NanoDrop Micro-UV/Vis Spectrophotometers

NanoDrop One

User Guide

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Thermo SCIENTIFIC

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For Research Use Only. This instrument or accessory is not a medical device and is not intended to be used for the prevention, diagnosis, treatment or cure of disease.

WARNING Avoid an explosion or fire hazard. This instrument or accessory is not designed for use in an explosive atmosphere.

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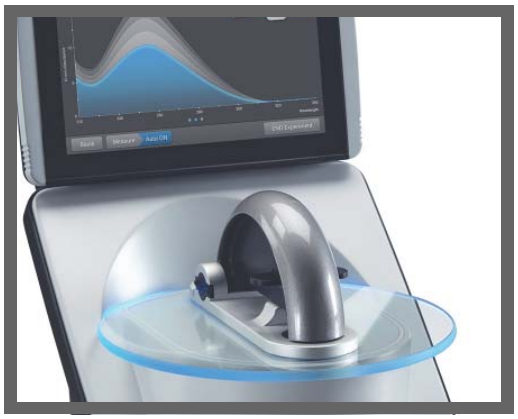
About the NanoDrop One Spectrophotometer



The Thermo Scientific™ NanoDrop™ One is a compact, stand-alone UV-Visible spectrophotometer developed for micro-volume analysis of purified nucleic acids and a wide variety of proteins. The patented sample retention system enables the measurement of highly concentrated samples without the need for dilutions.

The NanoDrop One system comes with preloaded software and a touchscreen display. The instrument can be connected to an optional USB label printer.

NOTICE Before operating a NanoDrop One instrument, please read the safety and operating precautions and then follow their recommendations when using the instrument.

Instrument Models and Features

There are two models available for the NanoDrop One spectrophotometer...

Optional Accessories

A number of accessories are available for the NanoDrop One instruments...

Register Your Instrument

Register your instrument to receive e-mail updates on software and...

Update Software

Quickly and easily download the latest NanoDrop One software...

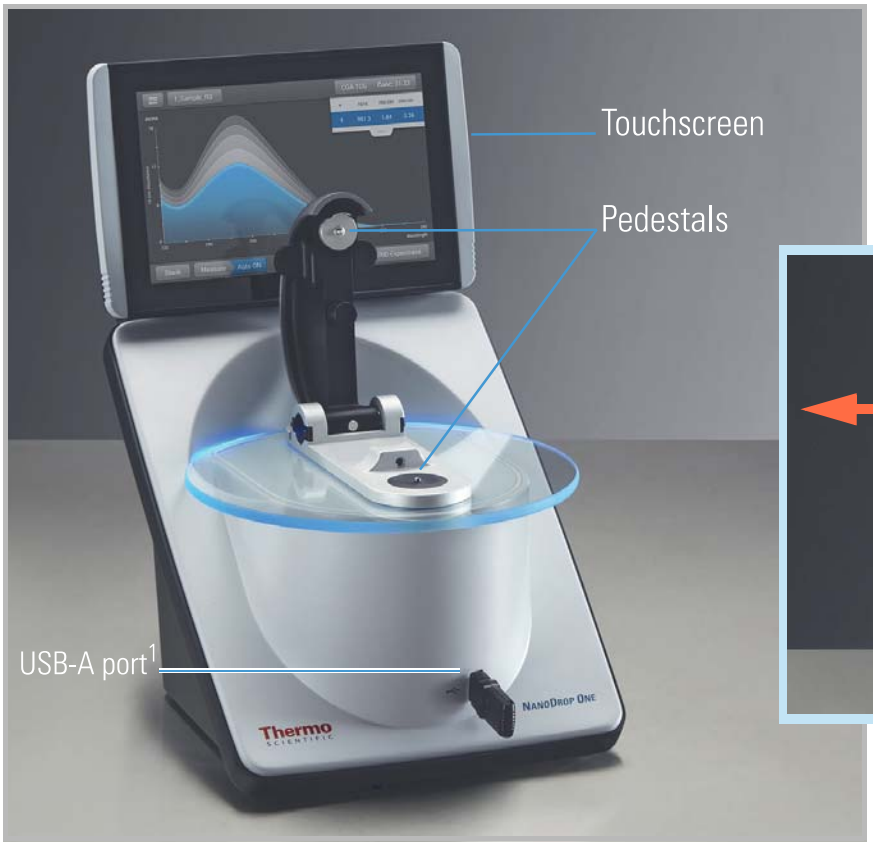
Instrument Models and Features

There are two models available for the NanoDrop One spectrophotometer—the NanoDrop One and the NanoDrop One^{C}. Both models include the patented micro-volume sample retention system and general features. The NanoDrop One^{C} model also features a cuvette holder for analyzing dilute samples using standard UV-visible cuvettes.

Both instruments come with a built-in, 7-inch Android high-resolution touchscreen preloaded with easy-to-use instrument control software. The NanoDrop One software is loaded with features to integrate with and simplify your daily workflows.

[1]Locate the instrument away from air vents and exhaust fans to minimize evaporation

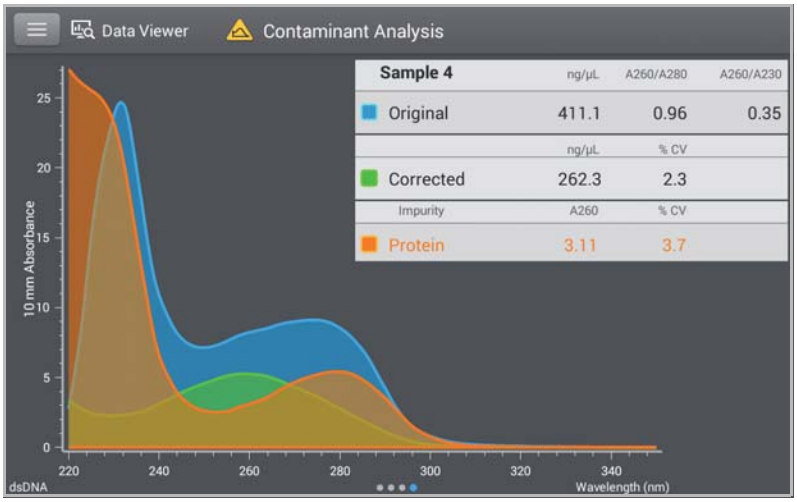
Touchscreen



Touchscreen can slide left or right to accommodate personal preference, and tilt forward or back for optimal viewing

