Custom Methods

Create and run a custom measurement method to display unique information for your samples.

Manage Custom Methods

Measure Custom Method

Delete Custom Method

Reported Results

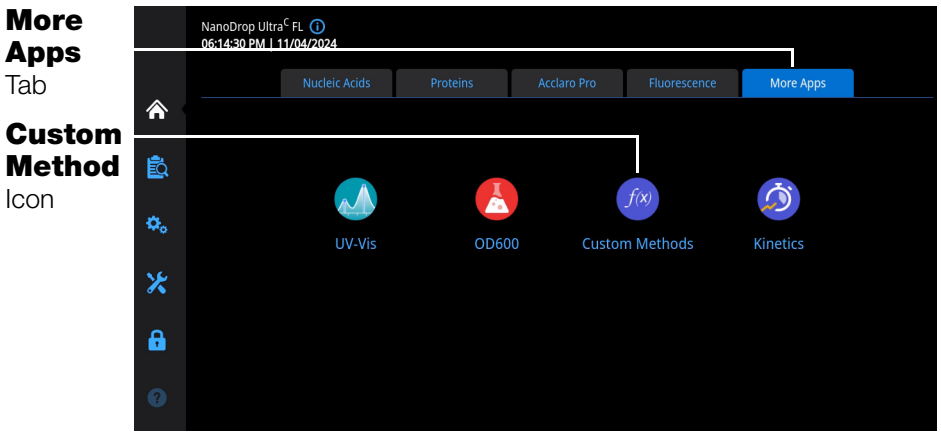
Manage Custom Methods

Use the Custom Methods application to run a user-defined method created using the NanoDrop Ultra local or PC control software. Custom methods can be made with or without standards.

Create custom method

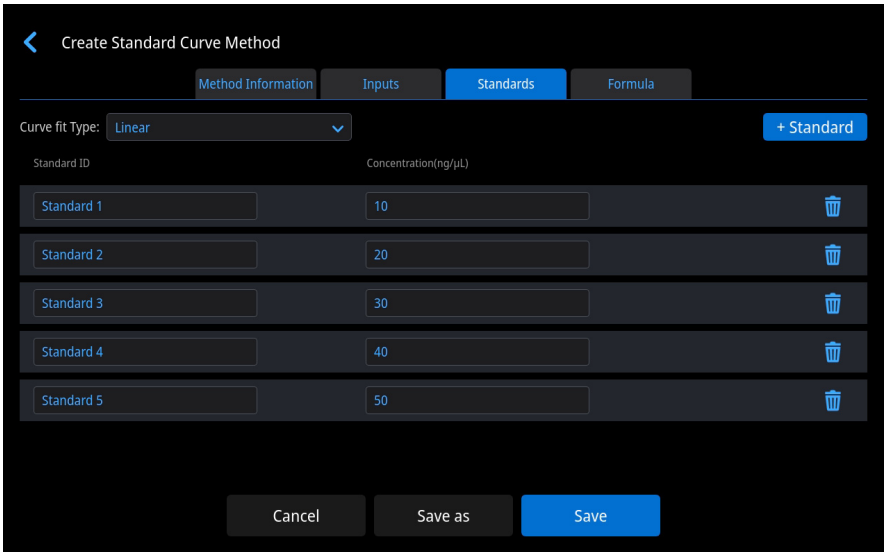
Create method to be used for sample measurements with user-defined settings.

1. From the home screen, select the **More Apps** tab, then select **Custom Methods**



2. Select + NEW METHOD and choose one of the following:
   - **Formula** (if your method will not have standards)
   - **Standard Curve** (if your method will have standards)

3. In the **Create Formula Method** window, enter **Method Name**.
4. Enter detailed **Description** of method, if desired.
5. Specify how to calculate and report the method results:
   - If method does not have standards, specify factor or extinction coefficient of analyte (enter "1" to report absorbance measurements only) within the **Inputs** tab.
   - If method has standards, enter name and concentration of each standard and select the curve fit type within the **Standards** tab.



   Alternatively, load a standard curve.
6. Enter or choose remaining custom settings as needed.
7. Choose **Save**

> **Note** Any errors in the method will be listed in orange text at the bottom of the method editor screen. Errors must be addressed before the method can be saved.

View or edit custom method

1. From the Custom Methods screen, select ⋮ next to the desired method to edit.

2. Select **Edit**
3. View and adjust the method settings as desired
4. Select **Save**

Copy custom method

To create a custom method that is similar to an existing one, open the existing method, make your changes, then select **Save As** and enter a new name.

Copy custom method

1. From the Custom Methods screen, select  next to the desired method to copy.
2. Select **Edit**
3. View and adjust the method settings as desired.
4. Enter new **Method name** and **Description**.
5. Select **Save As**.
6. Enter a new filename for the method and click **Save**.

Export custom method

Export a custom method in order to run it and store the measurement results on another NanoDrop Ultra instrument or another computer running the NanoDrop Ultra PC control software.

1. From the Custom Methods screen, select  next to the desired custom method.
2. Select **Export** (if method is invalid, an error message is displayed; errors must be fixed before method can be exported).
3. Select an available location to export to from the dropdown menu and then choose **Export** (method is exported to method file (*.method filename extension in proprietary format).

   To transfer the method to the NanoDrop Ultra instrument or NanoDrop PC control software, copy the method file to a USB memory device and then load the method (see Import a Custom Method for details)

Import custom method

Import a custom method back to a computer running the NanoDrop Ultra software in order to edit the method settings.

1. From the Custom Methods screen, select **Import**

2. Locate and select ".method" file
3. Choose **Import** (imported method is added to end of Select Method list)

Note Custom methods downloaded from the NanoDrop website have a .zip file-name extension and must be extracted using a third-party file decompression program before the software will recognize them as custom methods.

Delete custom method

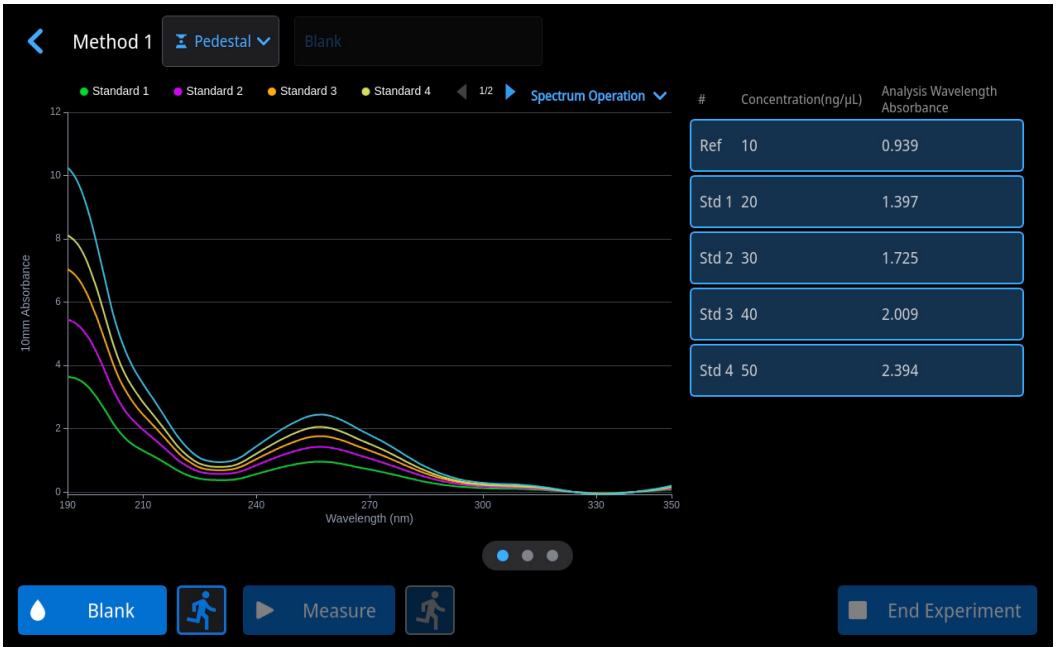
1. From the Custom Methods screen, select  next to the desired custom method.
2. Select **Delete**.
3. After the confirmation message, choose **Yes**.

Load standard curve

1. From the Custom Methods screen, select **Load Standard**.

2.Select the desired previously run standard curve, then select **Load**.

The measurement screen opens with the standard curve loaded. You can begin by blanking and then measuring samples.



Measure using a Custom Method

Use the Custom application to create and run a user-defined method created. For more information, see "Create custom method" on page 241.

Before you begin...

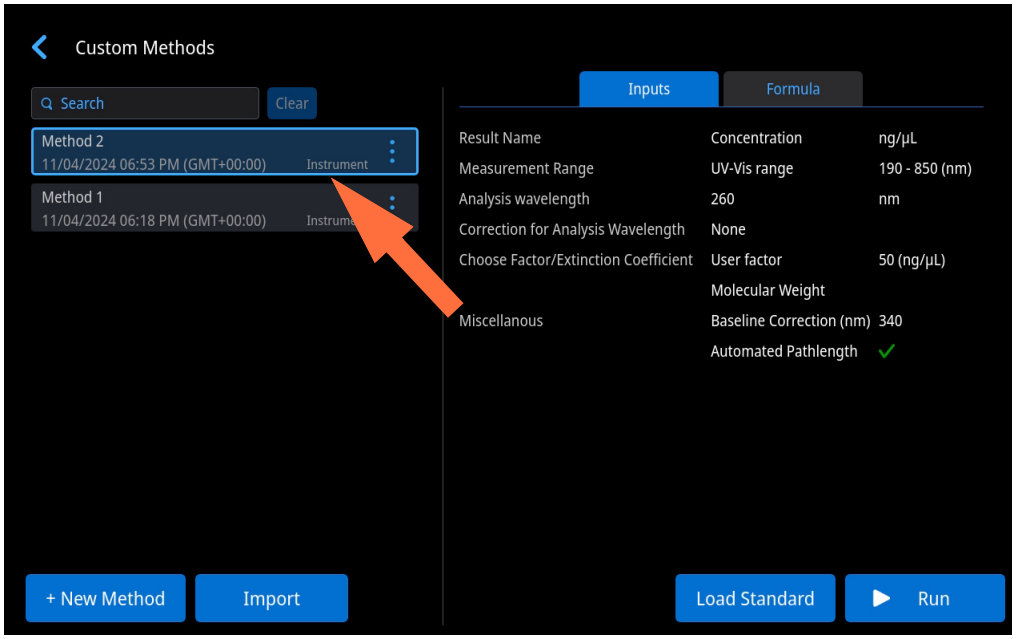
Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

NOTICE

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

Procedure

1. From the home screen, select the **More Apps** tab, then select **Custom Methods**.
2. In the method selection pane, select the method to run.



