

KAPA Library Quantification Kit

Illumina® Platforms

Technical Data Sheet

Product Description

KAPA Library Quantification Kits for Illumina platforms provide all the reagents needed for absolute, qPCR-based quantification of Illumina libraries flanked by the P5 and P7 flow cell oligo sequences. Kits contain:

- Library Quantification DNA Standards 1 – 6 (a 10-fold dilution series of a linear, 452 bp template)
- Library Quantification Primer Premix (10X), containing the following primers:
Primer 1: 5'-AAT GAT ACG GCG ACC ACC GA-3'
Primer 2: 5'-CAA GCA GAA GAC GGC ATA CGA-3'
- KAPA SYBR® FAST qPCR Master Mix (2X), available with various passive reference dyes (Table 1).

Library quantification is performed by amplifying the set of six pre-diluted DNA Standards and diluted library samples by qPCR, using the KAPA SYBR FAST qPCR Master Mix and primers targeting the Illumina P5 and P7 flow cell oligo sequences. The average Cq score for each DNA Standard is plotted against $\log_{10}$ (concentration in pM) to generate a standard curve. The concentrations of diluted library samples are then calculated against the standard curve, using absolute quantification.

KAPA Library Quantification Kits are rigorously tested to ensure minimal lot-to-lot variation. The kit contains the novel KAPA SYBR FAST DNA Polymerase, engineered through a process of directed evolution for high-performance SYBR Green I-based qPCR. The ability of the engineered polymerase to amplify diverse DNA fragments with similar efficiency enables the use of a universal standard for the reliable quantification of all Illumina libraries with an average fragment length of up to 1 kb, irrespective of library type or GC content.

KAPA SYBR FAST is an antibody-mediated hot start DNA polymerase formulation. KAPA Library Quantification Kits are therefore suitable for use with automated liquid handling systems for high-throughput sample quantification.

Product Applications

KAPA Library Quantification Kits for Illumina platforms are designed for the accurate and reproducible quantification of libraries prepared for Illumina sequencing. Any library with a concentration >0.0002 pM that contains sequences complementary to the primers in the Primer Premix (10X) can be quantified with the kit, irrespective of the library type, how it was constructed, or on which Illumina instrument it will be sequenced.

The kit supports quantification of libraries across a range of GC contents and average fragment lengths up to 1 kb.

In addition to NGS library quantification, the kit can also be used to detect library contamination in work spaces used during the preparation of Illumina libraries.

The KAPA Library Quantification assay consists of repetitive pipetting steps, which can easily be automated. The use of an automated liquid handling system is highly

recommended for high-throughput NGS pipelines. Please refer to the KAPA Library Quantification Technical Guide for more information.

Product Compatibility

KAPA Library Quantification Kits for Illumina platforms are suitable for the quantification of any NGS library (prepared for Illumina sequencing) which contains the P5 and P7 flow cell sequence motifs. Libraries constructed using full-length universal or indexed TruSeq™ adapters can be quantified after adapter ligation. Libraries prepared with partial or stem-loop adapters can only be quantified with this kit after library amplification, during which indexes (and flow cell sequences) are added. To ensure compatibility, compare the adapter sequences used during library preparation with the library quantification primer sequences in Product Description.

Ensure that the correct KAPA SYBR FAST qPCR Master Mix is used, in accordance with the reference dye requirements (if any) of the qPCR instrument to be used for library quantification (Table 1).

Product Specifications

Shipping and Storage

KAPA Library Quantification Kits are shipped on dry ice or ice packs, depending on the destination country. Upon receipt, immediately store all components at -15°C to -25°C in a constant temperature freezer. When stored under these conditions and handled correctly, the kit components will retain full activity until the expiry date indicated on the kit label.

Handling

Always ensure that components have been fully thawed and thoroughly mixed before use. The KAPA SYBR FAST qPCR Master Mix may not freeze solidly, even when stored at -15°C to -25°C.

