
   - Solutions cartridge
   - Enrichment Cartridge v2

7. Remove, then discard the following consumables from the Recovery centrifuges.
   - Recovery Station Disposable Lid v2
   - Recovery Tubes v2

8. Close the Chip-loading centrifuge cover.

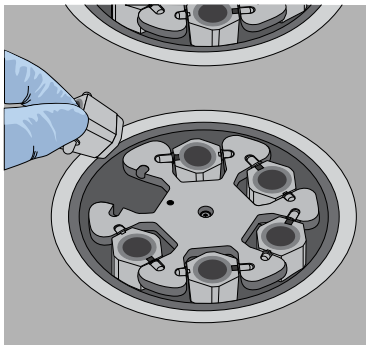
Inspect and clean the Recovery centrifuges and buckets

1. Inspect the Recovery centrifuges, then clean the components if excess liquid is present.

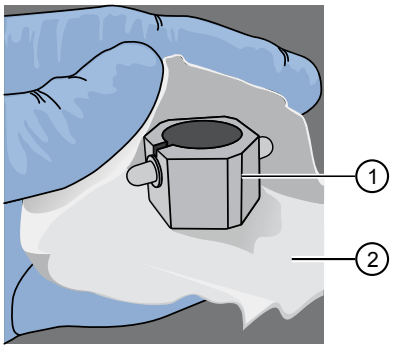
Is liquid present?	Action
No	Proceed to "Start the cleaning" on page 28.
Yes	Clean the centrifuge bowl and buckets as described below. **IMPORTANT!** Clean the Recovery centrifuges occasionally, only when excess liquid is noticeable in the bowl and/or buckets. You do *not* need to clean the centrifuges after every run.

IMPORTANT! Wear powder-free, nitrile gloves when cleaning the Recovery centrifuge.

2. Remove the buckets from the Recovery centrifuges. Clean the inside and outside of each bucket using a lint-free wipe, then place the buckets on a clean, dry surface while you clean the centrifuge.

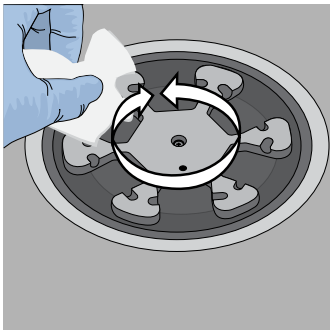


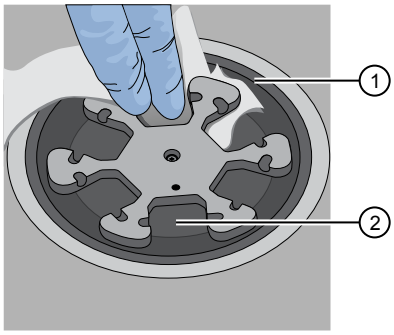
① Bucket



② Lint-free wipe

3. Use lint-free wipes to remove all fluid from inside the centrifuge bowl.





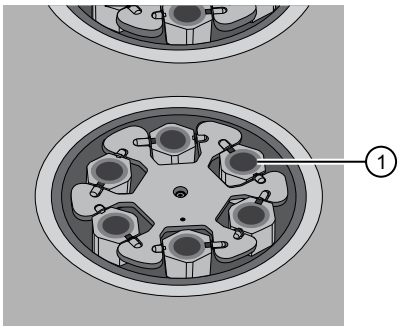
① Inside rim of the centrifuge

② Bottom of the centrifuge bowls

4. Use lint-free wipes treated with 70% isopropanol to clean the following parts.
   - Inside rim of the centrifuge.
   - Bottom of the centrifuge bowl.
   - Outside and inside of the centrifuge buckets.

5. Dry the centrifuge and buckets with lint-free wipes.

6. Install the centrifuge buckets, then close the Recovery centrifuge cover.



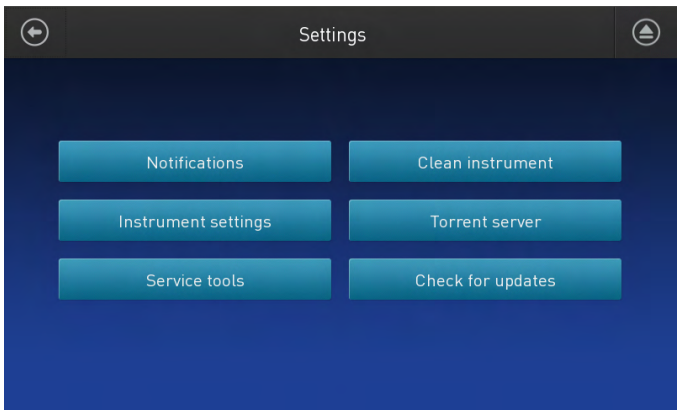
① Buckets (cleaned and installed)

Start the cleaning

1. Close the instrument door by first lifting it up slightly to disengage the locking mechanism, then pushing down on the door until the locks engage.

IMPORTANT! Before closing the door, ensure the covers of the Chip-loading and Recovery centrifuges are closed.

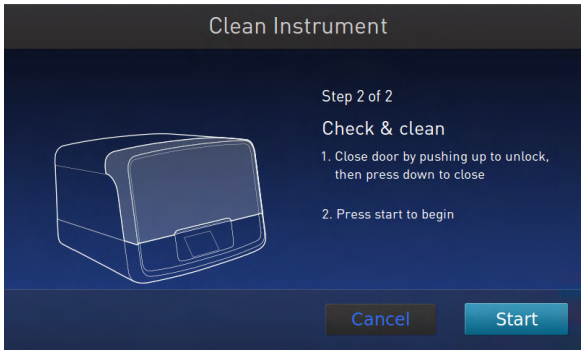
2. To start the cleaning, tap **Settings** ▸ **Clean instrument**.



Note: You can also clean the instrument after a run by tapping **Next** in the home touchscreen.

3. Confirm that you have removed all consumables from the Ion Chef™ Instrument, except the empty pipette tip rack in the waste tip position, then tap **Next**.

4. With the door closed, tap **Start**. The instrument performs a Deck Scan before starting the cleaning routine. The Ion Chef™ Instrument stops ventilation, then illuminates the ultraviolet (UV) light in the instrument for ~1 minute.

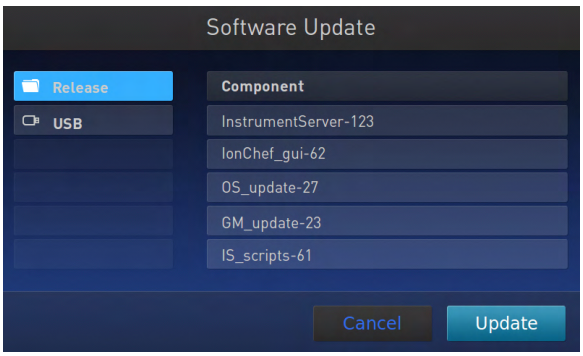


CAUTION! The Ion Chef™ Instrument emits UV light at 254 nm. Wear appropriate eye wear, protective clothing, and gloves when working near the instrument. Do not look directly at the UV light while it is illuminated during the cleaning routine.

Install a firmware update

To ensure proper operation of the Ion Chef™ Instrument, we recommend periodically confirming that your instrument is running the most current firmware. We occasionally release updates to the instrument firmware, which can include important changes to the Ion Chef™ System operation. To ensure that your instrument is running the most current firmware, check the firmware version, then update if needed.

1. On the Ion Chef™ Instrument touchscreen, tap **Settings**.
2. On the **Settings** screen, tap **Check for updates**.
3. On the **Software Update** screen, tap **Release** to search the server for updates to the Ion Chef™ Instrument firmware.



Note: Users are also notified of the availability of firmware updates on the Notifications screen.

4. Select the available component update, tap **Update**, then wait for the instrument to complete the update.

   Note: If the Ion Chef™ Instrument is operating on an isolated network and cannot connect to our website, you must transfer the firmware update manually. To perform a manual update, transfer the firmware files to a USB drive and insert the drive into the USB port at the rear of the instrument. Tap **USB** on the Software Update screen, select from the list of available software components, then tap **Update**.

   When finished, the Ion Chef™ Instrument displays the update status.

5. Power off and on the Ion Chef™ Instrument to complete the update.

IMPORTANT! If you are updating the firmware manually, remove the USB drive before powering on the Ion Chef™ Instrument.

Replace the ultraviolet bulb

Replace the ultraviolet (UV) bulb after approximately 500 hours of use.

Materials required

Item	Cat. No.
LAMP, 16W 2-PIN, G5T5 TUV	4486547

IMPORTANT! The Ion Chef™ Instrument is designed to use UV bulbs ordered from Thermo Fisher Scientific only. Replace only with same type of UV bulb.

Install a new UV bulb

IMPORTANT! Wear gloves when you handle the UV bulbs.

1. From the instrument touchscreen, tap ⏏, then open the instrument door.
2. Power off, then unplug the Ion Chef™ Instrument. Allow the instrument to cool for 15 minutes.

   WARNING! PHYSICAL INJURY HAZARD. The UV bulb in the Ion Chef™ Instrument can become hot when in use. Allow sufficient time for the bulb to cool and wear protective, powder-free gloves when handling it.