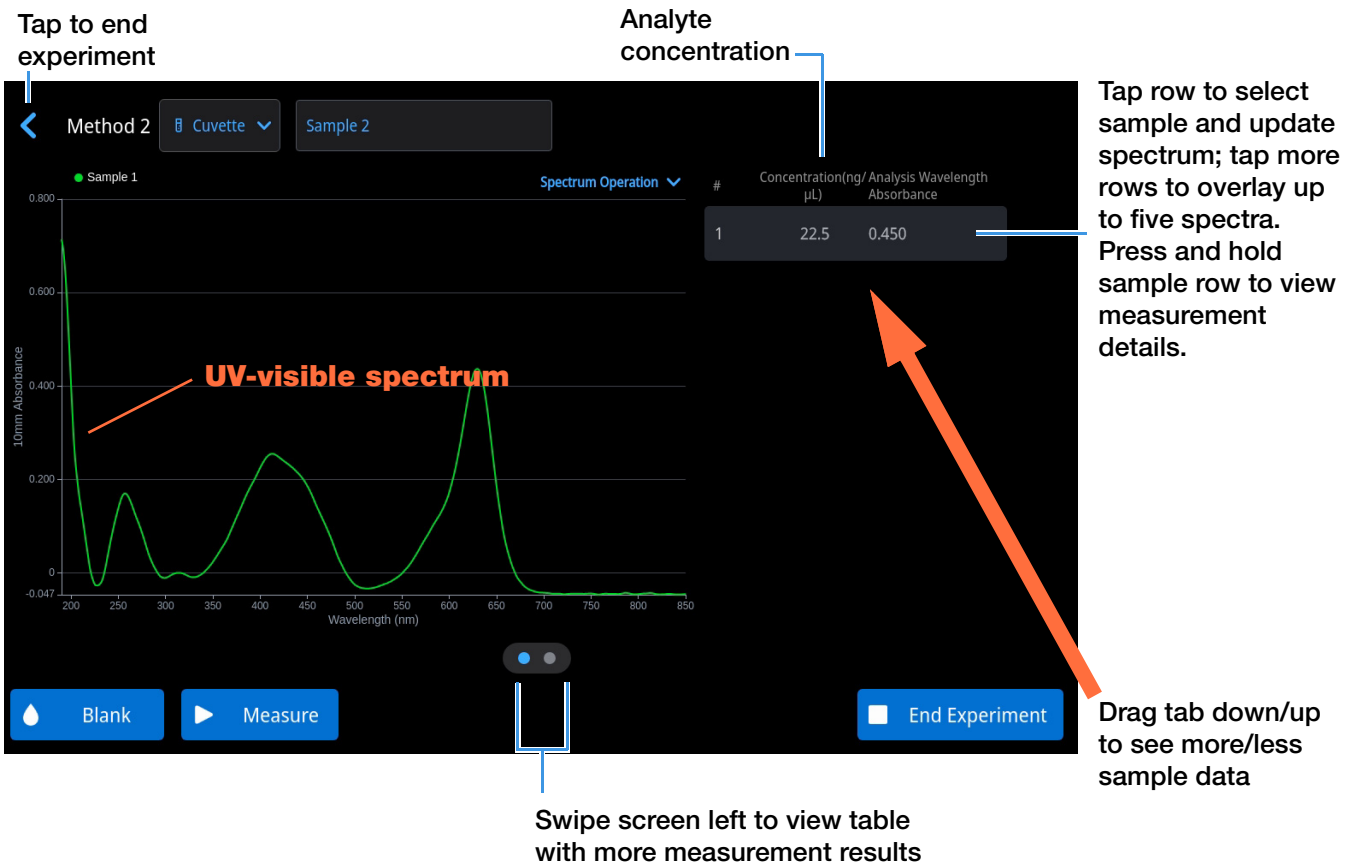
Information about the selected method appears in the Method Details pane.

3. Select Run.
4. If using a NanoDrop Ultra^{C} or NanoDrop Ultra^{C} FL model, select the correct measurement pathway.
   - When using a cuvette, select **Cuvette** from the drop-down menu at the top of the screen, this will present the cuvette settings. Select desired pathlength, stir speed, and heating then close the drop-down menu.
   - When using the pedestal for measurement, leave **Pedestal** as the selected setting at the top of the screen.
5. Follow the on-screen instructions to measure a sample.

Reported Results

Custom method measurement screen (local control)

For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example of the NanoDrop Ultra local control software screen:



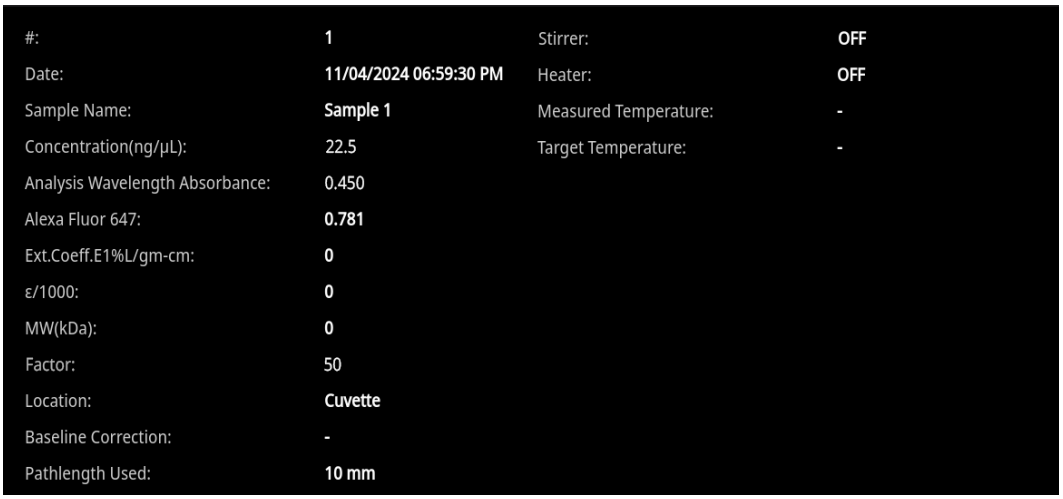
Note Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

Custom method measurement screen (PC control)

For each measured sample and standard, this application shows the absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See “Measurement Screen Display Options” on page 301 for an example.

Reported values

The initial screen that appears after each measurement (see previous image) shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:



#:	1	Stirrer:	OFF
Date:	11/04/2024 06:59:30 PM	Heater:	OFF
Sample Name:	Sample 1	Measured Temperature:	-
Concentration(ng/µL):	22.5	Target Temperature:	-
Analysis Wavelength Absorbance:	0.450		
Alexa Fluor 647:	0.781		
Ext.Coeff.E1%L/gm-cm:	0		
ε/1000:	0		
MW(kDa):	0		
Factor:	50		
Location:	Cuvette		
Baseline Correction:	-		
Pathlength Used:	10 mm		

Settings

These settings are available for creating custom methods.

Setting	Available Options
Result	Enter descriptive name for calculated concentration result (for example, “MTT Assay”) and use adjacent drop down list to select appropriate unit. Result name appears as column heading for reported concentration value.
Measurement range	Select spectral range in which method will acquire data. Available options: • Ultra-violet only (190 nm - 350 nm) • Visible only (350 nm - 850 nm) • Ultra-violet and visible (190 nm - 850 nm) • Custom (specify starting and ending point in nanometers) **Notes**: • If a Baseline correction and/or Analysis wavelength correction are used, make sure your selected spectral range includes your specified baseline correction and/or analysis correction wavelength. • For micro-volume absorbance measurements and measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent.
Analysis wavelength correction	Use this option to specify absorbance correction at analysis wavelength only. Available options: • **None.** No correction at analysis wavelength. • **Single point**. Enter wavelength for analysis correction. (Absorbance value at specified analysis correction wavelength is subtracted from absorbance value at analysis wavelength. Corrected value is used to calculate sample concentration.) • **Sloping baseline**. Enter two wavelengths that define sloping baseline for analysis correction. (Absorbance value of sloping baseline at analysis wavelength is subtracted from absorbance value at analysis wavelength. Corrected value is used to calculate sample concentration.)

Setting	Available Options
Factor or Extinction coefficient at 1 cm pathlength (Formula methods only)	Specify whether to use factor or extinction coefficient to calculate concentration result: • **User-defined factor**. Enter **factor** for 1 cm pathlength and use adjacent drop down list to select appropriate **unit**. Equation below shows how factor is used to calculate sample concentration: $c = (A * f) / b$ where: c = analyte concentration A = absorbance in absorbance units (A) **f** = factor (typically 1/ε, where ε = wavelength-dependent molar absorptivity coefficient, or extinction coefficient) b = pathlength in cm (determined at measurement time, then normalized to 10 mm (1 cm) pathlength equivalent) • **Extinction coefficient and molecular weight**. Enter **extinction coefficient** for 1 cm pathlength and use adjacent drop down list to select appropriate **unit**. Equation below shows how extinction coefficient is used to calculate sample concentration: $c = A / (\varepsilon * b)$ where: c = analyte concentration A = absorbance in absorbance units (A) ε = wavelength-dependent molar absorptivity coefficient (or extinction coefficient) b = pathlength in cm (determined at measurement time, then normalized to 10 mm (1 cm) pathlength equivalent)
	Notes: • Refer to product literature for information about factors and extinction coefficients for specific materials. • To set up a method that reports absorbance measurements only, select Factor or Extinction Coefficient with the factor or extinction coefficient set to “1”. • If specified unit for factor or extinction coefficient is based on mass (such as mg/mL) and specified unit for calculated result is based on molarity (such as pmol/µL) or vice versa, enter **molecular weight** and use adjacent drop down list to select appropriate **unit**.