[1]Two more USB-A ports are located on instrument back panel

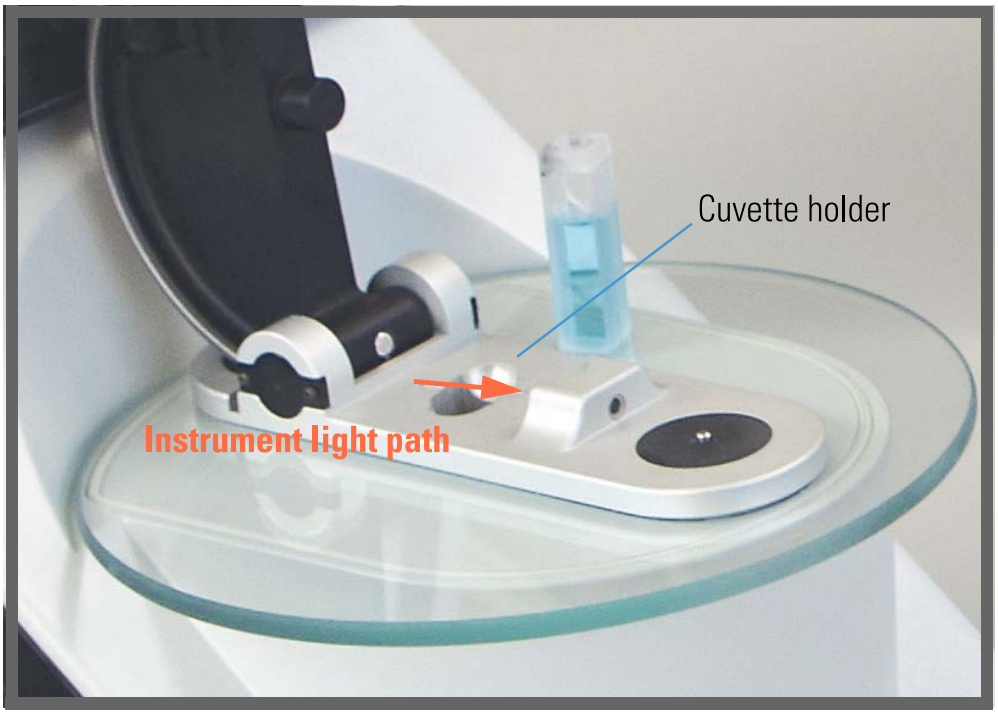
NanoDrop One Software with Acclaro Sample Intelligence Technology



The Thermo Scientific™ Acclaro™ Sample Intelligence technology built into the NanoDrop One instruments provides these exclusive features to help you assess sample integrity:

- contaminant analysis to help qualify a sample before use in downstream applications
- on-demand technical support for measurements that are atypical or very low concentration
- invalid result alerts (a column sensor monitors for the presence of bubbles or reflective particles that can compromise measurement results)

NanoDrop One^{C} Model Additional Features



The NanoDrop One^{C} model includes a cuvette holder for measuring dilute samples, colorimetric assays, cell cultures and kinetic studies. The cuvette system has these additional features:

- extended lower detection limits
- 37 °C heater option for temperature-sensitive samples and analyses
- micro-stirring option to ensure sample homogeneity and support kinetic studies

For details, see Measure a Sample using a Cuvette.

Optional Accessories

A number of accessories are available for the NanoDrop One instruments. To order an accessory, contact your local distributor or visit our website.

DYMO™ LabelWriter™ 450 USB Label Printer

Prints two 5/16-in x 4-in self-adhesive labels for transferring sample data directly into laboratory notebooks or posting on bulletin boards. The software allows printing of data from each sample measurement or from a group of samples logged and measured together.

The printer connects to the instrument (front or back) via a USB cable (included).

PR-1 Pedestal Reconditioning Kit

Specially formulated conditioning compound that can be applied to the pedestals to restore them to a hydrophobic state (required to achieve adequate surface tension for accurate sample measurements). The kit includes conditioning compound and applicators. For more information, see Reconditioning the Pedestals.

PV-1 Performance Verification Solution

Liquid photometric standard used to check instrument performance. For more information, see Performance Verification.

Register Your Instrument

Register your instrument to receive e-mail updates on software and accessories for the NanoDrop One instruments. An Internet connection is required for registration.

To register your instrument

1. Do one of the following:
   - From the NanoDrop One Viewer software running on a personal computer (PC) that is connected to the Internet, open the Help menu and choose NanoDrop One Website.
   - From any PC that is connected to the Internet, use any web browser to navigate to our website.
2. On the website, locate NanoDrop One Registration and follow the instructions to register the instrument.

Update Software

Quickly and easily download and install the latest NanoDrop One software and release notes from our website. Follow the steps to update or upgrade the software on your local instrument and/or install or update the NanoDrop One Viewer software on a personal computer (PC). An Internet connection is required to download software.

To install or update NanoDrop One Viewer software

1. Do one of the following:
   - To install the Viewer software on a computer for the first time, open any web browser and find the NanoDrop website.
   - To update or upgrade the Viewer software, from the Viewer Home screen, open the Help menu and choose NanoDrop One Website to open our website.
2. On the NanoDrop website, locate the software downloads page.
3. Select to download NanoDrop One (PC) Viewer software (English version) and follow the instructions to download and run the installer. (A computer restart is required after the installer completes.)
4. To add a language, including software and Help systems, download and run the language pack installer (English must be installed first). (No computer restart is required after a language installer completes.)

To update or upgrade NanoDrop One instrument software

1. Do one of the following:
   - From the NanoDrop One Viewer software, open the Help menu and choose NanoDrop One Website to open our website.
   - From any personal computer that is connected to the Internet, navigate to the NanoDrop website.
2. Insert a USB device such as a memory stick into a USB port on the computer.
3. On the NanoDrop website, locate the software downloads page, select to update or upgrade NanoDrop One operating software (English version) and follow the instructions to download the installer to the USB device.
4. To add a language, including software and Help systems, download the language pack installer(s) to the USB device.
5. Insert the USB device into any USB port on the NanoDrop One instrument.
6. From the instrument Home screen, tap  (Settings) > System > Update Software.

   If the USB device contains more than one version of the installer, a message is displayed. Select the version to install (English installer must be run first) and tap Update. (An instrument restart is required after the English installer completes.)

   When the installation is complete, a message similar to the following appears next to the Update Software button:

   Version: 1.2.0 (currently installed version of instrument operating software)
   Database version: 1 (version of NanoDrop One database on this instrument)
7. To add a language, including software and Help systems, tap Update Software again, select the language and version to install and tap Update. (No instrument restart is required after a language installer completes.)

Note: To change the language, tap Language, select an installed language and tap OK. (An instrument restart is required after you change the language.)

Applications

Detection Limits for All Applications

Note Detection limits provided in the tables below are approximate and apply to micro-volume measurements only; they are based on the instrument's photometric absorbance range (10 mm equivalent) of 0–550 A. For measurements with 10 mm pathlength cuvettes, the photometric absorbance range is 0–1.5 A.