3. Firmly grasp the UV light (by the ends), twist the bulb 90 degrees, then carefully pull downward, removing from the slotted mount. Dispose of the used bulb appropriately.

   Note: The UV bulb fixture is positioned above the deck chamber at center.

① UV bulb

4. Remove the new UV bulb from its packaging. While holding the bulb nearest the ends, insert the bulb into the slotted mounts in the instrument, then rotate it until the bulb locks into place.

   IMPORTANT! Do not handle the replacement UV bulb without powder-free gloves. Oils from fingerprints can shorten lamp life.

5. Close the instrument door, then plug in and power on the Ion Chef™ Instrument.

6. When the instrument touchscreen displays the main menu, tap **Clean** to test the installation of the UV bulb by performing a cleaning routine.

   While the instrument performs the cleaning routine, confirm that the UV bulb illuminates the instrument interior, indicating a successful installation. If the UV bulb fails to illuminate, or if the instrument displays an error:

   a. Power off, then unplug the instrument.

   b. Reseat the UV bulb, then plug in and power on the instrument.

   c. Repeat the preceding test. If the UV bulb does not illuminate after reseating it several times, contact Technical Support.

A

Troubleshooting

Ion Chef™ System setup and operation

Observation	Possible cause	Recommended action
Instrument does not display the home screen when powered on	Multiple causes are possible.	1. Power off the instrument, wait 30 seconds, then power on the instrument. 2. If the instrument fails again, contact Technical Support.
The instrument door cannot be opened	An obstruction was present on or around the door mechanism.	Remove the obstruction blocking the door, then operate the instrument normally.
	A hardware or software error occurred.	Contact Technical Support to report the problem and for further assistance.
The instrument stops during a run	The instrument encountered an internal error.	1. Record the error displayed on the instrument display, then tap **OK**. 2. Contact Technical Support to report the problem and for further assistance.
	A consumable was improperly loaded.	Ensure that all consumables are loaded according to the instructions provided.
Liquid droplets are present on the deck after a run	After normal instrument operation, a noticeable collection of liquid can be observed in the cartridge compartments, or on the deck.	Before the UV cleaning step, absorb any liquid residue with a dry lint-free laboratory wipe, then clean the area with a wipe moistened with 70% isopropanol.
Liquid residue is present in the Recovery centrifuge following a run	During normal instrument operation, a noticeable coating of liquid can collect on the bowl and buckets of the Recovery centrifuge following repeated runs.	Remove the residue as instructed in "Inspect and clean the Recovery centrifuges and buckets" on page 27.

Observation	Possible cause	Recommended action
Instrument will not begin a run	The instrument has encountered a Deck Scan error (one or more consumables are absent or loaded improperly).	1. Confirm that the touchscreen does not display any Deck Scan warnings. If alarms are present, note the error(s) displayed, replace the missing consumable as directed, tap **No** when prompted then tap **Next** to cancel the run. After returning to the home screen, restart the run. 2. If the error persists, confirm that: • All buckets are seated correctly in the rotors of the Recovery and Chip-Loading centrifuges. • All cartridges are loaded correctly and are level on the instrument deck. • The barcodes of the Ion Chef™ Library Sample Tubes are visible and positioned correctly. • All tubes are both present *and* uncapped on the Reagents cartridge (Library Sample Tubes, NaOH tube, and the empty tube). 3. If the error persists after you check the consumables on the instrument deck, do one of the following: • If you are confident that the Ion Chef™ Instrument is set up correctly and you are comfortable disregarding the warnings, tap **YES** following Deck Scan to proceed with the run. • If the instrument cannot begin the run, contact Technical Support for further assistance.
	The instrument has encountered an internal error.	1. Record the error displayed on the instrument display, then touch **OK**. 2. Contact Technical Support to report the problem and for further assistance.

Observation	Possible cause	Recommended action
Instrument displays one or more alerts during a run	The instrument detected one or more problems during the run.	After the instrument completes the run, contact Technical Support. If possible, capture an image of the alert or error message to help troubleshoot. **IMPORTANT!** The detected problem might impact the performance of the sequencing run.

	• Network connection to the server was interrupted.
	• User name or password was incorrect.
		1. Tap the Instrument status button to view the alert(s).
		2. In the Instrument status screen, confirm that the name of the Ion Torrent™ Server connection is red.
		3. Contact your network administrator to confirm that:
		  • The Ion Torrent™ Server can be accessed from the network port used by the Ion Chef™ Instrument. If not, troubleshoot the network connection.
		  • The user name and password used by the Ion Chef™ Instrument are valid. If not, contact the server administrator to renew the credentials.
		4. If the alert persists, capture an image of the alert or error message, if possible, to help troubleshoot, then contact Technical Support for further assistance.

Observation	Possible cause	Recommended action
Sequencing results are variable between samples in a run **Note:** Users can sometimes see a significant difference in loading and sequencing metrics between identical samples that are prepared in the same Ion Chef™ run. A 10–20% difference in loading density using the same library is acceptable. The two samples in an Ion Chef™ run are handled independently, and due to normal variation intrinsic to the templating and chip-loading processes, these samples can exhibit different run results. Extensive testing has been done to evaluate the effect of sample position on sequencing metrics. These studies have shown no correlation between sample position and run quality.	The two libraries or barcoded library pools were not identical.	Confirm that the library type and input was the same for both samples. Different libraries can require different titration points and can behave differently on the Ion Chef™ Instrument.
		Run a control library. Confirm that the control library shows the same disparity in performance as the sample.
	The library input was not optimal.	Confirm that the library input is correct. Use the Ion Sphere™ Assay on a Qubit™ Fluorometer to evaluate templating efficiency. We recommend a percentage of templated unenriched ISPs of 15–25%.
		Increase the library input. If the library input is borderline too low, the differences in processing of the two samples can cause the recovery of one sample to be below the desired enriched ISP yield. Processing can vary with templating conditions, library types and lengths, and ISP types.
	The Ion Chef™ Instrument needs deck calibration.	If you see systematic variation that is unrelated to library input, perform the XYΘ and Z calibration routines (see "Run the XYΘ calibration" on page 17 and "Run the Z calibration" on page 21).
		If the instrument continues to show systematic differences in throughput between the two sample and chip positions after a successful deck calibration, contact Technical Support to report the problem and for further assistance.

Expected throughput for Ion Torrent™ chips

The following table provides expected sequencing throughput specifications per chip type. For further information, see the specification sheets available at **thermofisher.com** at the web addresses listed after the table.

Chip	Number of reads	Total throughput[1]	
		200-base read	400-base read
Ion 314™ Chip v2 BC	400–500 thousand	30–50 Mb	60–100 Mb
Ion 316™ Chip v2 BC	2–3 million	0.3–0.6 Gb	0.6–1 Gb
Ion 318™ Chip v2 BC	4–5.5 million	0.6–1 Gb	1.2–2 Gb
Ion 510™ Chip	2–3 million	0.3–0.5 Gb	0.6–1 Gb
Ion 520™ Chip	3–5 million	0.6–1 Gb	1.2–2 Gb
Ion 530™ Chip	15–20 million	3–4 Gb	6–8 Gb
Ion 540™ Chip	60–80 million	8–10 Gb	—
Ion PI™ Chip v3	60–80 million	8–10 Gb	—
Ion 550™ Chip	100–130 million	20–25 Gb	—

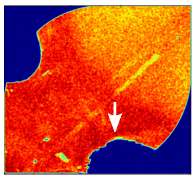
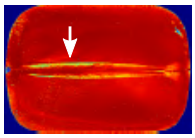
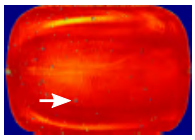
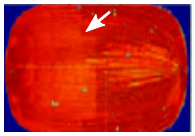
[1] Expected output with >99% aligned/measured accuracy. Output depends on read length and application.

System specification sheets

Ion PGM™ System	**https://tools.thermofisher.com/content/sfs/brochures/PGM-Specification-Sheet.pdf**
Ion Proton™ System	**http://tools.thermofisher.com/content/sfs/brochures/CO06326_Proton_Spec_Sheet_FHR.pdf**
Ion S5™ System/Ion S5™ XL System	**http://tools.thermofisher.com/content/sfs/brochures/Ion-S5-S5XL-SpecSheet.pdf**
Ion GeneStudio™ S5 Series System	**https://www.thermofisher.com/document-connect/document-connect.ion-genestudio-s5-seriesflyer.pdf**

Loading images for normal runs

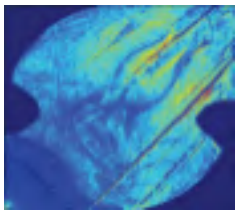
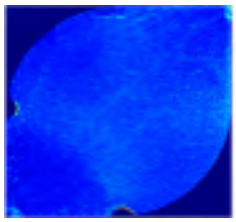
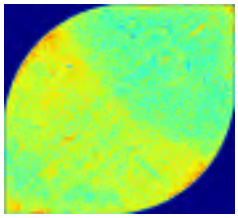
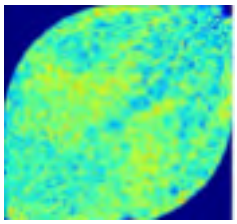
The following table shows loading images that depict typical loading phenotypes for normal runs. For each image type, causes and best practices are listed.