Setting	Available Options
Standards (Standard curve methods only)	Define the standards: • Enter name and analyte concentration of each standard and a reference, if desired: – Depending on the Curve Type setting, a standard curve can be generated using two or more standards. (The software **allows a reference and up to 15 standards**.) – **All reference and standards solutions** should be in the same buffer used to resuspend the samples plus the same volume of reagent added to the samples. – **First standard** can be a reference measurement. The reference solution should contain none of the analyte of interest. (The reference measurement is not the same as a blank measurement.) – **Concentration values for standards** can be entered in any order but the standards must be measured in the order in which they were entered; however, best practice dictates that standards be measured from the lowest concentration of the standard analyte stock to the highest. – **Concentration range of the standards** must cover the dynamic range of the assay and the expected range of the unknown samples. Sample analyte concentrations are not extrapolated beyond the concentration of the highest standard.

Setting	Available Options
	• Select curve fit type. Specify type of equation used to create standard curve from standard concentration values. Available options: – Linear: Draws the linear least squares line through all measured standards (requires reference measurement and at least one standard). Linear through zero is similar but with the constraint that the line passes through the origin (0,0) – Interpolation: Draws a series of straight lines to connect all measured standards (requires reference measurement ans at least one standard) – 2^{nd} order polynomial: Draws the 2^{nd} order least squares polynomial using all measured standards (requires reference measurement and at least standards). 2^{nd} order polynomial through zero is similar but with the constraint that the line passes through the origin (0,0) – 3^{rd} order polynomial: Draws the 3^{rd} order least squares polynomial using all measured standards (requires reference measurement and at least three standards). 3^{rd} order polynomial through zero is similar but with the constraint that the line passes through the origin (0,0)
Analysis wavelength (Standard curve methods only)	Monitor absorbance at specified wavelength (enter the wavelength in nanometers). **Note**: The specified wavelength must fall within the selected measurement range. The measurement results or the concentration will be calculated automatically using the absorbance value at the specified wavelength and applying the selected method type (factor or standard curve).
Baseline correction	Select this option to correct offset caused by light scattering particulates by subtracting the absorbance at a specified baseline point. Then specify wavelength for baseline correction. **Note**: Software subtracts absorbance value at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum. As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength.

Setting	Available Options
Automated pathlength	Affects micro-volume measurements only. • When Automated Pathlength is selected, software selects the optimal pathlength (between 1.0 mm and 0.03 mm) based on sample absorbance at the analysis wavelength. For example, when sample absorbance at the analysis wavelength is less than or equal to 12.5 (10 mm pathlength equivalent), the optimal longer pathlength is used. When sample absorbance is greater than 12.5, the optimal shorter pathlength is used. Recommended for samples that are highly absorbing at the analysis wavelength. (This option may cause reduced sensitivity when the sample spectra have a large absorbance peak that is not at the analysis wavelength.) **Note**: When the analysis wavelength is between 190 nm and 219 nm, the optimal longer pathlength is used when sample absorbance is less than or equal to 10 (10 mm pathlength equivalent), and the optimal shorter pathlength is used when sample absorbance is greater than 10. • When Automated Pathlength is deselected, the software uses a 1 mm pathlength regardless of the sample absorbance. This can cause detector saturation (resulting in jagged peaks) for highly absorbing samples (e.g., ~15 A at 10 mm pathlength equivalent).

Setting	Available Options

Formula table (optional)	Use the Formula table to specify additional reported results, such as a purity ratio, for each sample.
	
	Available options:
	
	• **Predefined**. Select from a list of predefined formulas, which can be used as is or edited, and choose **Save**. The predefined formula is listed in the Formula Table.
	
	• **+Formula**. Create formula for current method. Available options:
	
	  • **Formula Name**. Enter a name for the formula. After a measurement, the name is reported in Data Table and Sample Details screens.
	
	  • **Equation**. Enter valid formula (see below for rules and examples). After a measurement, the measured or calculated value is reported in Data Table and Sample Details screens.
	
	  • **Unit**. Select from a drop-down of available units for reported result. After a measurement, the unit is reported in Data Table and Sample Details screens.
	
	• **Delete**. Delete selected formula from current method by selecting  .

Formula rules	Custom formulas can include the following operators and functions: • **Path()**. Returns sample pathlength in cm. • **A(nm)**. Returns sample absorbance at specified wavelength (for example, enter A(650) to add the measured absorbance at 650 nm to your equation). • **Operators**: + (add), - (subtract), * (multiply), / (divide). • **Functions**: Log(x), Pow(x,y). **Notes**: Follow these additional rules for all languages: • Use period “.” decimal separators for floating point and double-floating point numbers. • Use comma “,” list separators (for example, “POW(2,8)”). • Do not use comma “,” group separators for large numbers (for example, enter 1000 rather than 1,000).

OD600

Measures the concentration of microbial cell cultures in solution by measuring scattered light at 600 nm.

Measure OD600

Reported Results

Settings

Calculations

Theory of OD600 application

The OD600 application measures light transmission and uses that value to calculate absorbance. In spectroscopy, transmitted light is defined as any light that is not absorbed by, reflected from and scattered off a sample.

In the case of living cells, most of the incident light is transmitted through the sample rather than scattered, reflected or absorbed. The amount of scattered light is low and can vary from instrument to instrument. As a result, calculated absorbance readings are typically very low.

The calculated absorbance values are used to determine the density of cells in solution in cells/mL. The physical concepts and formulas that relate optical properties of living cells to concentration include:

- Cells, which have a different index of refraction from the surrounding medium, randomly reflect and scatter light out of the incident light path. The amount of scattering is proportional to the density of cells in the sample.
- The Beer's Law equation is used to relate absorbance to concentration. See Calculations for OD600 Measurements for details.
- For cuvette reading with the NanoDrop Ultra^{C} and NanoDrop Ultra^{C} FL instruments, accurate absorbance readings are typically in the range between 0.04 A and 1.5 A. Serial dilutions of the sample are usually needed to bring the absorbance readings within this range.
- All measurements should be made on the same type of spectrophotometer and method (i.e., pedestal vs. cuvette) as the amount of scattered light captured varies based on the optical configuration. When using a different spectrophotometer or method, calculate and apply a conversion factor to the reported results. For example, to compare OD readings using the pedestal vs. a cuvette, a conversion factor can be calculated as follows:

  Conversion factor = Cuvette OD/Pedestal OD

Best practices for OD600 measurements

- Ensure the sample is within the instrument's absorbance detection limits.
- Blank with the growth or culture media the cells of interest are suspended in.
- Run a blanking cycle to assess the absorbance contribution of your media solution. If the media solution exhibits strong absorbance at or near the analysis wavelength (600 nm), you may need to choose a different media solution or application. See Choosing and Measuring a Blank for more information.
- Make dilutions as necessary to ensure sample cultures do not exceed the linear dynamic range of the assay before the culture reaches the stationary phase. The linear range depends largely on optical configuration and, therefore, differs for pedestal and cuvette measurements. To determine the linear range:
  - Measure a series of dilutions using a young overnight culture (~16 hrs) of the microbial strain
  - Graph the OD600 measurements against the dilution factor

  The upper detection limit is the measured OD600 value at which there ceases to be a linear correlation between dilution factors and OD600 readings.
- Mix samples gently but thoroughly immediately before taking an aliquot for measurement.
- For micro-volume measurements:
  - Ensure pedestal surfaces are properly cleaned and conditioned.
  - Avoid introducing bubbles when mixing and pipetting.
  - Start the measurement promptly to avoid settling or evaporation.
  - Follow best practices for micro-volume measurements.
  - Use 2 µL sample volume. See Recommended Sample Volumes for more information.
  - For dilute samples that exhibit low absorbance at 600 nm, use an alternative wavelength such as 400 nm to measure absorbance, or use cuvettes instead of micro-volume measurements.
- For cuvette measurements (NanoDrop Ultra^{C} and NanoDrop Ultra^{C} FL instruments only):
  - Use clean plastic, glass or quartz cuvettes.
  - Follow best practices for cuvette measurements.
  - Do not use the automatic stirring feature for this assay.

Measure OD600

Use the OD600 application to monitor the growth rate of bacterial or other microbial cell cultures by measuring the optical density (absorbance) of the culture in growth media at 600 nm. The Beer-Lambert equation and a user-entered conversion factor are used to correlate absorbance with concentration. Reported concentration values can be used to identify the phase of cultured cell populations, e.g., log or exponential and stationary.

The OD600 application reports cell concentration in cells/mL. A single-point absorbance correction can be used. This application does not require a standard curve.

Before you begin...

Before taking pedestal measurements with the NanoDrop Ultra instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

Note Due to the amount of scattered light present in this assay, absorbance readings are typically very low.

NOTICE

- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

Procedure

1. From the home screen, select the **More Apps** tab, then select **OD600**.
2. Configure any of the setup options if desired and select **Save**.
3. If using a NanoDrop Ultra^{C} or NanoDrop Ultra^{C} FL model, select the correct measurement pathway.
   - When using a cuvette, select **Cuvette** from the drop-down menu at the top of the screen, this will present the cuvette settings. Select desired pathlength, stir speed, and heating then close the drop-down menu.
   - When using the pedestal for measurement, leave **Pedestal** as the selected setting at the top of the screen.