Detection limits for standard applications

Sample Type	Lower Detection Limit	Upper Detection Limit	Typical Reproducibility[a]
dsDNA	2.0 ng/µL (pedestal) 0.20 ng/µL (cuvette)	27,500 ng/µL (pedestal) 75 ng/µL (cuvette)	±2.0 ng/µL for sample concentrations between 2.0 and 100 ng/µL samples; ±2% for samples >100 ng/µL
ssDNA	1.3 ng/µL (pedestal) 0.13 ng/µL (cuvette)	18,150 ng/µL (pedestal) 49.5 ng/µL (cuvette)	±2.0 ng/µL for sample concentrations between 2.0 and 100 ng/µL samples; ±2% for samples >100 ng/µL
RNA	1.6 ng/µL (pedestal) 0.16 ng/µL (cuvette)	22,000 ng/µL (pedestal) 60 ng/µL (cuvette)	±2.0 ng/µL for sample concentrations between 2.0 and 100 ng/µL samples; ±2% for samples >100 ng/µL

Sample Type	Lower Detection Limit	Upper Detection Limit	Typical Reproducibility[a]
DNA Microarray (ssDNA)	1.3 ng/µL (pedestal) 0.13 ng/µL (cuvette)	495 ng/µL (pedestal) 49.5 ng/µL (cuvette)	±2.0 ng/µL for sample concentrations between 2.0 and 100 ng/µL samples; ±2% for samples >100 ng/µL
Purified BSA by Protein A280	0.06 mg/mL (pedestal) 0.006 mg/mL (cuvette)	825 mg/mL (pedestal)	±0.10 mg/mL (for 0.10–10 mg/mL samples); ±2% for samples >10 mg/mL
IgG by Protein A280	0.03 mg/mL (pedestal) 0.003 mg/mL (cuvette)	402 mg/mL (pedestal)	
Purified BSA by Proteins & Labels	0.06 mg/mL (pedestal) 0.006 mg/mL (cuvette)	19 mg/mL (pedestal)	±0.10 mg/mL for 0.10–10 mg/mL samples
Protein BCA	0.2 mg/mL (20:1 reagent/sample volume) 0.01 mg/mL (1:1 reagent/sample volume)	8.0 mg/mL (pedestal) 0.20 mg/mL (cuvette)	2% over entire range 0.01 mg/mL over entire range
Protein Lowry	0.2 mg/mL (pedestal)	4.0 mg/mL (pedestal)	2% over entire range
Protein Bradford	100 µg/mL (50:1 reagent/sample volume) 15 µg/mL (1:1 reagent/sample volume)	8000 µg/mL 100 µg/µL	±25 µg/mL for 100–500 µg/mL samples ±5% for 500–8000 µg/mL samples ±4 µg/mL for 15–50 µg/mL samples ±5% for 50–125 µg/mL samples
Protein Pierce 660	50 µg/mL (15:1 reagent/sample volume) 25 µg/mL (7.5:1 reagent/sample volume)	2000 µg/mL 1000 µg/mL	±3 µg/mL for 50–125 µg/mL samples ±2% for samples > 125 µg/mL ±3 µg/mL for 25–125 µg/mL samples ±2% for samples >125 µg/mL

[a] Based on five replicates (SD=ng/µL; CV=%)

Note To minimize instrument error with highly concentrated samples, make dilutions to ensure that measurements are made within these absorbance limits:

- For micro-volume measurements, maximum absorbance at 260 nm (for nucleic acids) or 280 nm (for proteins) should be less than 62.5 A.
- For measurements with 10 mm pathlength cuvettes, maximum absorbance at 260 nm (or 280 nm for proteins) should be less than 1.5 A, which is approximately 75 ng/µL dsDNA.

Detection limits for pre-defined dyes

Sample Type	Lower Detection Limit	Upper Detection Limit[a]	Typical Reproducibility[b]
Cy3, Cy3.5, Alexa Fluor 555, Alexa Fluor 660	0.2 pmol/µL (pedestal)	100 pmol/µL (pedestal)	±0.20 pmol/µL for sample concentrations between 0.20 and 4.0 pmol/µL; ±2% for samples >4.0 pmol/µL
Cy5, Cy5.5, Alexa Fluor 647	0.12 pmol/µL (pedestal)	60 pmol/µL (pedestal)	±0.12 pmol/µL for sample concentrations between 0.12 and 2.4 pmol/µL; ±2% for samples >2.4 pmol/µL
Alexa Fluor 488, Alexa Fluor 594	0.4 pmol/µL (pedestal)	215 pmol/µL (pedestal)	±0.40 pmol/µL for sample concentrations between 0.40 and 8.0 pmol/µL; ±2% for samples >8.0 pmol/µL
Alexa Fluor 546	0.3 pmol/µL (pedestal)	145 pmol/µL (pedestal)	±0.30 pmol/µL for sample concentrations between 0.30 and 6.0 pmol/µL; ±2% for samples >6.0 pmol/µL

[a] Values are approximate

[b] Based on five replicates (SD=ng/µL; CV=%)

Measure dsDNA, ssDNA or RNA

Measures the concentration of purified dsDNA, ssDNA or RNA samples that absorb at 260 nm.

Measure dsDNA, ssDNA or RNA

Reported Results

Settings

Detection Limits

Calculations

Measure dsDNA, ssDNA or RNA

Use the dsDNA, ssDNA and RNA applications to quantify purified double-stranded (ds) or single-stranded (ss) DNA or RNA samples. These applications report nucleic acid concentration and two absorbance ratios (A260/A280 and A260/A230). A single-point baseline correction can also be used.

To measure dsDNA, ssDNA or RNA samples

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.

Before you begin...

Before taking pedestal measurements with the NanoDrop One 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.

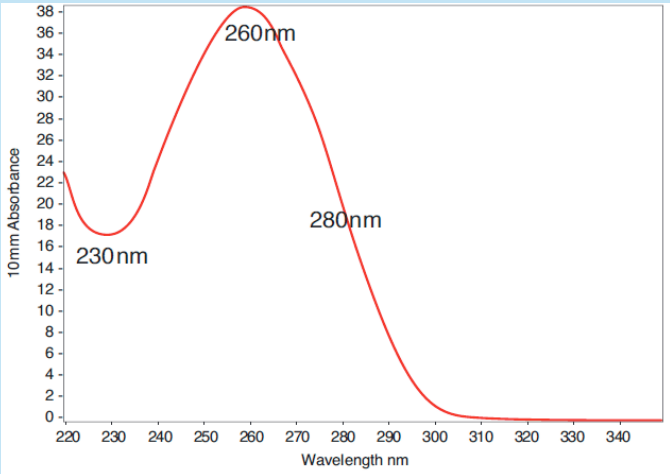
To measure nucleic acid

1. From the Home screen, select the Nucleic Acids tab and tap dsDNA, ssDNA or RNA, depending on the samples to be measured.
2. Specify a baseline correction if desired.
3. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder.