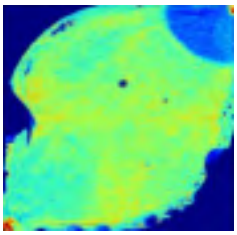
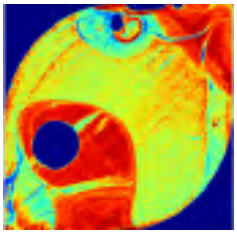
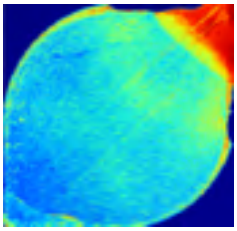
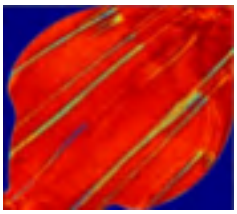
Description	Run details	Chip image	Causes and best practices
Areas of blue at the side of chip	Ion 314™ Chips; all kits		• Bubbles or debris that obstructs part of the chip is normal. This will cause a reduction in reads and useable wells. • This impact is lessened on larger chips like Ion 316™ and Ion 318™ Chips. • Ensure that you are centrifuging Ion 314™ Chips upside down for 5 seconds to empty the flowcell after the Ion Chef™ run is complete.
Low loading streaks through middle of the chip	Ion PI™/Ion 5xx series chips; all kits		• Known cosmetic issue • Bubbles introduced in loading can cause streaking throughout the chip. • Does not impact performance.
Low loading speckling throughout the chip	Ion PI™/Ion 5xx series chips		• Known cosmetic issue • Bead clumping or artifacts can cause speckles. • Does not impact performance.
Visualized blocks in loading heatmap	Ion 530™ Chips		• Known cosmetic issue • Does not impact performance.
	Ion 520™ Chips		

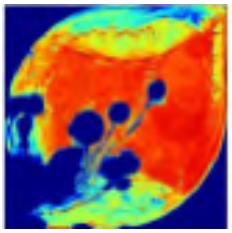
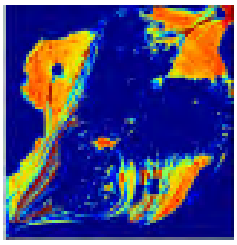
Troubleshoot common loading failure types with chip image phenotypes

The following tables show loading images for commonly seen failure types. For each type, a description and possible causes and recommended actions are listed. This information is intended to help focus troubleshooting efforts based on the loading image from the sequencing run report.

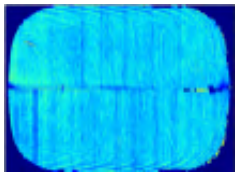
Ion PGM™ Chef runs

Observation	Possible cause	Recommended action
Poor or no loading   	Yield of template-positive Ion Sphere™ Particles (ISPs) was low, caused by • Low library input • Improper chip handling • Enrichment failure, caused, for example, by failure to remove cap of NaOH tube in Reagents cartridge before start of run	1. Review Test Fragment (TF) metrics. • If TFs are missing, or count or signal is low: – There was a problem with recovery. – Chips were handled improperly. • If TFs are present and have expected key signal and quality: – Check percentage of unenriched template-positive ISPs using the Ion Sphere™ Assay with a Qubit™ Fluorometer: <10% unenriched template-positive ISPs indicates library input was too low. – Requantify library concentration and repeat library preparation, if needed. 2. Repeat the Ion Chef™ templating run after verifying library quality, and/or remaking library, to rule out user error or a consumable problem. 3. If low library input and user error are ruled out, perform XYΘ and Z calibrations on the Ion Chef™ Instrument. 4. If the problem continues, contact Technical Support to report the problem and for further assistance.
Punctate yellow or blue dots, or uniform teal loading 	No or insufficient polymerase or primer was added to the sample.	1. Repeat the Ion Chef™ templating run to rule out user error, or a consumable or sequencer problem. 2. If failure repeats, run a control library to rule out a library preparation problem. 3. If the problem continues, contact Technical Support to report the problem and for further assistance.

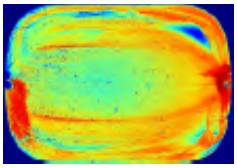
Observation	Possible cause	Recommended action
Low loading area upper right corner of chip 	Chip was installed in the Ion Chef™ Instrument in the incorrect orientation.	• See the kit user guide for proper chip installation instructions. • Place the adapter/chip assemblies into centrifuge buckets so that the chip barcode aligns above the white outline that is imprinted on the floor of the bucket.
Variable, low, or uneven loading  	Liquid was left in chip.	• See the kit user guide for proper chip handling after the run completes. Remove the chip and adapter from the Chip-loading centrifuge promptly. • If there is visible liquid in the Chip Adapter ports, it can be withdrawn with a pipette before removing the adapter from the chip. • To remove an adapter, hold the chip with the tab facing downward, then remove Chip Adapter the starting with the top hinge first. • If using Ion 314™ Chips, centrifuge upside down for 5 seconds after removing the adapter.
High loading or high polyclonality 	Too many ISPs were loaded.	1. Requantify library concentration and perform a QC check for percentage of template-positive ISPs in unenriched ISP samples using the Ion Sphere™ Assay with a Qubit™ Fluorometer: >30% template-positive ISPs indicates that library input is too high. 2. Repeat the Ion Chef™ templating run after verifying library concentration, and reduce library input.

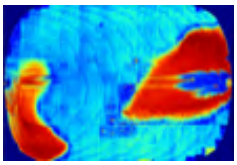
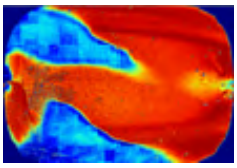
Observation	Possible cause	Recommended action
Bubbles  	• The Wash 2 Bottle had a leaky seal. • The sequencer waste line was clogged. • There was an internal line leak in the sequencer.	Contact Technical Support for service on the Ion PGM™ Sequencer.

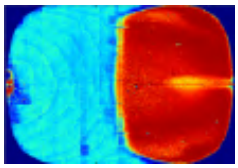
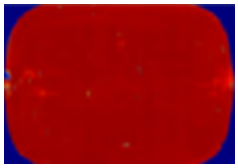
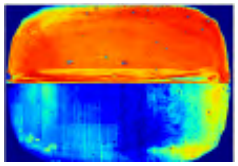
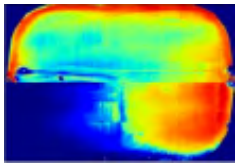
Ion Proton™, Ion S5™/Ion S5™ XL, and Ion GeneStudio™ S5 Chef runs

Observation	Possible cause	Recommended action
No loading (blue chip) 	Yield of template-positive Ion Sphere™ Particles (ISPs) was low, caused by • Low library input • Enrichment failure, caused, for example, by failure to remove cap of NaOH tube in Reagents cartridge before start of run	1. Review Test Fragment (TF) metrics. • If TFs are missing, or count or signal is low, there may be a problem with consumables, ISP enrichment, or recovery on the Ion Chef™ Instrument or with the sequencer. • If TFs are present and have expected key signal and quality: – Check percentage of unenriched template-positive ISPs using the Ion Sphere™ Assay with a Qubit™ Fluorometer: <10% unenriched template-positive ISPs indicates that library input was too low. – Requantify library concentration and repeat library preparation, if needed. 2. Repeat the Ion Chef™ templating run after verifying library quality, and/or remaking library, to rule out user error or a consumable problem. 3. If the problem continues, contact Technical Support.

Observation	Possible cause	Recommended action

Low loading
 
	• The Chip Adapter was improperly installed.
	• The Chip Adapter was defective.
	• The yield of template-positive ISPs was low due to low library input.
		1. Review Test Fragment (TF) metrics.
		  • If TFs are missing, or count or signal is low, there may be a problem with consumables, ISP enrichment, or recovery on the Ion Chef™ Instrument or with the sequencer.
		  • If TFs are present and have expected key signal and quality:
		  – Check percentage of unenriched template-positive ISPs using the Ion Sphere™ Assay with a Qubit™ Fluorometer: <10% unenriched template-positive ISPs indicates that library input was too low.
		  – Requantify library concentration and repeat library preparation, if needed.
		2. Carefully inspect Chip Adapters for defective gaskets and presence of foreign material before use.
		3. Repeat the Ion Chef™ templating run after verifying library quality, and/or remaking library, to rule out user error or a consumable problem.
		4. If low library input and user error are ruled out, perform XYΘ and Z calibrations on the Ion Chef™ Instrument.
		5. If the problem continues, contact Technical Support to report the problem and for further assistance.

Uneven loading—Ion S5™ chips  	Loading was uneven due to a defective Ion S5™ Chip Adapter.	Carefully inspect Chip Adapters for defective gaskets and presence of foreign material before use.

Observation	Possible cause	Recommended action
Poor loading on the left side of the chip 	The sample volume that was loaded in the chip was low.	Contact Technical Support to report the problem and for further assistance.
High loading or high polyclonality 	Too many ISPs were loaded.	1. Requantify library concentration and perform a QC check for percentage of template-positive ISPs in unenriched ISP samples using the Ion Sphere™ Assay with a Qubit™ Fluorometer: >25% template-positive ISPs indicates that library input is too high. 2. Repeat the Ion Chef™ templating run after verifying library concentration, and reduce library input.
Missing chip blocks, blocky heat map  	• There was a chip problem. • The sequencer has a pogo pin or chip clamp problem.	• Save the chip for possible diagnosis by support team. • Contact Technical Support to report the problem and for further assistance.