4. Pipette 2 µL blanking solution (i.e., the media solution the cells of interest are suspended in) onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder.

   Tip: If using a cuvette, make sure to align the cuvette light path with the instrument light path.

5. Select **Blank** and wait for the measurement to complete.

   Tip: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements.)

6. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.

7. Pipette 2 µL sample solution onto the pedestal and lower the arm, or insert the sample cuvette into the cuvette holder.

8. Start the sample measurement:

   – Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is off, lower arm and select **Measure**.

   – Cuvette: select **Measure**

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).

9. When you are finished measuring samples, select **End Experiment**.

10. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.

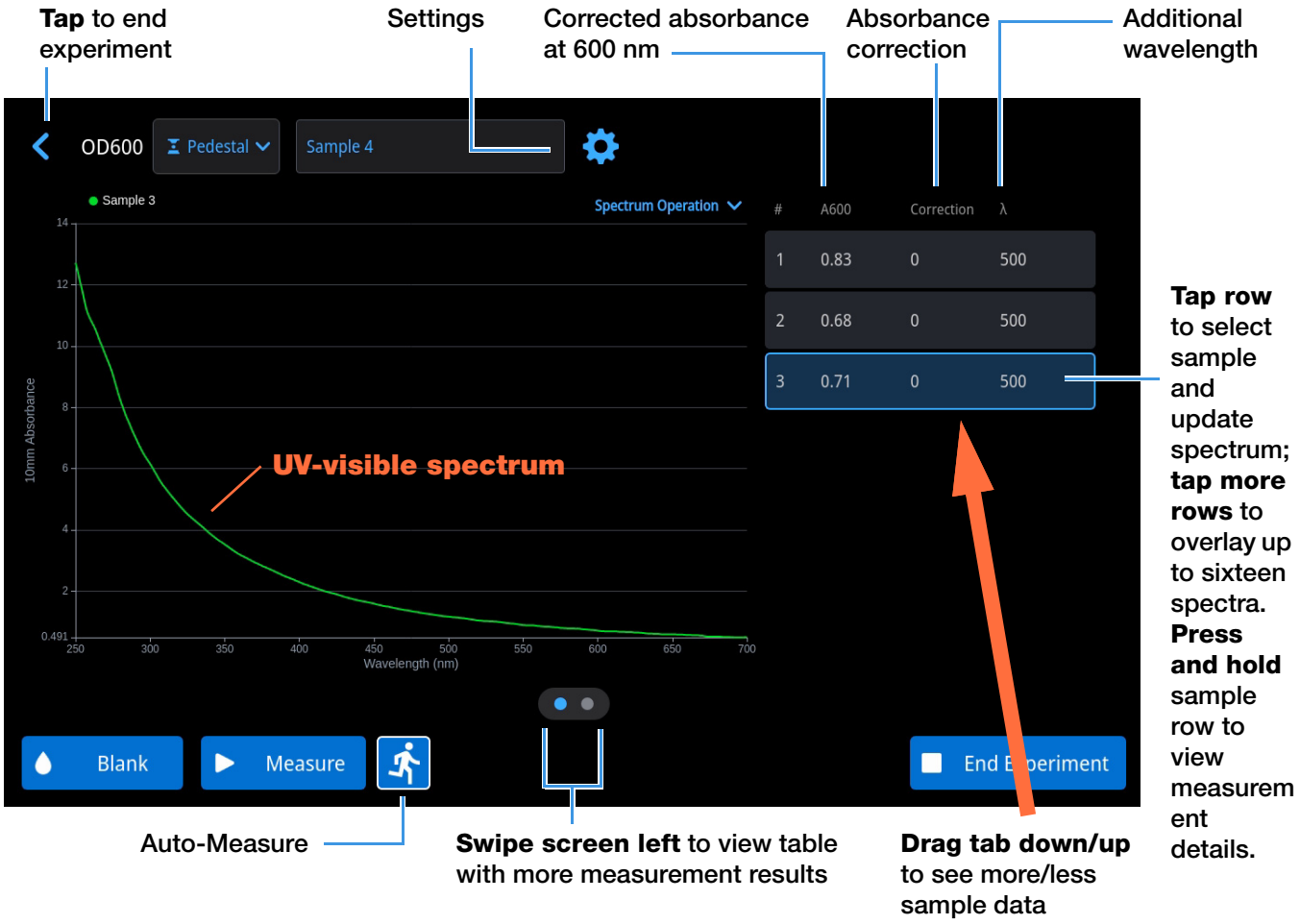
Related Topics

- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette
- Prepare Samples and Blanks
- Basic Instrument Operations

Reported Results

OD600 measurement screen (local control)

For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example of the NanoDrop Ultra local control software:



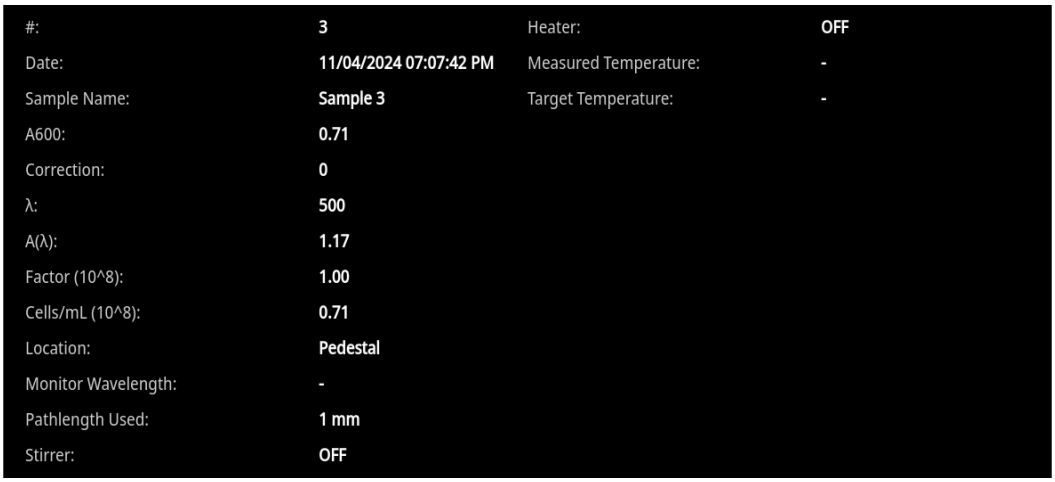
Note Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

OD600 measurement screen (PC control)

For each measured sample and standard, this application shows the absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See "Measurement Screen Display Options" on page 301 for an example.

Reported values

The initial screen that appears after each measurement shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:

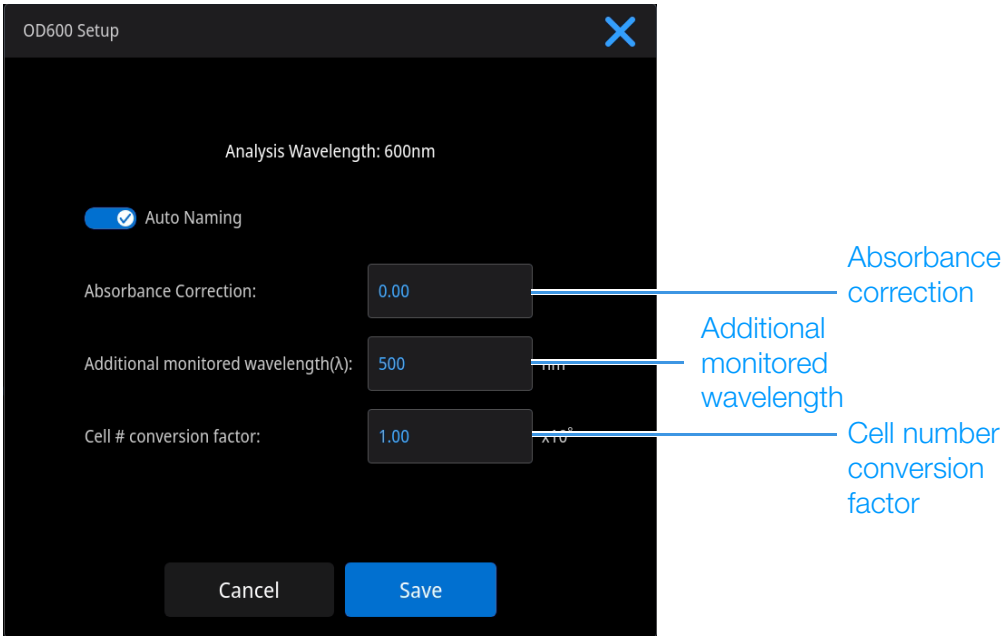


Related Topics

- Basic Instrument Operations
- OD600 Calculations

Settings

The OD600 setup screen appears after you select the OD600 application from the More Apps tab on the home screen. To show the OD600 settings from the OD600 measurement screen, select .



Setting	Available Options	Description
Auto Naming	On or off	When enabled, each sample is given a default base name “sample” followed by the number sample in the sequence. For example, the first sample would be named “Sample 1” followed by “Sample 2,” etc. You can edit the default base name and overwrite any sample name.
Absorbance correction	Absorbance value between 0 and 300 A	User-defined absorbance correction. Enter absorbance correction for displayed spectrum. This can be useful, for example, to correct baseline offset caused by any difference between the media solution used to blank the instrument and media used to suspend the cell culture sample, and because scattered light generally produces an offset. Absorbance correction value is subtracted from absorbance values at all wavelengths in sample spectrum. (All displayed absorbance values are corrected values.)