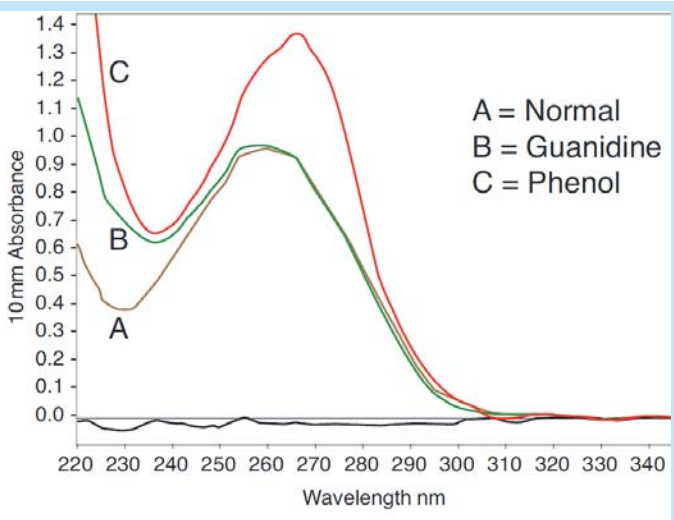
   Tip: If using a cuvette, make sure to align the cuvette light path with the instrument light path.
4. Tap Blank and wait for the measurement to complete.

   Tip: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements.)
5. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.
6. Pipette 1-2 µL sample solution onto the pedestal and lower the arm, or insert the sample cuvette into the cuvette holder.
7. Start the sample measurement:
   - Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is off, lower arm and tap Measure.
   - Cuvette: Tap Measure.

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).
8. When you are finished measuring samples, tap End Experiment.
9. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.



Typical nucleic acid spectrum



Comparison of nucleic acid spectra with and without two common contaminants

Best practices for nucleic acid measurements

- Isolate and purify nucleic acid samples before measurement to remove impurities. Depending on the sample, impurities could include DNA, RNA, free nucleotides, proteins, some buffer components and dyes. See Preparing Samples for more information.

  Note Extraction reagents such as guanidine, phenol, and EDTA contribute absorbance between 230 nm and 280 nm and will affect measurement results if present in samples (even residual amounts).

- Ensure the sample absorbance is within the instrument's absorbance detection limits.
- Blank with the same buffer solution used to resuspend the analyte of interest. The blanking solution should be a similar pH and ionic strength as the analyte solution.
- Run a blanking cycle to assess the absorbance contribution of your buffer solution. If the buffer exhibits strong absorbance at or near the analysis wavelength (typically 260 nm), you may need to choose a different buffer or application. See Choosing and Measuring a Blank for more information.
- For micro-volume measurements:
  - Ensure pedestal surfaces are properly cleaned and conditioned.
  - If possible, heat highly concentrated or large molecule samples, such as genomic or lambda DNA, to 63 °C (145 °F) and gently (but thoroughly) vortex before taking a measurement. Avoid introducing bubbles when mixing and pipetting.
  - Follow best practices for micro-volume measurements.
  - Use a 1-2 µL sample volume. See Recommended Sample Volumes for more information.
- For cuvette measurements (NanoDrop One^{C} instruments only), use compatible cuvettes and follow best practices for cuvette measurements.

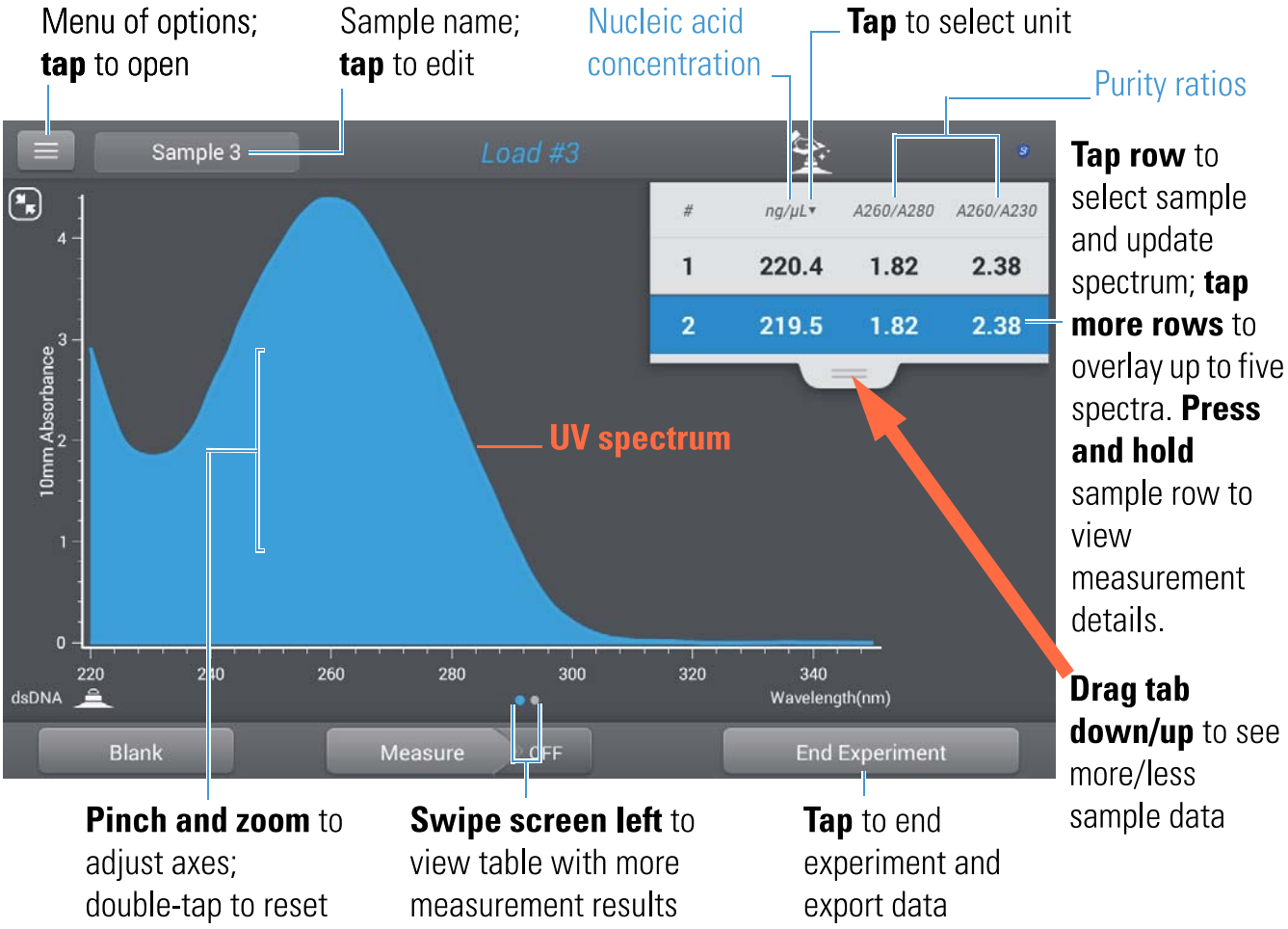
Related Topics

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

Nucleic Acid Reported Results

dsDNA measurement screen

For each measured sample, the dsDNA, ssDNA and RNA applications show the UV absorbance spectrum and a summary of the results. Here is an example:



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

dsDNA, ssDNA and RNA 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:

- sample details (application and sampling method used, i.e., pedestal or cuvette)
- sample name
- created on (date sample measurement was taken)
- nucleic acid concentration
- A260/A280
- A260/A230
- A260
- A280
- factor
- baseline correction

Related Topics

- Basic Instrument Operations
- Nucleic Acid Calculations

Setting for Nucleic Acid Measurements

To show the dsDNA, ssDNA or RNA settings, from the dsDNA, ssDNA or RNA measurement screen, tap  > Nucleic Acid Setup.