Ion Chef™ Instrument user issues

The following is a list of common errors that are made by Ion Chef™ users.

- Tip Cartridge locking bracket is not closed securely before the start of a run.
  - The Tip Cartridge can be dislodged during a run if it is not closed securely.
  - The lever of the locking bracket can interfere with pipette arm and cause run to abort or complete with chips with no ISP loading.
- Cap of NaOH tube in Position C of Reagents cartridge has not been not removed before the start of a run.

  Deck scan should detect a capped tube and report it to the user. If this is does not happen, the following failure modes are possible:
  - Ion Chef™ run aborts—no samples created
  - Ion Chef™ run completes but chips do not have any ISPs (Melt off solution for enrichment is not created; can have bent 1000-uL pipette tip in waste location or on deck).
- Recovery tubes have not been fully seated in buckets.
  - During a run, pipette can dislodge bucket causing centrifuge fault, and causing a run to abort.
- Recovery centrifuge lids have not been snapped into place firmly.
  - Pipette can lift centrifuge lid, causing the lid to interfere with downstream pipette movement and causing a run to abort.
- The Ion Chef™ Instrument is power-cycled before a firmware update is complete.
  - Files can become corrupted and Ion Chef™ Instrument may not run, or may run an incorrect program.
- User does not recognize that the door spring is damaged.
  - Door cannot open during QC pause or at end of timed run, causing a run to abort.
- The door is incompletely latched.
  - Ion Chef™ Instrument will not proceed. The stall sometimes occurs at the QC pause.

B

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words:

- **CAUTION!** – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!** – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!** – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

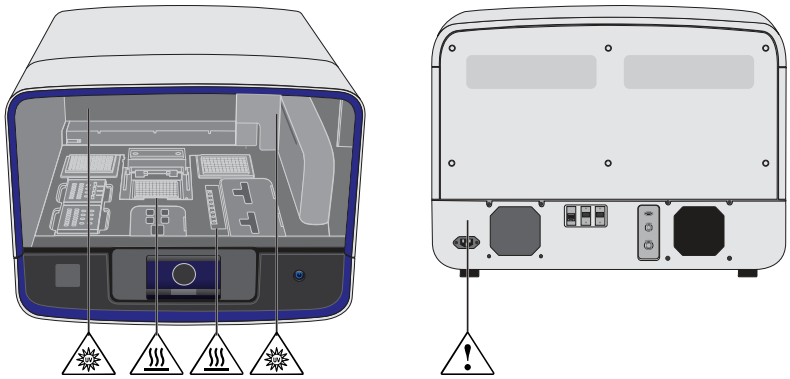
Symbol	English	Français
	Caution, risk of danger Consult the manual for further safety information.	Attention, risque de danger Consulter le manuel pour d'autres renseignements de sécurité.
	Moving parts	Parties mobiles
	Caution, hot surface	Attention, surface chaude

Symbol	English	Français
	Ultraviolet light	Rayonnement ultraviolet
	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)
	Do not dispose of this product in unsorted municipal waste  **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.	Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif.  **MISE EN GARDE !** Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable.

Conformity symbols

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with European Union Low Voltage Directive 2006/95/EC, EMC Directive 2014/30/EU, and R&TTE Directive 1999/5/EC.
	Indicates conformity with Australian standards for electromagnetic compatibility.

Location of safety labels on this instrument



Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

CAUTION! Solvents and Pressurized fluids. Wear eye protection when working with any pressurized fluids. Use caution when working with any polymeric tubing that is under pressure:

- Extinguish any nearby flames if you use flammable solvents.
- Do not use polymeric tubing that has been severely stressed or kinked.
- Do not use polymeric tubing with tetrahydrofuran or nitric and sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause polymeric tubing to swell and greatly reduce the rupture pressure of the tubing.
- Be aware that high solvent flow rates (~40mL/min) may cause a static charge to build up on the surface of the tubing and electrical sparks may result.

Physical injury

CAUTION! Moving and Lifting Injury. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:

- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time. Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.

CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Electrical safety

WARNING! Fuse Installation. Before installing the instrument, verify that the fuses are properly installed and the fuse voltage matches the supply voltage. Replace fuses only with the type and rating specified for the unit. Improper fuses can damage the instrument wiring system and cause a fire.

WARNING! Voltage Selector Switch. Before installing the instrument, verify that the voltage selector switch is set for the supply voltage. This will prevent damage to the instrument, reduce risk of fire, and enable proper operation.

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.

WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

Cleaning and decontamination

CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods that are specified in the manufacturer user documentation. It is the responsibility of the operator (or other responsible person) to ensure that the following requirements are met:

- No decontamination or cleaning agents are used that can react with parts of the equipment or with material that is contained in the equipment. Use of such agents could cause a HAZARD condition.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) before the instrument is serviced at your facility or is sent for repair, maintenance, trade-in, disposal, or termination of a loan. Request decontamination forms from customer service.
- Before using any cleaning or decontamination methods (except methods that are recommended by the manufacturer), confirm with the manufacturer that the proposed method will not damage the equipment.

Ultraviolet (UV) Safety

The Ion Chef™ System uses a UV lamp which emits light at 254 nm. Under normal operating conditions, the UV lamp is powered on when performing the cleaning protocol. Safety interlocks are used to ensure that the UV lamp is not powered when the door is open.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety compliance

Reference	Description
EU Directive 2006/95/EC	European Union "Low Voltage Directive"
IEC 61010-1 EN 61010-1 UL 61010-1 CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-010 EN 61010-2-010	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials*
IEC/EN 61010-2-020	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-020: Particular requirements for laboratory centrifuges*
IEC 61010-2-081 EN 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*

EMC

Reference	Description
Directive 2014/30/EU	*European Union "EMC Directive"*
FCC Part 15	*U.S. Standard "Industrial, Scientific, and Medical Equipment"*
AS/NZS 2064	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-001, Issue 3	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*
US FCC CFR Title 47 Part 15.225, Subpart C	*Operation within the band 13.110–14.010 MHz.*
Industry Canada RSS 210, Issue 8, Annex 2 EN 302 291-1/2 V1.1.1	*Licence-Exempt Radio Apparatus: Category I Equipment*

Environmental design

Reference	Description
Directive 2012/19/EU	European Union "WEEE Directive" – Waste electrical and electronic equipment
Directive 2011/65/EU	European Union "RoHS Directive" – Restriction of hazardous substances in electrical and electronic equipment

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

The following Ion Chef™ reagent kit user guides and quick references, and related guides are available for download on the instrument and kit product pages at **thermofisher.com**.

Document	Publication number
Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide	MAN0013432
Ion AmpliSeq™ Library Preparation on the Ion Chef™ System Quick Reference	MAN0013433
Ion PGM™ Hi-Q™ View Chef Kits User Guide	MAN0014571
Ion PGM™ Hi-Q™ View Chef Kits Quick Reference	MAN0014572
Ion PI™ Hi-Q™ Chef Kit User Guide	MAN0010967
Ion PI™ Hi-Q™ Chef Kit Quick Reference	MAN0010968
Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef User Guide	MAN0016854
Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef Quick Reference	MAN0016855
Ion 520™ & Ion 530™ ExT Kit – Chef User Guide	MAN0015805
Ion 520™ & Ion 530™ ExT Kit – Chef Quick Reference	MAN0015806
Ion 540™ Kit – Chef User Guide	MAN0010851
Ion 540™ Kit – Chef Quick Reference	MAN0014119
Ion 550™ Kit – Chef User Guide	MAN0017275
Ion 550™ Kit – Chef Quick Reference	MAN0017276
Ion Chef™ System Site Preparation Guide	MAN0007956
Ion Chef™ Instrument Network Connection User Guide	MAN0013444
Ion Sphere™ Quality Control Kit User Guide	MAN0017531

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

invitrogen

Qubit fluorometers and assays

Accurate, specific, and sensitive quantification of DNA, RNA, and protein samples

ThermoFisher SCIENTIFIC

Qubit fluorometers

Intuitive user interface coupled with accurate measurements

Invitrogen™ Qubit™ 4 and Qubit™ Flex fluorometers are benchtop microvolume fluorometers designed to accurately measure DNA, RNA, or protein quantity. Whether you are an expert or a novice, the easy-to-use touchscreen menus make it easy to perform assays, with accurate and reliable results displayed in just a few seconds. Both instruments provide flexible data exportation using a USB drive, Wi-Fi cloud connectivity, or direct USB cable connection so your quantification data is easily accessed.

Figure 1. Qubit Flex and Qubit 4 fluorometers with intuitive touchscreens and applications for specific assays.

Key benefits of Qubit fluorometers

- **High sensitivity**—more sensitive than UV absorbance–based quantification
- **Accuracy and speed**—accurately quantifies DNA, RNA, or protein in less than 3 seconds
- **Ideal for precious samples**—requires as little as 1 µL of sample
- **Optimized reagents and tubes**—Invitrogen™ Qubit™ reagents and assay tubes work best with Qubit fluorometers

Qubit fluorescence technology

Qubit fluorometers and assay kits are designed to measure the intensity of the signal from fluorescent dyes bound to specific biological molecules. These optimized dyes bind selectively to DNA, RNA, or protein and only emit a fluorescent signal when bound to the target.