Setting	Available Options	Description
Additional monitored wavelength (λ)	Any wavelength between 250 nm and 700 nm	User-defined wavelength. Enter an additional wavelength to measure if desired (useful for dilute samples that exhibit low absorbance at 600 nm). If an alternative wavelength is specified, use this equation to calculate cell concentration: $c = A(\lambda) * factor(\lambda)$ where: c = analyte concentration in cells/mL A(λ) = UV-visible absorbance at specified wavelength in absorbance units (A) factor(λ) = 1/(ε(λ) * b) in mL/cell-cm where: ε(λ) = molar absorption coefficient (or extinction coefficient) at specified wavelength b = pathlength in cm (1.0 cm for the NanoDrop Ultra instruments)
Cell number conversion factor (10^8)	Any number	User-defined factor. Generally accepted factor for measured cell type, or one derived empirically using a solution of study cells at known concentration using the same media. Default value is $1x10^8$ which is the generally accepted factor for most bacterial cell suspensions such as E. coli. **Tip**: The factor is wavelength specific for each cell type and can be affected by the type of media used for the measurements. Ideally, the factor should be determined empirically using a solution of the study cells at a known concentration using the same media.

Related Topics

- Instrument Settings

Calculations

Similar to the nucleic acid applications, the OD600 application uses a modification of the Beer-Lambert equation to calculate sample concentration where the extinction coefficient and pathlength are combined and referred to as a "factor."

The OD600 application offers a user-specified factor, to be used in conjunction with Beer's Law to calculate sample concentration. If the factor is known, enter the factor. Otherwise, use $1x10^8$, which is the generally accepted factor for most bacterial cell suspensions such as E. coli.

Calculated cell concentrations are based on the absorbance value at 600 nm, the entered factor and the sample pathlength. A single-point absorbance correction may be applied.

Measured Values

A600 absorbance

Note: For micro-volume absorbance measurements and measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent.

- Cell culture absorbance values are measured at 600 nm using the normalized spectrum. If no Absorbance Correction is specified, this is the reported A600 value and the value used to calculate cell concentration.
- If an Absorbance Correction is specified, the normalized and (absorbance) corrected absorbance value at 600 nm is reported and used to calculate cell concentration.

A(λ) absorbance

- Normalized and (absorbance) corrected (if used) absorbance value at any specified Additional Monitored Wavelength (λ) is also reported.

Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1.0 mm and 0.03 mm) based on sample absorbance at the analysis wavelength.
- For cuvette measurements, the pathlength selected after switching to cuvette mode is utilized. (see Cuvette Settings).
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.

Reported Values

Cell concentration. Reported in cells/mL. Calculations are based on Beer-Lambert equation using corrected A600 absorbance value.

Kinetics

Make time-based kinetic measurements using the cuvette holder (NanoDrop Ultra^{C} and NanoDrop Ultra^{C} FL model instruments only).

Manage Kinetics Methods

Measure Kinetics Method

Reported Results

Settings

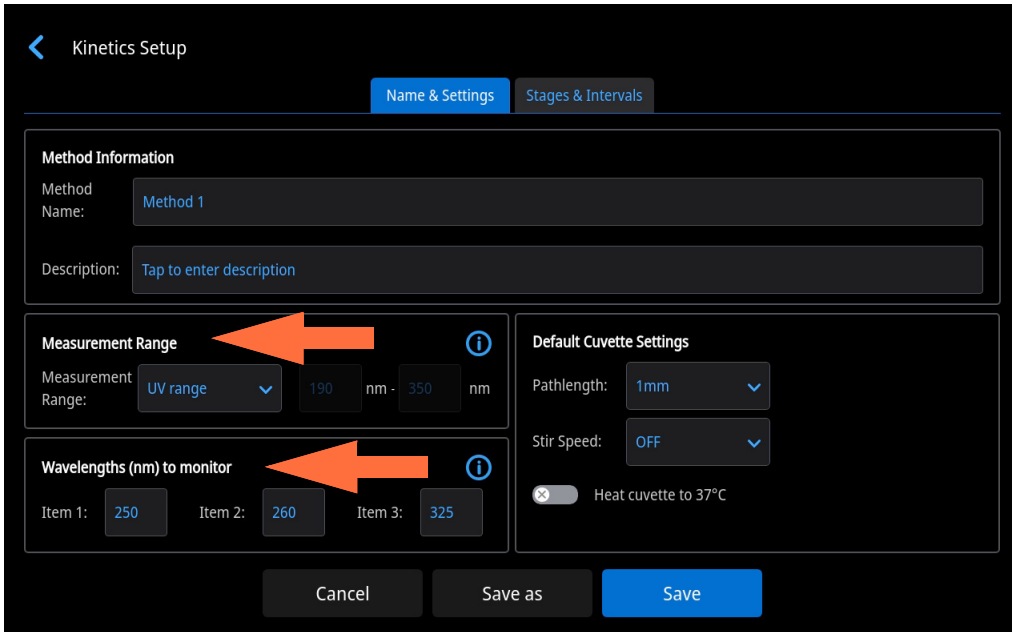
Detection Limits

Manage Kinetics Methods

Create kinetics method

1. From the home screen, select the **More Apps** tab, then select **Kinetics**.
2. Select **+ New Method**.
3. Enter **Method Name** and **Description** (if desired), select **Measurement range** (use the drop down to select ranges, shown below) and specify up to three **Wavelengths to monitor.**

   Select all applicable cuvette specific settings (pathlenth, stir speed, and heating)



4. Select the Stages and Intervals tab. The stages and intervals settings are displayed.

5. Select **Number of stages** and **Time unit** (minutes or seconds)
6. For each stage, specify **# intervals**, **Interval times** and any **Delays** between stages

   The colored rows and boxes at the right visually represent the specified stages. The colored **rows** show the start and end times for each stage; the colored **boxes** correspond with the specified delay and number of intervals for each stage.

7. To save the method and return to the Kinetics menu, select **Save**.

Note The **Method Name**, **Interval Time**, and **# Intervals** must be defined before you are able to save the method.

View or edit kinetics method

1. From the Kinetics screen, select ⋮ next to the desired method to edit.
2. Select **Edit**.
3. View and adjust the method settings as desired.
4. Select **Save**.

Copy kinetics method

To create a kinetics method that is similar to an existing one, open the existing method, make your changes, then select Save As and enter a new name.

1. From the Kinetics screen, select  next to the desired method to copy.
2. Select **Edit**.
3. View and adjust the method settings as desired.
4. Enter new **Method Name** and **Description**.
5. Select **Save As**.
6. Enter a new name for the method and click **Save**.

Export kinetics method

Export a kinetics method in order to run it and store the measurement results on another NanoDrop Ultra instrument or another computer running the NanoDrop Ultra PC control software.

1. From the Kinetics screen, select  next to the desired method to export.
2. Select **Export** (if method is invalid, an error message is displayed; errors must be fixed before method can be exported).
3. Select an available export location from the drop-down menu and choose **Export** (method is exported to method file (*.method filename extension) in proprietary format).

To transfer the method to another NanoDrop Ultra instrument or NanoDrop Ultra PC control software, copy the method file to a USB memory device and then load the method (see Import kinetics method for details).

Import kinetics method

Import a kinetics method back to a NanoDrop Ultra instrument or a computer running the NanoDrop Ultra PC control software to run or edit the method.

1. From the **Kinetics** screen, select **Import**.
2. Locate and select ".method" file.
3. Select **Import** (imported method is added to end of the method selection pane).

Delete kinetics method

1. From the **Kinetics** screen, select  next to the desired method to delete.
2. Select **Delete**.
3. After the confirmation message, select **Yes**.

Measure Kinetics

The NanoDrop Ultra^{C} and NanoDrop Ultra^{C} FL model instruments can be used to make time-based kinetic measurements on samples in cuvettes. Up to 3 wavelengths between 190 nm and 850 nm can be designated for continuous absorbance monitoring at user-defined intervals in up to 5 stages. Cuvette measurements offer an extended lower detection limit and an optional 37 °C heater and micro-stirrer.

Note The instrument arm can be up during cuvette measurements, which allows you to add reagents to the sample solution if desired.

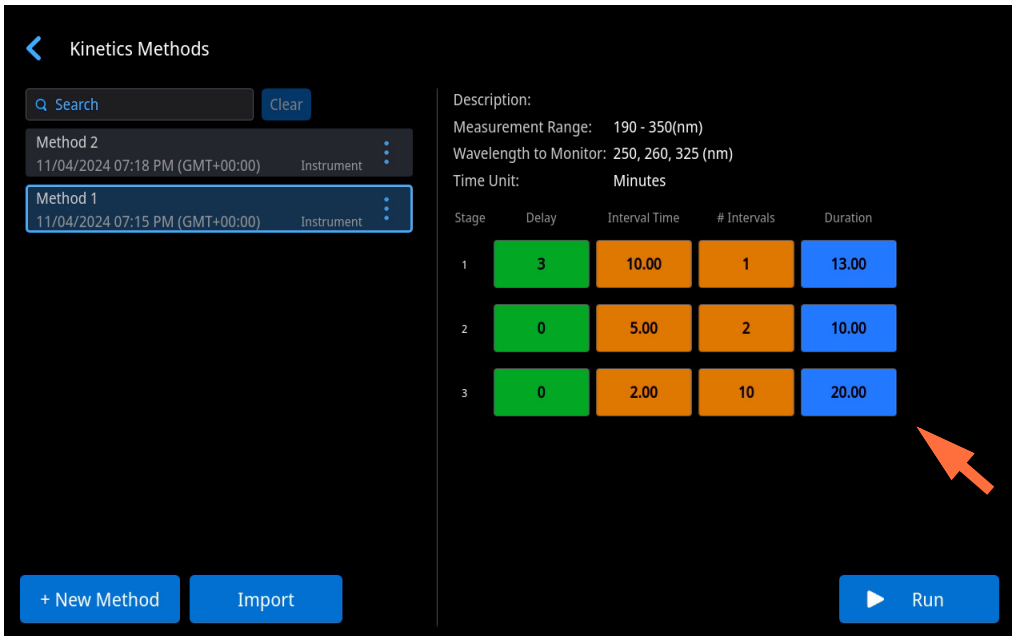
To make kinetic measurements

NOTICE

- To prevent damage from spills, keep containers of liquids away from the instrument.
- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.