Setting	Available Options	Description
Baseline Correction	On or off Enter baseline correction wavelength in nm or use default value (340 nm)	Optional user-defined baseline correction. Can be used to correct for any offset caused by light scattering particulates by subtracting measured absorbance 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.

Related Topics

- Instrument Settings

Calculations for Nucleic Acid Measurements

The nucleic acid applications use the Beer-Lambert equation to correlate absorbance with concentration. Solving Beer's law for concentration yields the equation at the right.

Beer-Lambert Equation (solved for concentration)

$$c = A / (\varepsilon b)$$

where:

A = UV absorbance in absorbance units (AU)

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)

Note: Dividing the measured absorbance of a sample solution by its molar extinction coefficient yields the molar concentration of the sample. See Published Extinction Coefficients for more information regarding molar vs. mass concentration values.

The Nucleic Acid applications use a modification of the Beer-Lambert equation (shown at right) to calculate sample concentration where the extinction coefficient and pathlength are combined and referred to as a "factor."

For the dsDNA, ssDNA and RNA applications, the generally accepted factors for nucleic acids are used in conjunction with Beer's Law to calculate sample concentration. For the Custom Factor application, the user-specified factor is used.

Extinction Coefficients vs Factors

Using the terms in the Beer-Lambert equation, factor (f) is defined as:

$$\text{factor (f)} = 1/(\varepsilon b)$$

where:
ε = wavelength-dependent molar extinction coefficient in ng-cm/µL
b = sample pathlength in cm

As a result, analyte concentration (c) is calculated as:

$$c = A * [1/(\varepsilon b)]$$

or

$$c = A * f$$

where:
c = analyte concentration in ng/µL
A = absorbance in absorbance units (A)
f = factor in ng-cm/µL (see below)

Factors Used

- dsDNA (factor = 50 ng-cm/µL)
- ssDNA (factor = 33 ng-cm/µL)
- RNA (factor = 40 ng-cm/µL)
- Custom Factor (user entered factor between 15 ng-cm/µL and 150 ng-cm/µL

Calculated nucleic acid concentrations are based on the absorbance value at 260 nm, the factor used and the sample pathlength. A single-point baseline correction (or analysis correction) may also be applied.

Concentration is reported in mass units. Calculators are available on the Internet to convert concentration from mass to molar units based on sample sequence.

Absorbance values at 260 nm, 280 nm and sometimes 230 nm are used to calculate purity ratios for the measured nucleic acid samples. Purity ratios are sensitive to the presence of contaminants in the sample, such as residual solvents and reagents typically used during sample purification.

Measured Values

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.

A260 absorbance

- Nucleic acid absorbance values are measured at 260 nm using the normalized spectrum. This is the reported A260 value if Baseline Correction is not selected.
- If Baseline Correction is selected, the absorbance value at the correction wavelength is subtracted from the absorbance at 260 nm. The corrected absorbance at 260 nm is reported and used to calculate nucleic acid concentration.

A230 and A280 absorbance

- Normalized and baseline-corrected (if selected) absorbance values at 230 nm and 280 nm are used to calculate A260/A230 and A260/A280 ratios.

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, pathlength is determined by the cuvette Pathlength setting in the software (see General Settings).
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.

Reported Values

- **Nucleic acid concentration.** Reported in selected unit (i.e., ng/µL, µg/uL or µg/mL). Calculations are based on modified Beer's Law equation using corrected nucleic acid absorbance value.
- **A260/A280 purity ratio.** Ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm. An A260/A280 purity ratio of ~1.8 is generally accepted as "pure" for DNA (~2.0 for RNA). Acidic solutions may under represent the reported value by 0.2-0.3; the opposite is true for basic solutions.
- **A260/A230 purity ratio.** Ratio of corrected absorbance at 260 nm to corrected absorbance at 230 nm. An A260/A230 purity ratio between 1.8 and 2.2 is generally accepted as "pure" for DNA and RNA.

Note: Although purity ratios are important indicators of sample quality, the best quality indicator quality is functionality in the downstream application of interest (e.g., real-time PCR).

Measure Microarray

Measures the concentration of purified nucleic acids that have been labeled with up to two fluorescent dyes for use in downstream microarray applications.

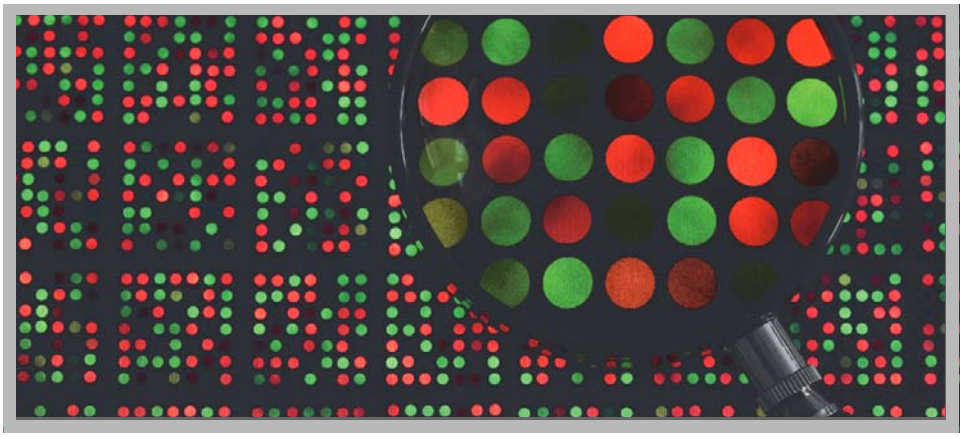
Measure Microarray Samples

Reported Results

Settings

Detection Limits

Calculations



Measure Microarray Samples

Use the Microarray application to quantify nucleic acids that have been labeled with up to two fluorescent dyes. The application reports nucleic acid concentration, an A260/A280 ratio and the concentrations and measured absorbance values of the dye(s), allowing detection of dye concentrations as low as 0.2 picomole per microliter.

To measure microarray samples

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.

Before you begin...

Before taking pedestal measurements with the NanoDrop One 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.