Qubit fluorometers use specialized curve-fitting algorithms to develop a calibration curve using standard samples with a known concentration. An unknown sample concentration of DNA, RNA, or protein is calculated by comparing the relative fluorescence units (RFUs) of the sample to the RFUs of the standards used in calibration. The detection limits of the measurements are specific to each assay.

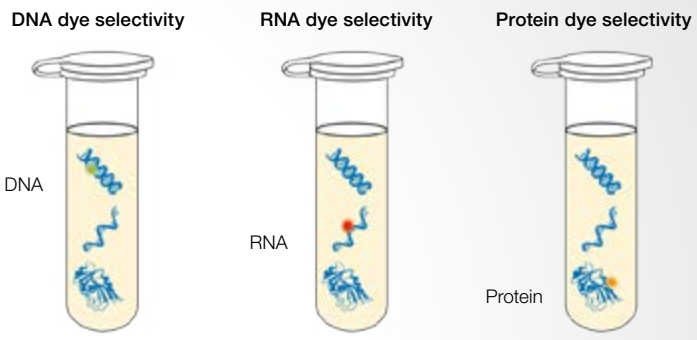


Figure 2. Fluorescent dyes selectively bind to DNA, RNA, or protein. Dyes only emit signal when bound to the target.

Convenient, easy-to-use onboard calculators

Reagent calculator

The reagent calculator conveniently calculates how much working solution to prepare based on the number of samples to quantify. Available in both the Qubit 4 and Qubit Flex fluorometer models.

Assay range calculator

The assay range calculator displays the core sample concentration range based on the sample volume, as well as the extended low and high ranges. This aids in the selection of the appropriate Qubit assay for the most accurate quantification based on your sample volume and estimated sample concentration. This calculator is only available with the Qubit Flex Fluorometer.

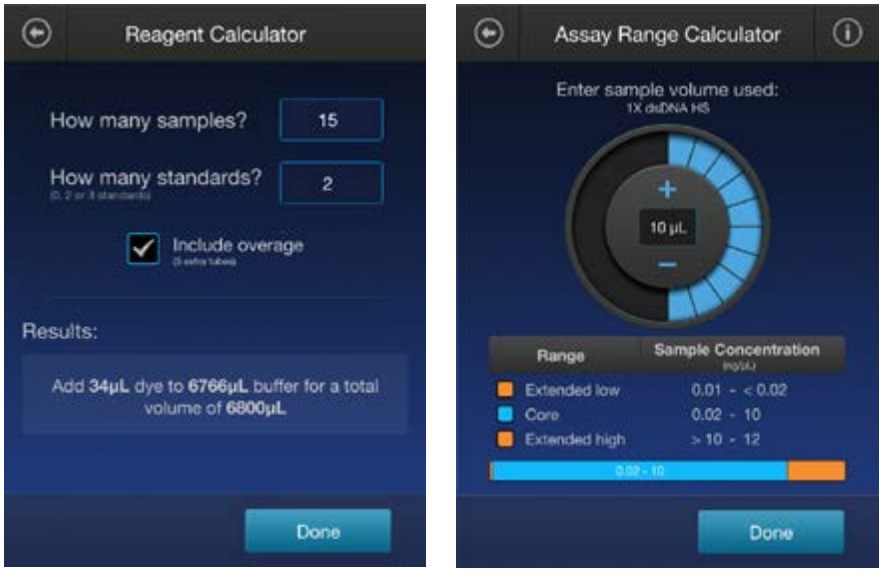


Figure 3. Reagent and assay range calculators. Easily make working solutions for all assays that are not in 1X format using the reagent calculator. The assay range calculator aids in the determination of sample volume requirements based on required accuracy.

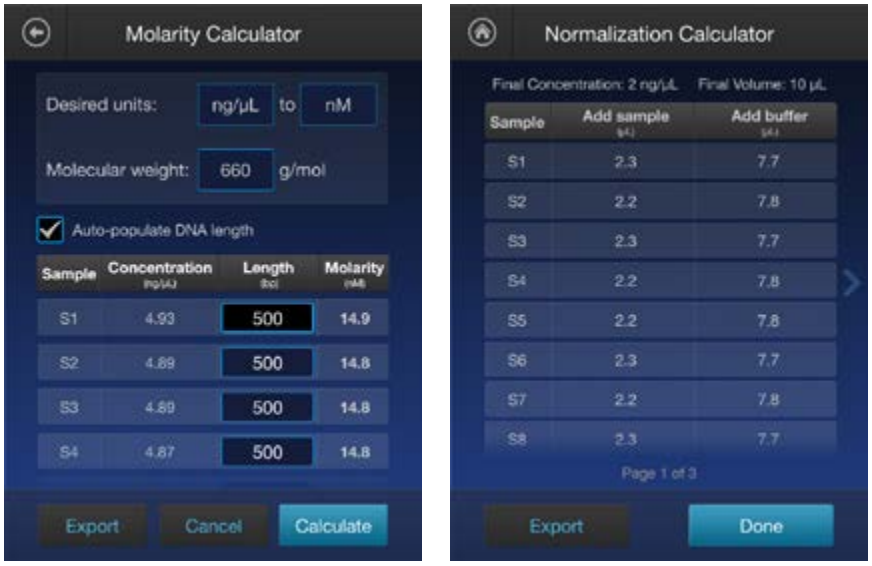


Figure 4. Integrated post-results molarity and normalization calculators. Use the molarity calculator to convert values to molarity based on nucleic acid length. Use the normalization calculator to determine how to dilute the samples to the same concentration.

Calculators for next-generation sequencing (NGS) workflows

Molarity calculator

Quickly calculate the molarity of your samples based on nucleic acid length and the measured concentration. The molarity calculator is only available on the Qubit Flex Fluorometer.

Normalization calculator

Easily normalize to a desired mass, concentration, or molarity with the normalization calculator. This replaces spreadsheet calculations for standard normalization during library preparation for sequencing. The normalization calculator is only available on the Qubit Flex Fluorometer.

Personalized workflows

Envision and create custom assays for the Qubit 4 Fluorometer

MyQubit functionality brings your favorite fluorescence assays right to your benchtop, providing a reliable platform for many quantitation needs—from laboratory research and quality control to process monitoring and beyond. Any fluorescent reagent or assay that is spectrally compatible with the Qubit hardware can be adapted for use with the Qubit 4 Fluorometer.

Compare Qubit fluorometers

	Qubit 4 Fluorometer	Qubit Flex Fluorometer
Sample throughput	1 sample in 3 seconds	1 to 8 samples in 3 seconds
User interface	5.7 in. color touchscreen	8 in. color touchscreen
Onboard calculators	Reagent calculator	Reagent calculator Assay range calculator Molarity calculator Normalization calculator
Informs where the sample concentration resides within the assay range	Provides quantification data for samples that are within the core and the extended range of the standard curve. Sample concentrations that are out of range are not given a measurement.	
System check	Qubit 4 System Verification Assay Kit	Qubit Flex System Verification Assay Kit
Fluorometer mode	Yes	No
Programable open format	Yes—MyQubit	No
Instrument footprint (W x L x H)	13.6 x 25 x 5.5 cm 5.4 x 10 x 2.2 in.	1.86 x 28.2 x 10.3 cm 7.3 x 11.1 x 4.1 in.
Sample data storage	1,000 samples	10,000 samples
Data export	Wi-Fi USB drive Direct to computer via USB or ethernet cable	Wi-Fi USB drive Direct to computer via USB or ethernet cable
Light sources	Blue LED (peak ~470 nm), Red LED (peak ~635 nm)	Blue LED (peak ~460–480 nm), Red LED (peak ~620–640 nm)
Excitation filters	Blue LED (430–495 nm), Red LED (600–645 nm)	Blue LED (456–484 nm), Red LED (612–644 nm)
Emission filters	Green (510–580 nm), Red (665–720 nm)	Green (513–563 nm), Far-red (671–693 nm)

Qubit assays

Qubit assays are designed to work with Qubit fluorometers. Common contaminants such as salts, free nucleotides, RNA, solvents, detergents, and proteins are well tolerated in Qubit assays.

Qubit 4 and Qubit Flex kits for system verification

The Invitrogen™ Qubit™ 4 System Verification Assay Kit and the Invitrogen™ Qubit™ Flex System Verification Assay Kit are fast, easy-to-use, reagent-based assays that test the performance of Qubit fluorometers. Each kit consists of three components: a blank reagent solution, a green fluorescent reagent, and a far-red fluorescent reagent. Paired with a hardware functionality test, the assay is designed to test the internal components of the instrument to help ensure proper functionality.

Qubit RNA quantification assays

There are three RNA assay kits, which offer differing detection ranges, and one microRNA assay kit:

- **Invitrogen™ Qubit™ RNA HS Assay Kit**—high sensitivity
- **Invitrogen™ Qubit™ RNA BR Assay Kit**—broad range
- **Invitrogen™ Qubit™ RNA XR Assay Kit**—extended range
- **Invitrogen™ Qubit™ microRNA Assay Kit**—highly selective for miRNA over rRNA or large mRNA (>1,000 bp)

The RNA assays are accurate for initial sample concentrations from as little as 250 pg/µL to 10,000 ng/µL. These kits are highly selective for RNA over dsDNA. Unlike other RNA assays, they do not require DNase if DNA is present in the sample for an accurate measurement.