Procedure

1. From the home screen, select the **More Apps** tab, then select **Kinetics**.
2. In the method selection pane, select the method to run.



Information about the selected method appears in the method details pane.

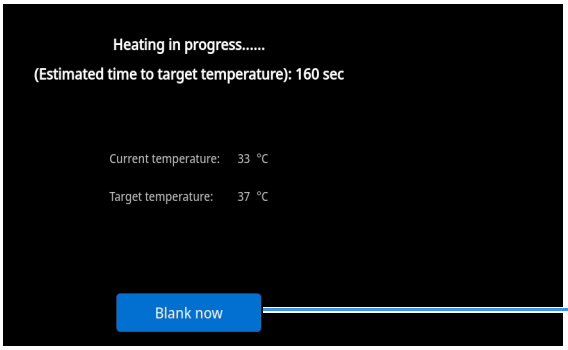
3. Select 

.

   Note: If your cuvette pathlength is not 10 mm, specify the correct pathlength in Kinetics Settings.

4. Measure a blank:
   - Fill clean, dry cuvette with enough blanking solution to cover instrument optical path
   - Lift instrument arm and insert blanking cuvette into cuvette holder, making sure to align light path of cuvette with light path of instrument
   - Select **Blank**

     If **Heat Cuvette to 37 °C** is selected in see Kinetics Settings, a message tells you the current temperature and waits for the heater to reach the target temperature before starting the measurements:



To override the wait and start the blank measurement immediately, tap Blank Now.

   - Wait for blank measurement to complete and then remove cuvette

     Note: The heater target temperature is not adjustable.

5. Measure a sample:
   - Fill clean, dry cuvette with enough sample solution to cover optical path
   - Insert sample cuvette into cuvette holder, making sure to align light paths
   - Select **Measure**

     If **Heat Cuvette to 37 °C** is selected in Kinetics Settings, a message tells you the current temperature and waits for the heater to reach the target temperature before starting the measurements:

     Note: You may add reagents to the sample solution at any time during the measurement

     Use the **Pause and Resume** button at the bottom of the measurement screen to pause and resume the experiment (if you need to end the experiment early, select **Stop**)

- Wait for all measurement stages to complete
- Remove cuvette and clean it according to manufacturer specifications

Results for each measurement in each interval are displayed in real time. When all stages are completed, the spectra and reported values for the entire experiment are displayed.

6. When you are finished reviewing the data, select **End Experiment**. Each saved experiment contains one complete set of kinetic measurements based on the selected method.

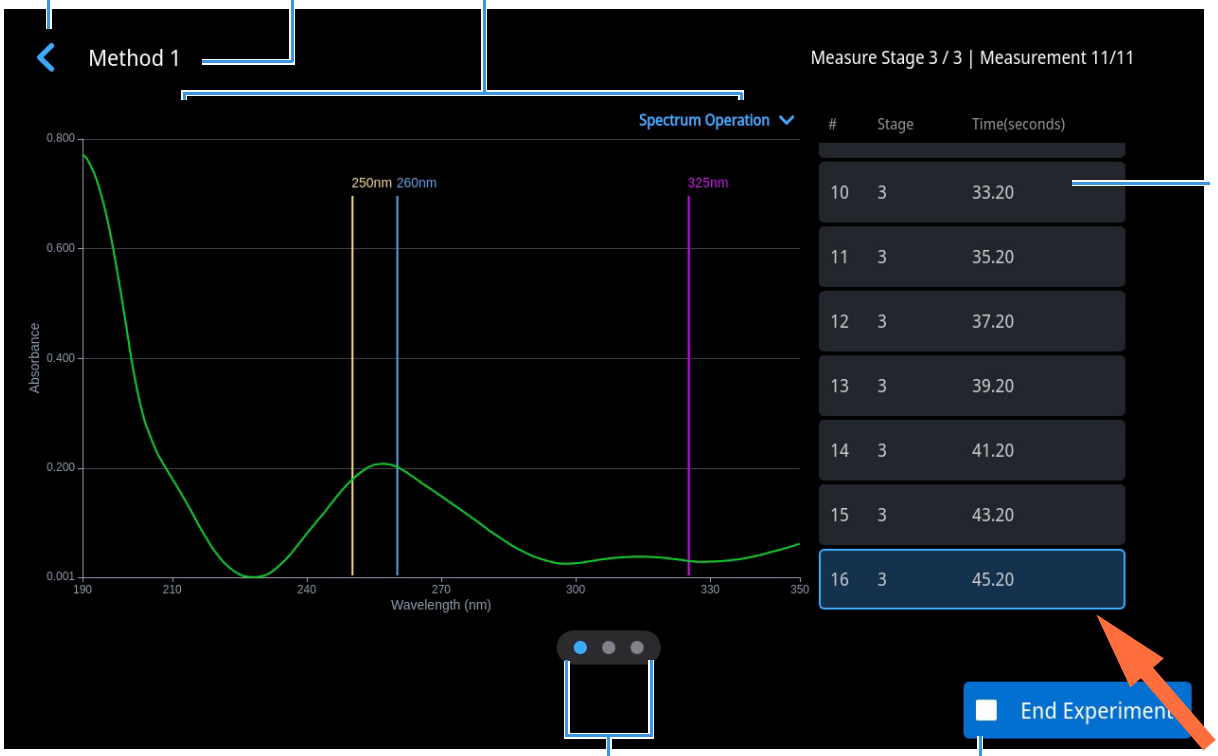
Related Topics

- Measure a Sample Using a Cuvette
- Best Practices for Cuvette Measurements
- Prepare Samples and Blanks
- Basic Instrument Operations

Reported Results

Absorbance measurement screen (local control)

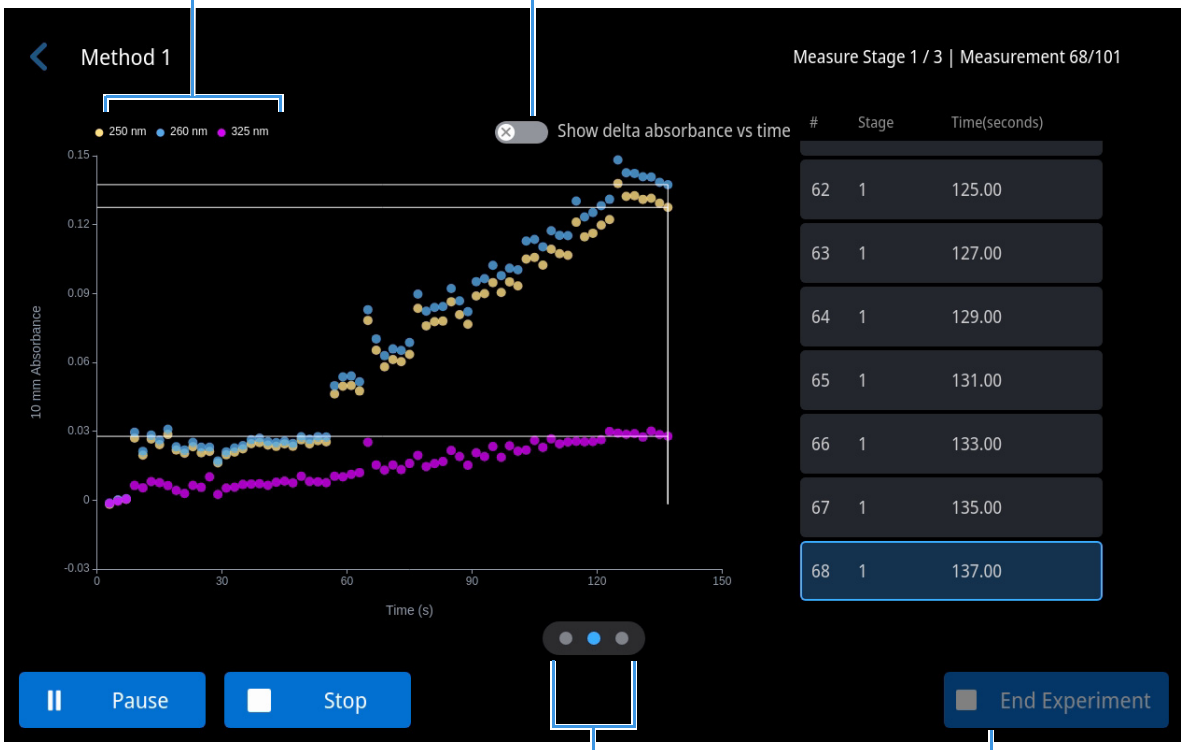
The absorbance measurement screen appears immediately after you select **Measure** in the kinetics experiment. This screen shows the absorbance spectrum for each measurement, with wavelength on the X-axis and absorbance on the Y-axis. Vertical lines indicate the specified wavelengths to monitor. The list at the right shows the time each measurement was taken in each specified stage (drag the tab down and up to see more entries). Each item in the list at the right has a corresponding absorbance spectrum at the left. The following image highlights the available features on the NanoDrop Ultra local control software.