To measure a microarray sample

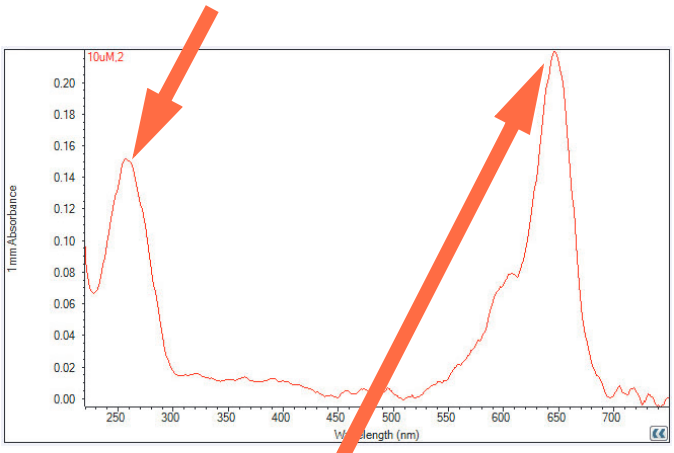
1. From the Home screen, select the Nucleic Acids tab and tap Microarray.
2. Specify the sample type and factor and the type of dye(s) used.

   Tip: Select a dye from the pre-defined list or add a custom dye using the Dye/Chromophore Editor.
3. Pipette 1–2 µL blanking solution 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.
4. Tap 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.)
5. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.
6. Pipette 1-2 µL sample solution onto the pedestal and lower the arm, or insert the sample cuvette into the cuvette holder.
7. Start the sample measurement:
   - Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is off, lower arm and tap Measure.
   - Cuvette: Tap Measure.

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).
8. When you are finished measuring samples, tap End Experiment.
9. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.



Typical microarray spectrum

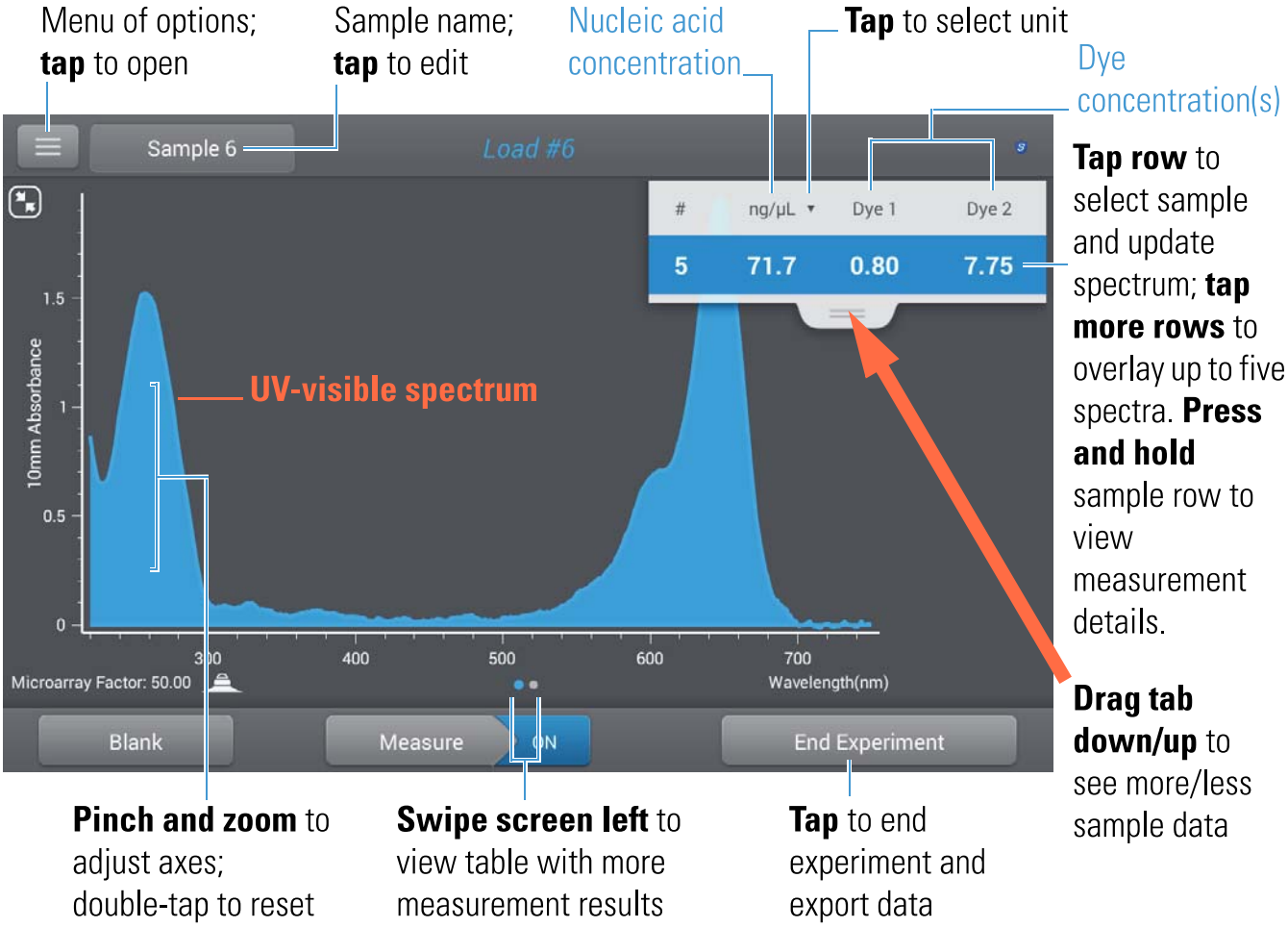
Related Topics

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

Microarray Reported Results

Microarray measurement screen

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



Note

- A baseline correction is performed at 750 nm (absorbance value at 750 nm is subtracted from absorbance values at all wavelengths in sample spectrum).
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

Microarray 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:

- sample details (application used and pedestal or cuvette)
- sample name
- created on (date sample measurement was taken)
- nucleic acid concentration
- A260
- A260/A280
- dye 1/dye 2 concentration
- sample type
- analysis correction
- factor

Related Topics

- Basic Instrument Operations
- Microarray Calculations

Settings for Microarray Measurements

Microarray settings

The Microarray Setup screen appears after you select the Microarray application from the Nucleic Acids tab on the Home screen. To show the Microarry settings from the Microarray measurement screen, tap  > Microarray Setup.