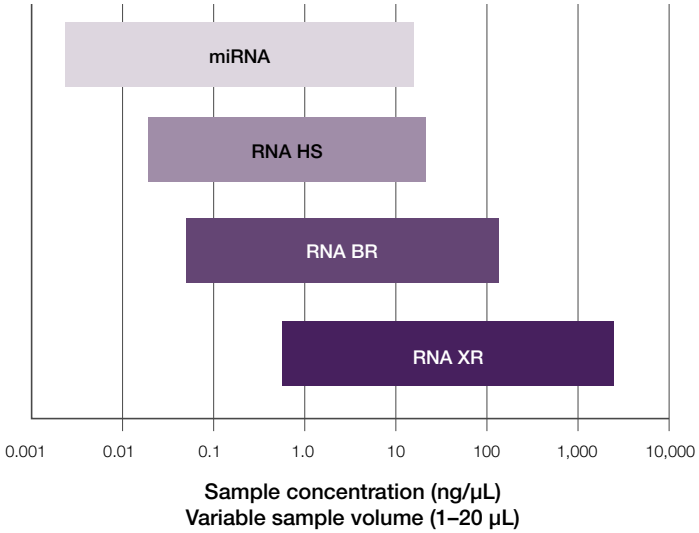


Figure 5. Quantification ranges of Qubit RNA assay kits.

Qubit RNA integrity and quality (IQ) assay

The Invitrogen™ Qubit™ RNA IQ Assay was developed to quickly assess the quality and integrity of an RNA sample. This assay allows assessment of RNA quality at a lower cost and with an easier, simpler, and faster workflow than other solutions currently on the market.

The Qubit RNA IQ Assay utilizes two unique dyes—one that binds to large, intact, and/or structured RNA, and another that selectively binds to small, degraded RNA. Together, they are able to quickly assess the quality and integrity of an RNA sample. To use, simply add your samples to the Qubit RNA IQ working solution, then measure on the Qubit 4 or Qubit Flex Fluorometer.

Results are presented as a total value for the RNA sample integrity and quality, or RNA IQ number, and as the calculated percentage of large and small RNA in the sample. The RNA IQ number is based on a scale of 1 to 10, wherein a high IQ number indicates the majority of the sample consists of large and/or structured RNA. Conversely, a small IQ number indicates the sample comprises mainly small RNA with limited tertiary structure.

Figure 6. A proprietary algorithm is used to report a quality score representative of the ratio of small and large and/or structured RNA in the sample.

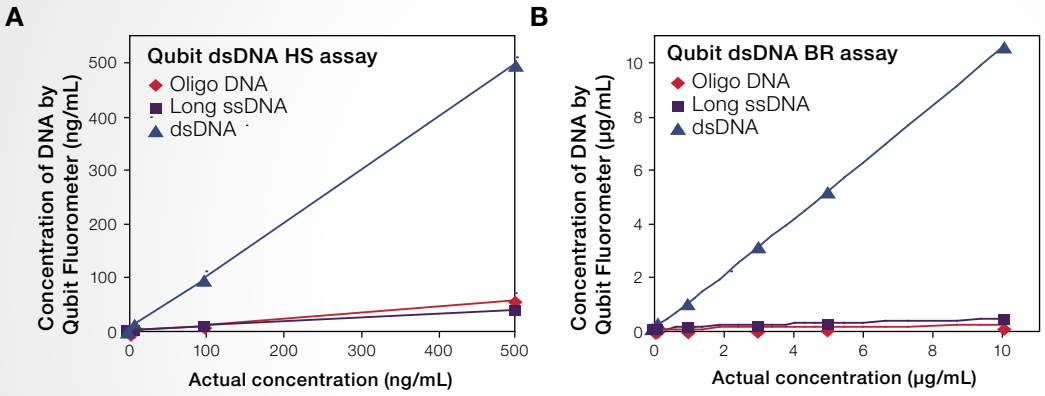
Qubit DNA quantification assays

Invitrogen™ Qubit™ DNA assay kits are broadly categorized as double-stranded DNA (dsDNA) assays or single-stranded DNA (ssDNA) assays.

Qubit dsDNA assay kits—available in two detection ranges and two formats

Detection ranges: high-sensitivity and broad-range assays

- The Invitrogen™ Qubit™ dsDNA High-Sensitivity (HS) Assay Kit is for samples with a low concentration of dsDNA, making it ideal for precious samples. It has a detection range between 0.1 and 120 ng.
- The Invitrogen™ Qubit™ dsDNA Broad-Range (BR) Assay Kit is ideal for a broad range of DNA concentrations and applications. It can detect between 4 and 4,000 ng.



Use high-sensitivity (HS) assays for low concentrations and broad-range (BR) assays for high concentrations of dsDNA

Figure 7. Detection of double-stranded DNA by the Qubit dsDNA HS (A) and BR (B) assay kits. Duplicate samples of long ssDNA, oligo DNA, or lambda dsDNA at concentrations of 0.5 to 500 ng/mL in the assay tube were quantified using the Qubit dsDNA HS assay, and at concentrations of 0.01 to 10 µg/mL in the assay tube using the Qubit dsDNA BR assay according to kit protocols.

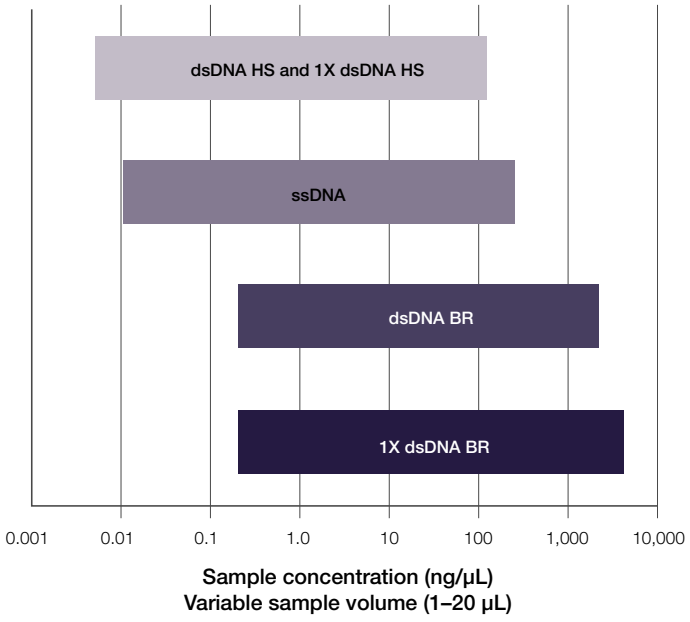


Figure 8. Quantification ranges of Qubit DNA assay kits.

Formats: standard assay and 1X assay

- Invitrogen™ Qubit™ standard assays require same-day mixing of the buffer with the reagent to create the working solution prior to preparing standards and samples for quantification.
- Invitrogen™ Qubit™ 1X assays eliminate the step of preparing the working solution.
  - The Invitrogen™ Qubit™ 1X dsDNA HS Assay Kit provides the same dynamic range and limit of detection as the standard assay, while the Invitrogen™ Qubit™ 1X dsDNA BR Assay Kit has a wider dynamic range than the standard assay, achieving 4,000 ng/µL in the extended range.
  - This format offers a simplified workflow while reducing the tubes in the kit, therefore reducing the amount of plastic used.
  - Simply add your sample or standard to the premixed solution, incubate, and read your results.