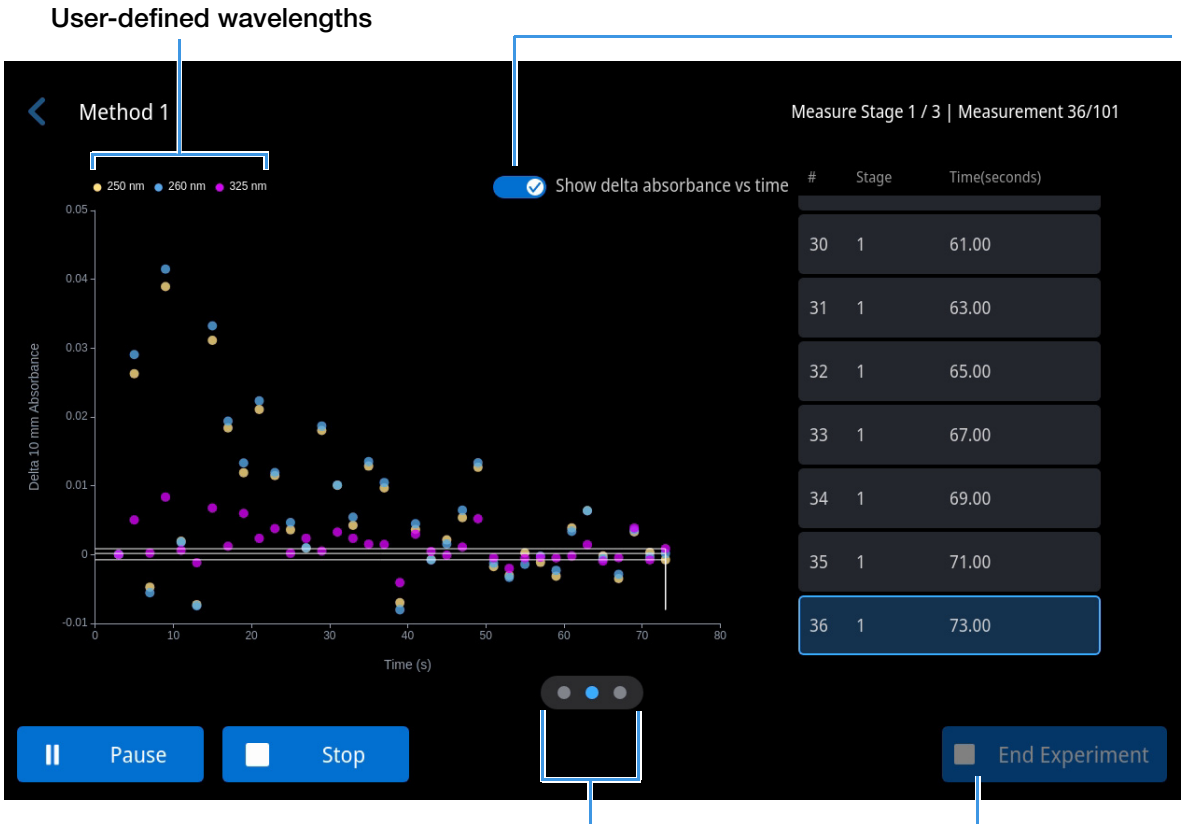
Note For measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent.

Rate measurement screen (local control)

To see the Rate measurement screen, swipe the absorbance measurement screen (see above) to the left. The Rate measurement screen shows a sample's absorbance measured at each user-defined wavelength over time, with time on the X-axis and absorbance on the Y-axis. Measurements taken at each specified wavelength are presented in a unique color. A key showing the monitored wavelengths and their assigned colors appears in the upper left corner of the screen.

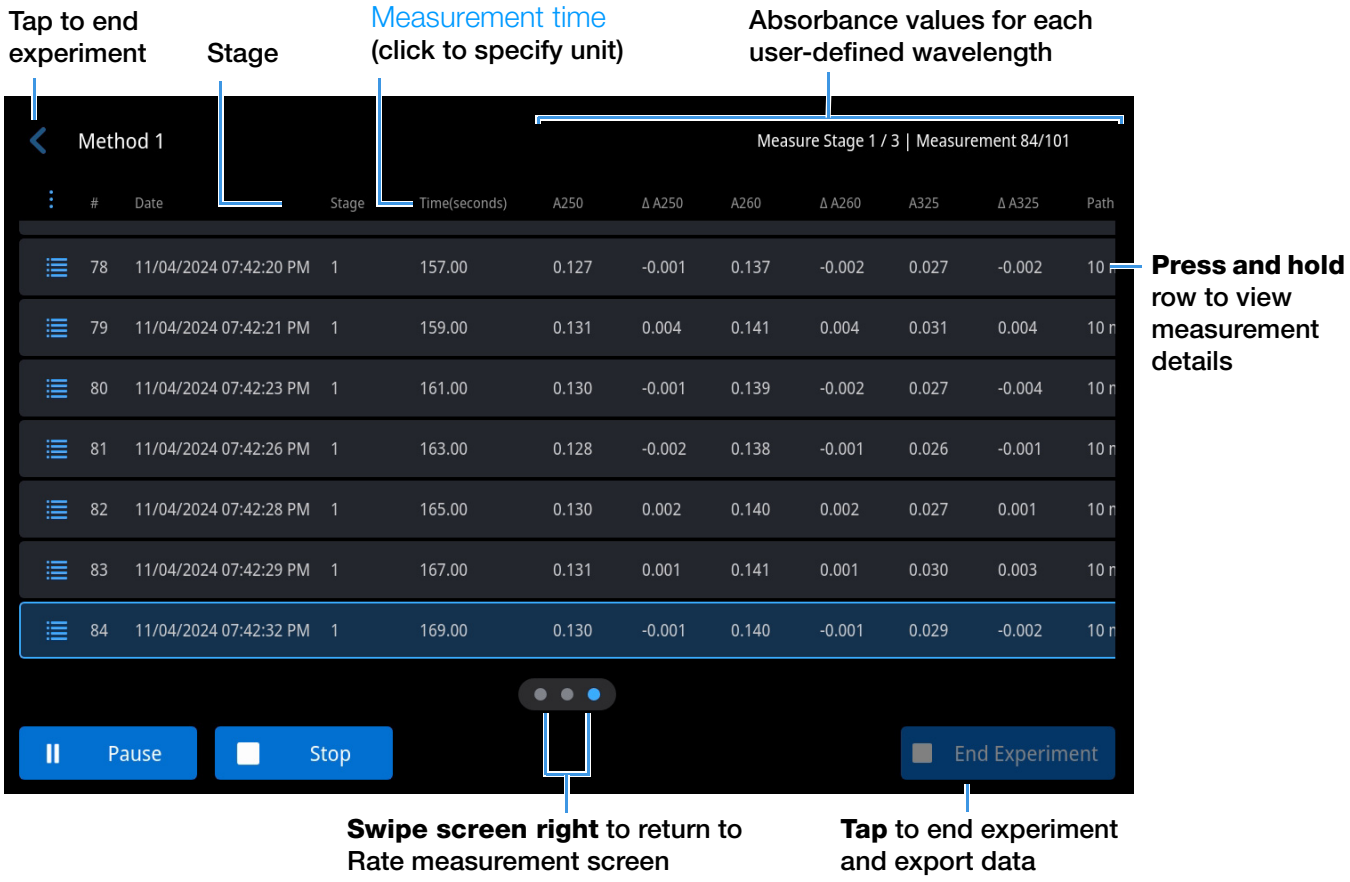


Use the slider button next to **Show Delta Absorbance Vs Time** to show the change in measured absorbance over time, where each data point is the difference in absorbance from the previous measurement.



Data Table (local control)

To see the data table, in local control, swipe the rate measurement screen to the left. Each row in the table shows the absorbance values at all user-defined wavelengths at a given stage and time. Scroll down to see measurement information that is out of view. The image below highlights the available features.

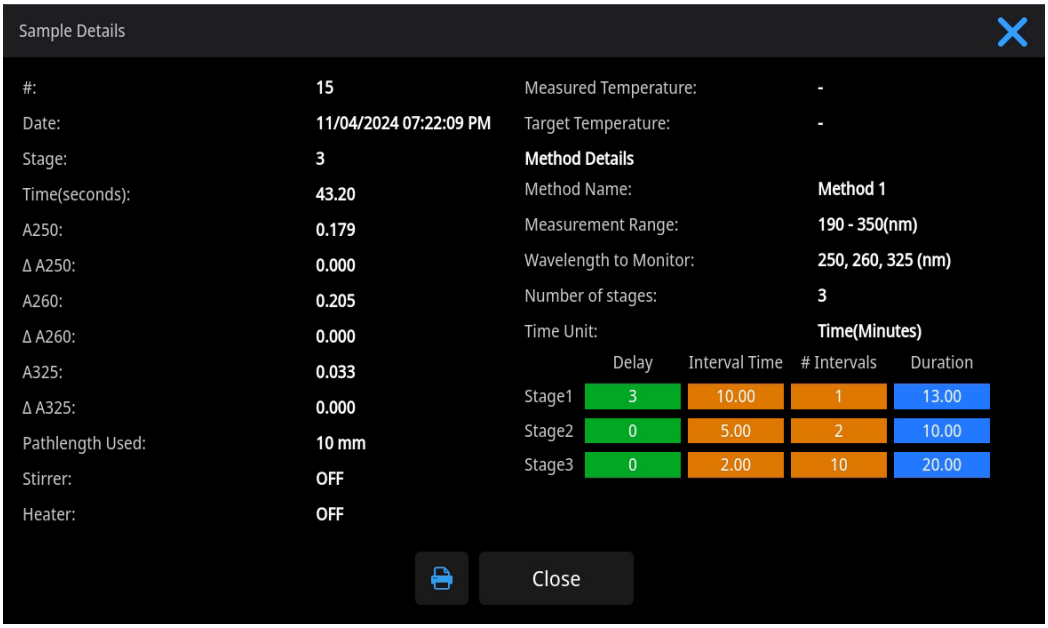


Absorbance measurement screen (PC control)

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. The layout of the measurement screen of the PC control software differs slightly from the local control. See “Measurement Screen Display Options” on page 301 for an example.

Reported values

The initial screen that appears after each measurement shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:



Related Topics

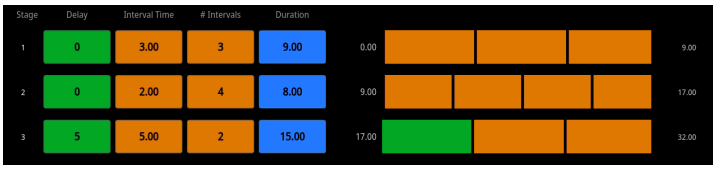
- Basic Instrument Operations

Settings

These settings are available for creating kinetics methods.