Setting	Available Options	Description
Sample type and Factor	dsDNA (with non-editable factor of 50 ng-cm/µL)	Widely accepted value for double-stranded DNA
	ssDNA (with non-editable factor of 33 ng-cm/µL)	Widely accepted value for single-stranded DNA
	RNA (with non-editable factor of 40 ng-cm/µL)	Widely accepted value for RNA
	Oligo DNA with non-editable calculated factor in ng-cm/µL	Factor calculated from user-defined DNA base sequence. When selected, available DNA base units (i.e., G, A, T, C) appear as keys. Define sequence by tapping appropriate keys. Factor is calculated automatically based on widely accepted value for each base unit.
	Oligo RNA with non-editable calculated factor in ng-cm/µL	Factor calculated from user-defined RNA base sequence. When selected, available RNA base units (i.e., G, A, U, C) appear as keys. Define sequence by tapping appropriate keys. Factor is calculated automatically based on widely accepted value for each base unit.
	Custom (with user-specified factor in ng-cm/µL)	Enter factor between 15 ng-cm/µL and 150 ng-cm/µL
Dye 1/Dye 2 Type[a]	Cy3, 5, 3.5, or 5.5, Alexa Fluor 488, 546, 555, 594, 647, or 660	Select pre-defined dye(s) used to label sample material, or one that has been added using Dye/Chrom. Editor.
Dye 1/Dye 2 Unit	picomoles/microliter (pmol/uL), micromoles (uM), or millimoles (mM)	Select unit for reporting dye concentrations
Analysis Correction[b]	On or off Enter analysis correction wavelength in nm or use default value (340 nm)	Corrects sample absorbance measurement for any offset caused by light scattering particulates by subtracting absorbance value at specified analysis correction wavelength from absorbance value at analysis wavelength. Corrected value is used to calculate sample concentration. Tip: If the sample has a modification that absorbs light at 340 nm, select a different correction wavelength or turn off Analysis Correction.

[a] To add a custom dye or edit the list of available dyes, use the Dye/Chromophore Editor.

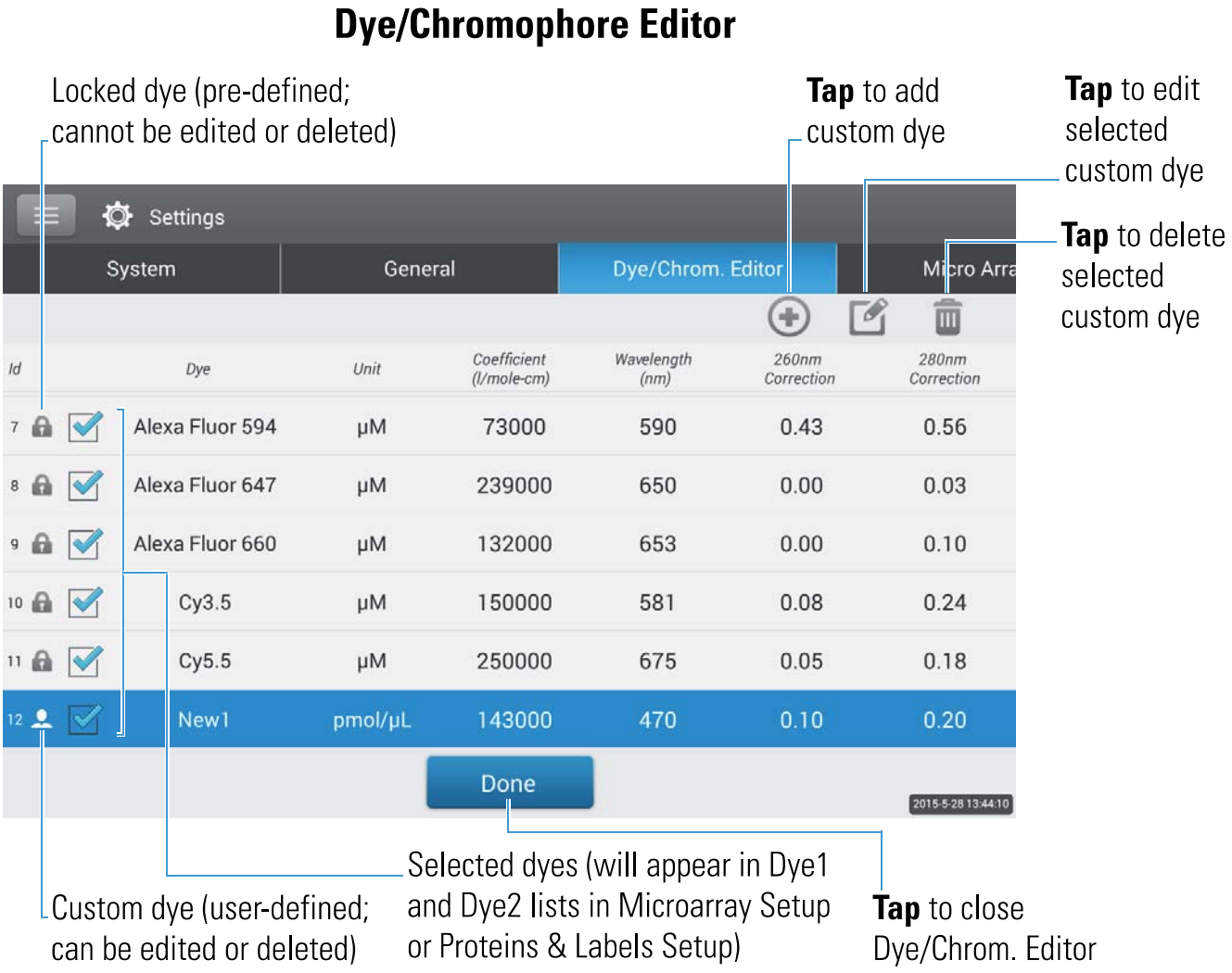
[b] The Analysis Correction affects the calculation for nucleic acid concentration only.

Dye/chromophore editor

Use the Dye/Chromophore Editor to add a custom dye to the list of available dyes in Microarray Setup or Proteins & Labels Setup. You can also specify which dyes are available in that list.

To access the Dye/Chromophore Editor:

- from the Home screen, tap > Dye/Chrom. Editor
- from the Microarray or Proteins & Labels measurement screen, tap > Settings > Dye/Chrom. Editor



These operations are available from the Dye/Chromophore Editor:

Add or remove a dye

To add or remove a dye from the Dye1 or Dye2 drop-down list in Microarray Setup or Proteins & Labels Setup:

– select or deselect corresponding checkbox

Add custom dye

– tap ⊕ to show New Dye box

– enter unique **Name** for new dye (tap field to display keyboard, tap **Done** key to close keyboard)

– select default **Unit** that will be used to display dye concentration

– enter dye's **Extinction Coefficient** (or molar absorptivity constant) in L/mole-cm (typically provided by dye manufacturer)

– specify **Wavelength** in nm (between 450 nm and 700 nm) that will be used to measure dye's absorbance

– specify dye's correction values at 260 nm and 280 nm

– tap **Add Dye**

Note To determine dye correction values (if not available from dye manufacturer):

– use instrument to measure pure dye and note absorbance at 260 nm, 280 nm and at analysis wavelength for dye (see above)

– calculate ratio of $A_{260}/A_{\text{dye wavelength}}$ and enter that value for 260 nm Correction

– calculate ratio of $A_{280}/A_{\text{dye wavelength}}$ and enter that value for 280 nm Correction

When a custom dye is selected before a measurement, the dye's absorbance and concentration values are reported and the corrections are applied to the measured sample absorbance values, and to the resulting sample concentrations and purity ratios.