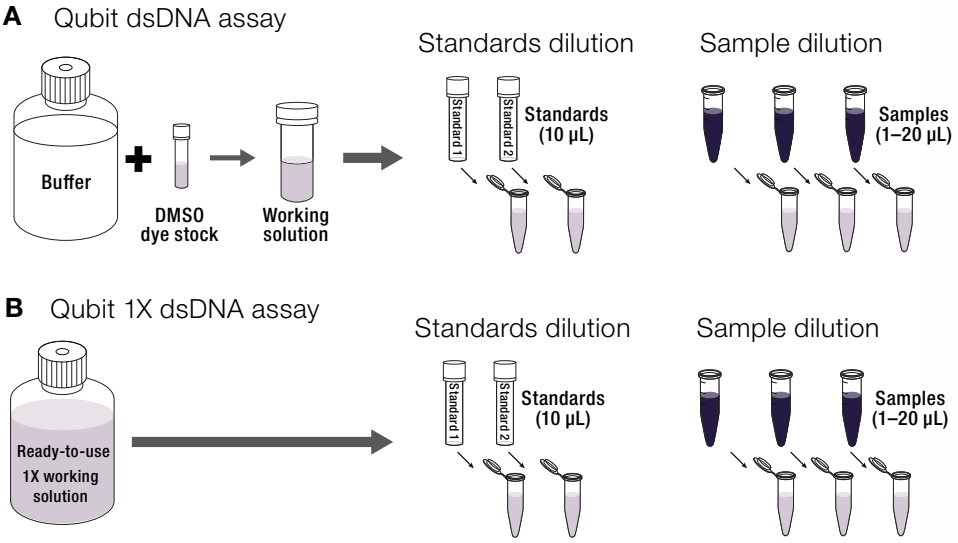


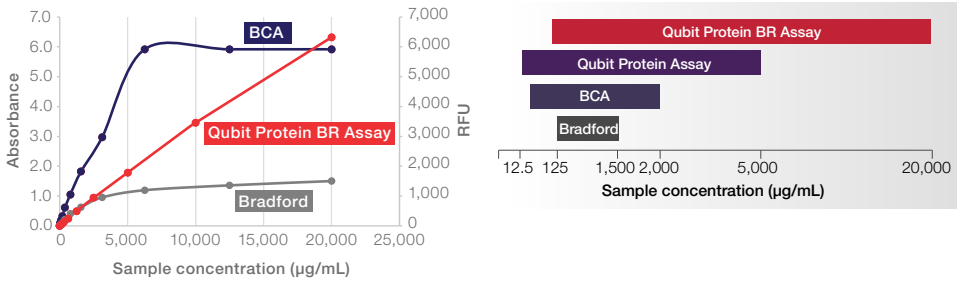
Figure 9. Workflow comparison for the (A) Qubit dsDNA and (B) Qubit 1X dsDNA assays. Standard Qubit dsDNA High Sensitivity (HS) and Qubit dsDNA Broad Range (BR) assay kits include a fluorogenic dye, buffer, and dsDNA standards. Prior to each assay, a fresh aqueous working solution needs to be prepared by diluting the dye stock in the provided buffer in a 1:200 ratio. Qubit 1X dsDNA assay kits eliminate this step by providing a ready-to-use working solution.

Qubit ssDNA and oligos quantification assays

The Invitrogen™ Qubit™ ssDNA Assay Kit is ideal for quantifying single-stranded DNA or oligonucleotides. It is accurate for initial sample concentrations from 50 pg/µL to 200 ng/µL, providing an assay range of 1–200 ng.

Qubit protein quantification assays

Invitrogen™ Qubit™ protein assay kits are designed to make protein quantification easy and fast. The assays provide low protein variability, rapid quantitation, and high sensitivity. Common contaminants, such as reducing reagents (DTT, β-mercaptoethanol), salts, free nucleotides, amino acids, solvents, DNA, and detergents (Invitrogen™ Qubit™ Protein BR Assay only), are well tolerated in the assays. The assays' wide dynamic ranges make it easy to determine the concentrations of a wide range of samples compared to standard colorimetric protein assays.



	Qubit Protein BR Assay	Qubit Protein Assay
Platform	Qubit 4	Qubit 4, Qubit Flex
Compatibility	Detergents, reducing agents	Reducing agents
Quantitation range	100 µg/mL to 20 mg/mL	12.5 µg/mL to 5 mg/mL

Figure 10. Quantification ranges of protein assays.

Need higher throughput for your nucleic acid or protein samples?

Qubit assays are ideal when the number of samples you measure at one time is low enough not to warrant a microplate reader. With larger sample batches requiring a fluorescence microplate reader, use Invitrogen™ Quant-iT™ assay kits and reagents, which are designed to use with microplate readers for nucleic acid or protein quantification.

Learn more about Quant-iT assays at **thermofisher.com/quantit**

Learn more about microplate readers at **thermofisher.com/platereaders**

Frequently asked questions

Q. I already have a Thermo Scientific™ NanoDrop™ instrument; why should I use a Qubit fluorometer?

A. NanoDrop instruments use UV absorbance to measure DNA and RNA concentrations. Absorbance-based measurements have limitations in distinguishing between DNA, RNA, and free nucleotides, which absorb at 260 nm.

Qubit assays are fluorescence-based. They are designed to only quantify the target analyte. Additionally, fluorescence-based nucleic acid quantification provides a more sensitive dynamic range than absorbance-based instruments.

When used with Qubit assays, Qubit fluorometers can accurately measure low concentrations of sample, while NanoDrop spectrophotometers can detect the presence of common contaminants.

Q. Do I have to use new standards every time?

A. For each assay, you have the choice to run a new calibration or to use the values from the previous calibration. As you first use the instrument, perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, you can determine the level of comfort you have using the calibration data stored from the last time the instrument was calibrated. We do recommend running a new calibration curve every time you prepare a new working solution.

Q. Is there a difference in signal between supercoiled and relaxed plasmid DNA when using a Qubit fluorometer?

A. Yes, we have seen a 20–30% difference. For the different forms of plasmid DNA, we recommend using a standard that more closely represents the composition of the plasmid DNA in the sample.

Q. Does the Qubit Protein Assay or Qubit Protein BR Assay work well in the presence of detergents?

A. The Qubit Protein BR Assay is compatible with samples that contain up to 5% detergents. The Qubit Protein Assay is not recommended if detergent is present.

Q. Why are some of the instrument setting menu options not available on my Qubit 4 or Qubit Flex device?

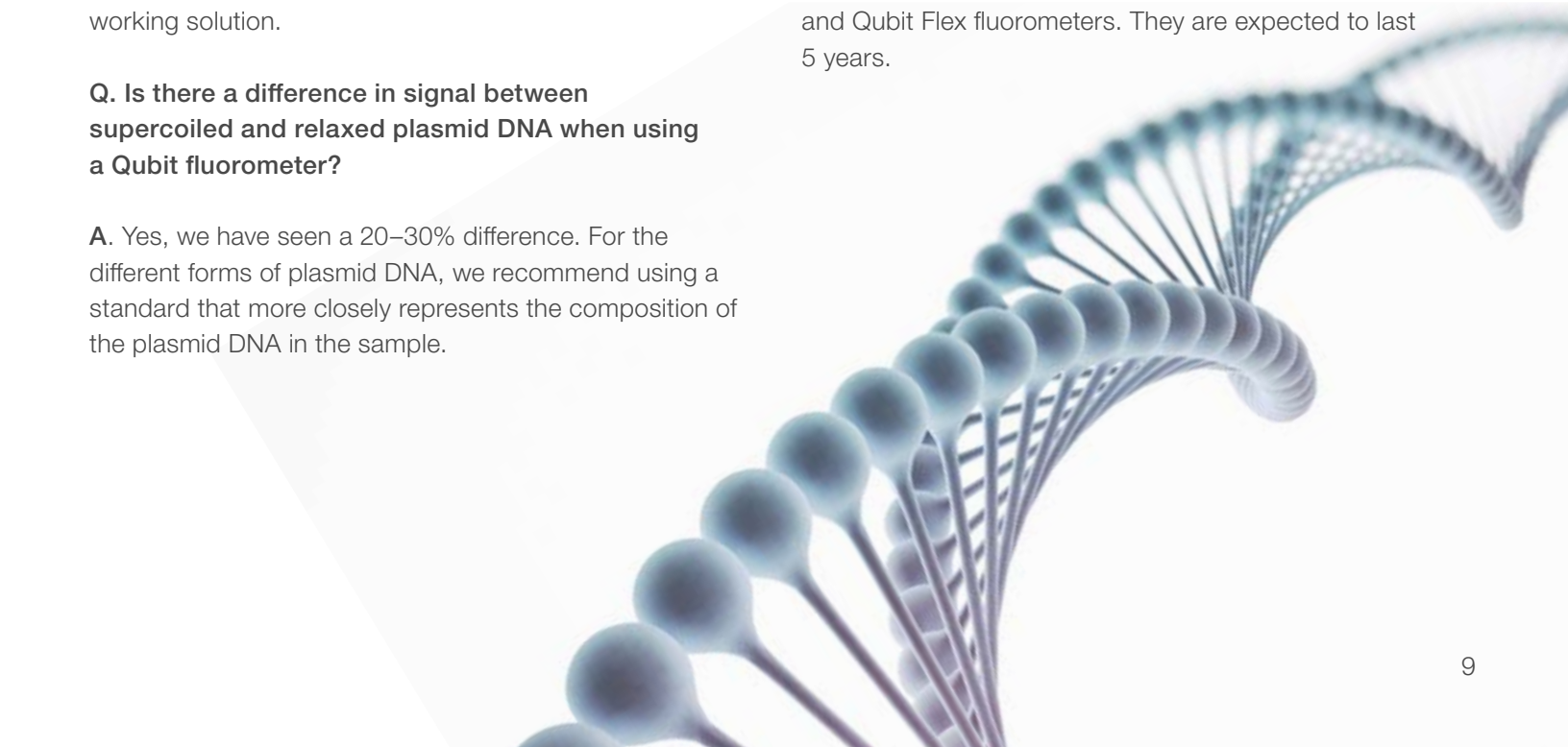
A. To adhere to cybersecurity legal standards, the latest firmware for Qubit 4 and Qubit Flex fluorometers will require users to log in to access certain menu options (such as instrument settings, software update, and system verification). When not logged into a user profile, these menu options will appear to be inactive.

Q. Is there a way to verify that my Qubit 4 or Qubit Flex fluorometer is functioning properly?

A. Qubit 4 and Qubit Flex System Verification Assay Kits offer a fast, easy-to-use, reagent-based method to test the internal components of a Qubit fluorometer. Perform the system verification when a problem with the instrument is suspected. It is not necessary to perform verification regularly.