Tab	Setting	Description
Name and Settings	Method name	**Enter a name** for this method (this name appears in the Kinetics Setup box after the method has been saved).
	Description	Enter a detailed **description** for this method, if desired, such as the type of samples, added reagents, etc.
	Measurement range	**Select the spectral range** in which this method will acquire data. Available options: • Ultra-violet only (190 nm - 350 nm) • Visible only (350 nm - 850 nm) • Ultra-violet and visible (190 nm - 850 nm) • Custom range (specify starting and ending point in nanometers) **Note**: For measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent.
	Monitored wavelengths	**Enter up to 3 wavelengths** to be measured and reported at run time. **Note**: All specified wavelengths must fall within the selected measurement range.
	Pathlength:	Enter cuvette pathlength (width) before taking blank or sample measurements with cuvettes (see cuvette manufacturer for cuvette specifications)
	Stir Speed:	If using automatic stirring, drop micro-stir bead into sample cuvette and set Stir Speed (levels 1 through 9 correspond with range from 10 RPM to 850 RPM with controlled ramping from zero)
	Heat cuvette to 37 °C:	Select this option if sample cuvettes require heating. Cuvette heater increases from room temperature to 37 °C at rate of 5 °C/minute

Tab	Setting	Description
Stages and Intervals	Number of Stages	**Specify up to 5 stages** for kinetic measurements. Each stage can have unique Delay, Interval Time and # Intervals settings. **Note**: Many kinetic measurements include only one stage. Additional stages are necessary only when a variation in stage interval or duration is needed.
	Time Unit	Select the **unit** for time-based measurements (seconds or minutes).
	Stage 1, 2, etc.	Specify the available settings for each stage: • **Delay**. Specify a delay before a stage starts. • **Interval Time**. Specify the length of time between measurements taken during this stage (minimum is 2 seconds). The first measurement occurs when the stage starts (or after the delay is completed if a Delay is specified). **Note**: If two or more stages are specified with Delay set to zero, two measurements occur at the same time (the measurement at the beginning of the new stage directly overlaps the one at the end of the previous stage). • **# Intervals**. Specify the number of absorbance measurements to take in this stage. **Note**: Since the first measurement is taken when the stage starts, the number of measurements reported for each stage will be the **# Intervals** setting plus 1. • **Duration**. Readout shows the total time required for this stage, including any delay and all specified intervals.

Tab	Setting	Description
		The colored **rows** at the right (see image below) show the start and end times for each stage; the colored **boxes** at the right correspond with the specified delay and number of intervals for each stage.  If no delay is specified, absorbance measurements are taken at the start and end of each stage and after each specified interval. If a delay is specified, as in stage 3 above, the first measurement occurs at the start of the first interval. If the unit is seconds in the example above, a total of 11 measurements are taken at the following times over a period of 32 seconds:
		• Stage 1: 0, 3, 6 and 9 seconds • Stage 2: 9, 11, 13, 15 and 17 seconds • Stage 3: 22, 27 and 32 seconds **Note**: Kinetic experiments are limited to 1000 measurements. This means the total number of measurements from all intervals in all stages must be less than 1000. Consider available instrument or computer disc space for lengthy experiments.

Related Topics

- Instrument Settings

9

Learning Center

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- PC Control Software 353

Micro-Volume Sampling—How it Works

Surface Tension

Absorbance Spectrum

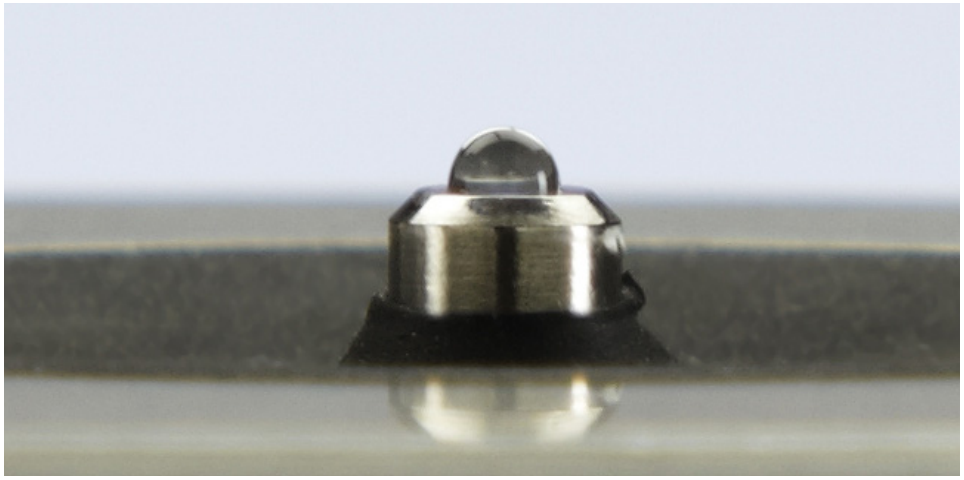
Sample Absorbance

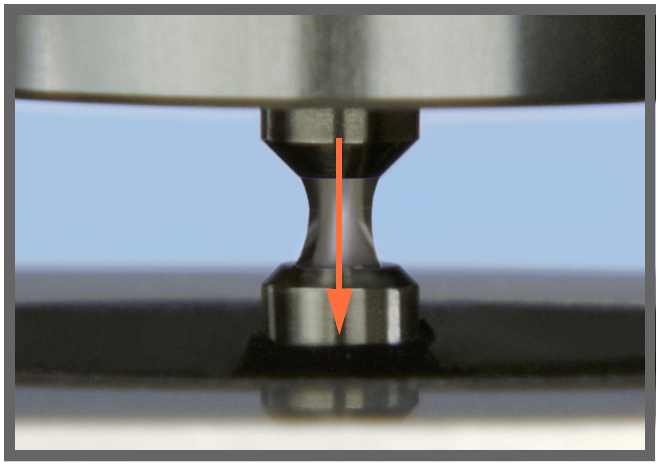
Sample Concentration

Baseline Correction

Sample Fluorescence

Fluorescence Sample Concentration

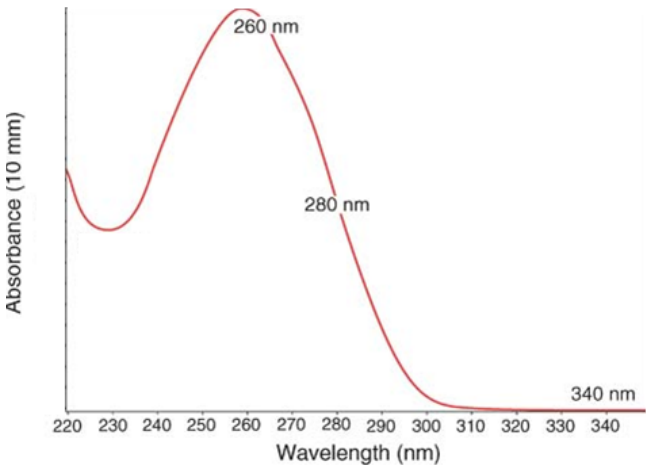




Surface Tension

The NanoDrop Ultra Spectrophotometers and Fluorometers use surface tension to hold a small volume of sample between two pedestals. The patented sample retention system enables the measurement of highly concentrated samples without the need for dilutions.

A fiber optic cable embedded in the upper pedestal leads to a xenon light source. A second cable embedded in the lower pedestal leads to a detector. When the instrument arm is down, the sample forms a liquid column, essentially bridging the gap between the two fiber optic cables.



$$\text{Absorbance} = -\log\left[\frac{\text{intensity}_{\text{sample}}}{\text{intensity}_{\text{blank}}}\right]$$

Beer-Lambert equation

$$A = \varepsilon \cdot b \cdot c$$

where:

A = absorbance in absorbance units (A)

ε = wavelength-dependent molar absorptivity coefficient (or extinction coefficient) in liter/mol-cm

b = pathlength in cm

c = analyte concentration in moles/liter or molarity (M)

Absorbance Spectrum

The light passes through the liquid column to the detector, which generates a spectrum of absorbance versus wavelength. The spectrum shows the amount of light absorbed by the molecules of the sample at each measured wavelength.

Note: To prevent evaporation, which affects measurement accuracy, close the arm quickly after you finish loading a sample or blank.

The example at the left shows a typical absorbance spectrum taken of a nucleic acid sample. The spectrum is measured from 190 nm to 850 nm. The displayed range may vary for each application.

Sample Absorbance

When the instrument is blanked, a reference spectrum is taken of the blanking solution and stored in memory. For each sample measurement, the sample intensities along with the blank intensities are used to calculate the total absorbance of the sample according to the equation at the left.

Sample Concentration

The Beer-Lambert equation (Beer's law) shown at the left is used to correlate sample absorbance with concentration.

The pathlength is the distance between the two pedestals, which varies in real time during each measurement. This auto-ranging pathlength technique produces accurate concentration results over a wide dynamic range.

Baseline Correction

For some applications, the instrument can be set up to apply a baseline correction to each measurement to minimize any offset caused by light scattering particulates in the sample spectra. The correction subtracts the absorbance value at a reference wavelength that is close to zero from the absorbance value at each wavelength across the spectrum, essentially “anchoring” the spectrum to zero absorbance units at the reference wavelength.

Sample Relative Fluorescence

The Red or Blue LED is used as the light source, the light passes through an excitation filter, through the liquid column, through an emission filter to the detector, which records relative fluorescence of the sample.

Fluorescence Sample Concentration

A curve-fitting algorithm which requires a minimum of two standards is used in the calculation of concentration data. The NanoDrop Ultra dsDNA BR, dsDNA HS, and RNA HS Fluorescence Assays all use a modified Hill plot.