The SYBR Green I dye (contained in the KAPA SYBR FAST qPCR Master Mix) and passive reference dyes are light sensitive. Exposure to direct light for an extended period of time will result in loss of fluorescent signal intensity.

All components of KAPA Library Quantification Kits, as well as the combined KAPA SYBR FAST/Primer Premix solution, are stable through 30 freeze-thaw cycles. Ensure that all reagents are stored protected from light at -15°C to -25°C when not in use. When protected from light, reagents are stable in the dark at 2°C to 8°C for long-term storage, and may be stored at this temperature for short-term use, provided that they do not become contaminated with microbes and/or nucleases.

Quality Control

All kit components are subjected to stringent functional quality control, are free of detectable contaminating exo- and endonuclease activity, and meet strict requirements with respect to DNA contamination. Please contact Technical Support at sequencing.roche.com/support for more information.

KAPA bibliotekų kiekybinio įvertinimo rinkiniuose *Illumina* sekoskaitos platformoms pateikiami visi reagentai, kurių reikia absoliučiam kiekybiniam su *Illumina* sekoskaitos platformomis suderinamų bibliotekų, apsuptų P5 ir P7 specifinėmis sekomis, įvertinimui atliekant qPCR. Rinkinyje yra:

- dvigrandės DNR standartai (1–6) bibliotekų kiekybiniam įvertinimui (452 bp dydžio fragmento 10-kartiniai skiedimai);
- Bibliotekos kiekybinio įvertinimo pradmenų mišinys (10X), kuriame yra toliau išvardyti pradmenys:

Pradmuo 1: 5'-AAT GAT ACG GCG ACC GA-3',

Pradmuo 2: 5'-CAA GCA GAA GAC GGC ATA CGA-3';

- KAPA SYBR® FAST *qPCR Master Mix* (2X), kartu su įvairiais pasyviais referentiniais dažais (1 lentelė).

Bibliotekos kiekybinis įvertinimas atliekamas amplifikuojant šešių praskiestų DNR standartų ir praskiestų bibliotekos mėginių rinkinį atliekant qPCR, naudojant KAPA SYBR FAST *qPCR Master Mix* ir pradmenis, komplementarius *Illumina* P5 ir P7 oligosekoms. Vidutinė Cq vertė kiekvienam dvigrandės DNR standartui nurodoma $\log_{10}$ grafike (koncentracija išreikšta pM) sudarant standartinę kreivę. Tada, naudojantis standartine kreive, absoliučiomis vertėmis apskaičiuojamos praskiestų bibliotekos mėginių koncentracijos.

KAPA bibliotekos kiekybinio įvertinimo rinkiniai yra griežtai tiriami, kad būtų užtikrinti minimalūs svyravimai tarp gaminių partijų. Rinkinyje naudojama inovatyvi KAPA SYBR FAST DNR polimerazė. Sukurtos polimerazės gebėjimas amplifikuoti įvairius DNR fragmentus tolygiu efektyvumu suteikia galimybę naudojant universalų standartą patikimai įvertinti su *Illumina* platformomis suderinamas bibliotekas su vidutinio 1 kb ilgio fragmentu, nepaisant bibliotekos tipo ar GC sancaupų DNR sekose.

KAPA rinkiniai kiekybiniam bibliotekų vertinimui gali būti naudojami su automatizuotomis skysčių išpilstymo sistemomis mėginių didelio našumo kiekybiniam vertinimui.

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[lietuvių kalbą išvertė vertėja Loreta Grigonytė, kuri yra susipažinusi su baudžiamąja atsakomybe pagal Lietuvos Respublikos baudžiamojo kodekso 235 straipsnį. Šis dokumentas, kuris yra vertimas iš anglų kalbos į lietuvių kalbą, yra ortografiškai teisingas ir perteikia teksto originalo esmę.

Vertėja Loreta Grigonytė

(parašas)



(signature)

Susiūta ir sunumeruota 2 lapų (lapai)