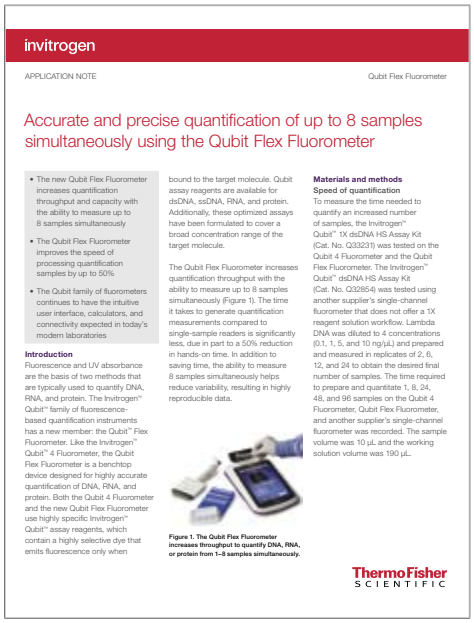
Q. How long does the lamp last?

A. There are two LED light sources in the Qubit 4 and Qubit Flex fluorometers. They are expected to last 5 years.

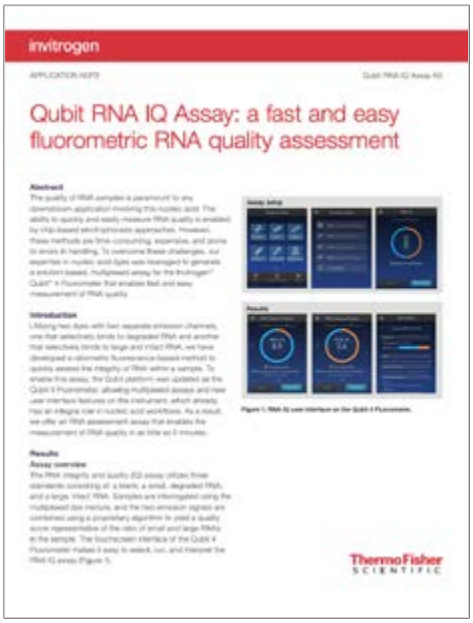


Support documents

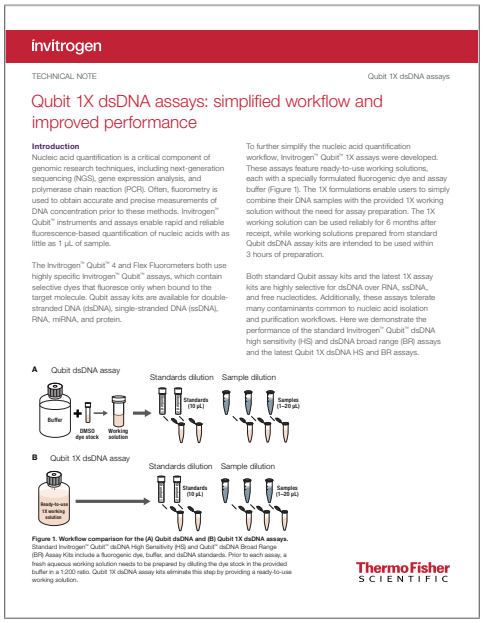
Qubit fluorometer technical resources are below—gain quick access to user guides, technical and application notes, and citations. Learn more at **thermofisher.com/qubitresources**.



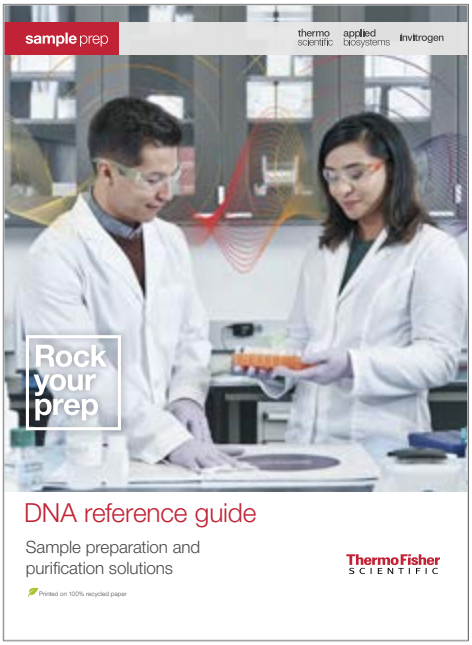
Application note: Accurate and precise quantification of up to 8 samples simultaneously using the Qubit Flex Fluorometer >



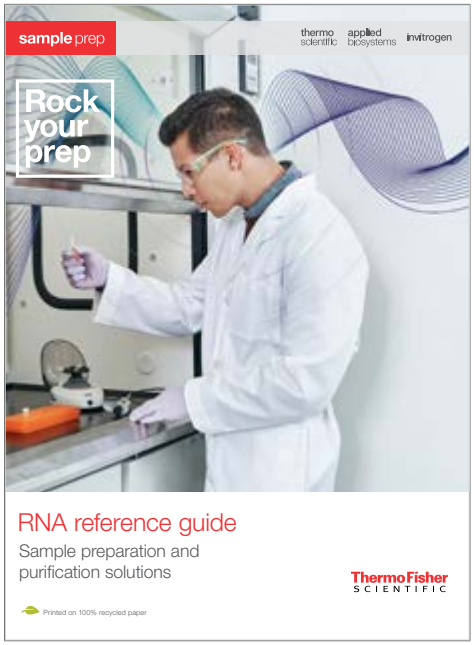
Application note: Qubit RNA IQ Assay: a fast and easy fluorometric RNA quality assessment >



Technical note: Qubit 1X dsDNA assays: simplified workflow and improved performance >



DNA reference guide: Sample preparation and purification solutions >



RNA reference guide: Sample preparation and purification solutions >

Technical note: Understanding library quantification assays for next-generation sequencing applications >

Ordering information

Instruments and accessories	Unit size	Cat. No.
Qubit 4 Fluorometer		
Qubit 4 Fluorometer (w/ Wi-Fi)	1 instrument	**Q33238**
Qubit 4 Quantitation Starter Kit (w/ Wi-Fi)	1 kit	**Q33239**
Qubit 4 NGS Starter Kit (w/ Wi-Fi)	1 kit	**Q33240**
Qubit 4 RNA IQ Starter Kit (w/ Wi-Fi)	1 kit	**Q33241**
Qubit 4 Protein BR Assay Starter Kit (w/ Wi-Fi)	1 kit	**A51292**
Qubit Assay Tubes	500 tubes	**Q32856**
Qubit 4 System Verification Assay Kit	50 assays	**Q33237**
Qubit Flex Fluorometer		
Qubit Flex Fluorometer	1 instrument	**Q33327**
Qubit Flex NGS Starter Kit	1 kit	**Q45893**
Qubit Flex Quantitation Starter Kit	1 kit	**Q45894**
Qubit Flex Assay Tube Strips	125 tube strips	**Q33252**
Qubit Flex Assay Reservoirs	100 reservoirs	**Q33253**
Qubit Flex System Verification Assay Kit	50 assays	**Q33254**

Product	Initial sample concentration (ng/µL)	Quantitation range (ng)	No. of assays	Cat. No.
DNA quantification assays				
dsDNA HS assays				
Qubit 1X dsDNA HS Assay Kit	0.005–120	0.1–120	100	**Q33230**
			500	**Q33231**
Qubit dsDNA HS Assay Kit	0.005–120	0.1–120	100	**Q32851**
			500	**Q32854**
Qubit 1X dsDNA HS Assay Lambda Standard	-	-	-	**Q33233**
dsDNA BR assays				
Qubit 1X dsDNA BR Assay Kit	0.2–4,000	4–4,000	100	**Q33265**
			500	**Q33266**
Qubit dsDNA BR Assay Kit	0.2–2,000	4–2,000	100	**Q32850**
			500	**Q32853**
Qubit 1X dsDNA BR Assay Lambda Standards	-	-	-	**Q33263**
ssDNA and oligos assay				
Qubit ssDNA Assay Kit	0.05–0.2	1–200	100	**Q10212**

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Product name	Initial sample concentration	Quantification range	No. of assays	Cat. No.
RNA quantification assays				
Qubit RNA HS Assay Kit	250 pg/µL and 100 ng/µL	5–100 ng	100	**Q32852**
			500	**Q32855**
Qubit RNA BR Assay Kit	1 ng/µL to 1 µg/µL	20–1,000 ng	100	**Q10210**
			500	**Q10211**
Qubit RNA XR Assay Kit	10 ng/µL and 10,000 ng/µL	200–10,000 ng	100	**Q33223**
			500	**Q33224**
Qubit microRNA Assay Kit	50 ng/mL to 100 µg/mL	1–1,000 ng	100	**Q32880**
			500	**Q32881**

Product name	Size	Cat. No.
RNA IQ assays		
Qubit RNA IQ Assay Kit	75 assays	**Q33221**
	275 assays	**Q33222**
Qubit RNA IQ Standards	1 set	**Q33235**

Product name	Instrument	Initial sample concentration	Size	Cat. No.
Protein assays				
Qubit Protein Assay Kit	Qubit Flex, Qubit 4	12.5 µg/mL to 5 mg/mL	100	**Q33211**
			500	**Q33212**
Qubit Protein BR Assay Kit	Qubit 4	100 µg/mL to 20 mg/mL	100	**A50668**
			500	**A50669**

Find out more at **thermofisher.com/qubit**

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