Edit custom dye

Tip Dyes pre-defined in the software cannot be edited.

– tap to select custom dye

– tap ✎

– edit any entries or settings
– tap Save Dye

Delete custom dye

Tip Dyes pre-defined in the software cannot be deleted.

– tap to select custom dye
– tap 

NOTICE Deleting a custom dye permanently removes the dye and all associated information from the software.

Related Topics

- Instrument Settings

Calculations for Microarray Measurements

As with the other nucleic acid applications, the Microarray 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 Microarray application offers six options (shown at right) for selecting an appropriate factor for each measured sample, to be used in conjunction with Beer's Law to calculate sample concentration.

If the factor is known, choose the Custom Factor option and enter the factor in ng-cm/µL. Otherwise, choose the option that best matches the sample solution.

Tip: Ideally, the factor or extinction coefficient should be determined empirically using a solution of the study nucleic acid at a known concentration using the same buffer.

Available Options for Factors

- dsDNA (factor = 50 ng-cm/µL)
- ssDNA (factor = 33 ng-cm/µL)
- RNA (factor = 40 ng-cm/µL)
- Oligo DNA (calculated from user entered DNA nucleotide sequence)
- Oligo RNA (calculated from user entered RNA nucleotide sequence)
- Custom Factor (user entered factor between 15 ng-cm/µL and 150 ng-cm/µL

Note: See Sample Type for more information.

Calculated nucleic acid concentrations are based on the absorbance value at 260 nm, the factor used and the sample pathlength. A single-point baseline correction (or analysis correction) may also be applied.

Concentration is reported in mass units. Calculators are available on the Internet to convert concentration from mass to molar units based on sample sequence.

Absorbance values at 260 nm, 280 nm and sometimes 230 nm are used to calculate purity ratios for the measured nucleic acid samples. Purity ratios are sensitive to the presence of contaminants in the sample, such as residual solvents and reagents typically used during sample purification.

Dye concentrations are calculated from the absorbance value at the dye's analysis wavelength, the dye's extinction coefficient, and the sample pathlength. A sloped-line dye correction may also be used.

Measured Values

A260 absorbance

Note: The absorbance value at 750 nm is subtracted from all wavelengths in the spectrum. As a result, the absorbance at 750 nm is zero in the displayed spectra. Also, 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.

- Nucleic acid absorbance values for all Microarray sample types are measured at 260 nm using the 750-corrected and normalized spectrum.
- If Analysis Correction is selected, the absorbance value at the correction wavelength is subtracted from the absorbance at 260 nm.
- If one or more dyes are selected, the dye correction values at 260 nm are also subtracted from the absorbance at 260 nm.
- The final corrected absorbance at 260 nm is reported and used to calculate sample concentration.

A280 absorbance

- 750-corrected and normalized absorbance value at 280 nm (minus the A280 dye correction) is used to calculate an A260/A280 ratio.

Dye absorbance

- Dye absorbance values are measured at specific wavelengths. See Dye/Chromophore Editor for analysis wavelengths used.
- If Sloping Dye Correction is selected, a linear baseline is drawn between 400 nm and 750 nm and, for each dye, the absorbance value of the sloping baseline is subtracted from the absorbance value at each dye's analysis wavelength. Baseline-corrected dye absorbance values are reported and used to calculate dye concentrations.

Dye correction

- Pre-defined dyes have known correction values for A260 and A280. See Dye/Chromophore Editor for correction values used.
- A260 dye corrections are subtracted from the A260 absorbance value used to calculate nucleic acid concentration, and from the A260 absorbance value used to calculate the A260/A280 purity ratio.

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, pathlength is determined by the cuvette Pathlength setting in the software (see General Settings).
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.
-

Reported Values

- Nucleic acid concentration. Reported in selected unit (i.e., ng/µL, µg/uL or µg/mL). Calculations are based on modified Beer's Law equation using corrected nucleic acid absorbance value.
- A260/A280 purity ratio. Ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm. An A260/A280 purity ratio of ~1.8 is generally accepted as "pure" for DNA (~2.0 for RNA). Acidic solutions may under represent the reported value by 0.2-0.3; the opposite is true for basic solutions.
- Dye1/Dye2 concentration. Reported in pmol/µL. Calculations are based on Beer's Law equation using (sloping) baseline-corrected dye absorbance value(s).

Note: Although purity ratios are important indicators of sample quality, the best indicator of DNA or RNA quality is functionality in the downstream application of interest (e.g., microarray).

Related Topics

- Calculations for Nucleic Acid Measurements

Measure using a Custom Factor

Measures the concentration of purified nucleic acids using a custom factor for the calculations.

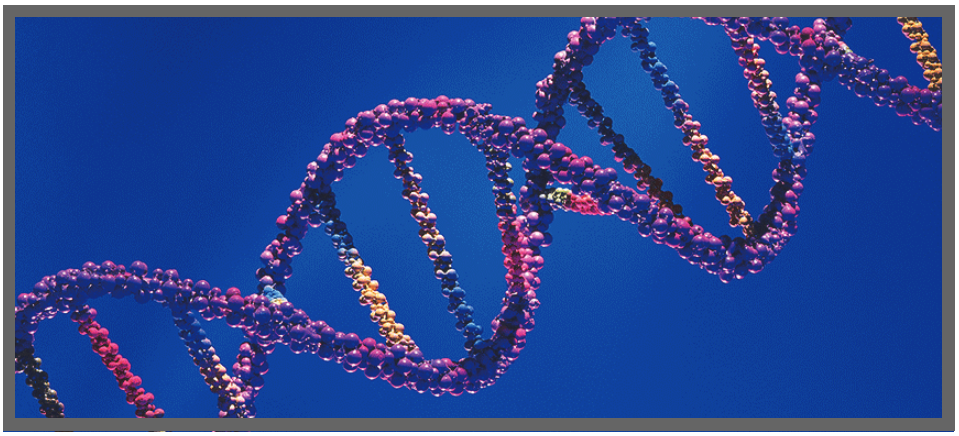
Measure using Custom Factor

Reported Results

Settings

Detection Limits

Calculations



Measure Nucleic Acid using a Custom Factor

Use the Custom Factor application to quantify purified DNA or RNA samples that absorb at 260 nm with a user-defined extinction coefficient or factor. The application reports nucleic acid concentration and two absorbance ratios (A260/A280 and A260/A230). A single-point baseline correction can also be used.

To measure nucleic acid samples using a custom factor

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.

Before you begin...

Before taking pedestal measurements with the NanoDrop One 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.

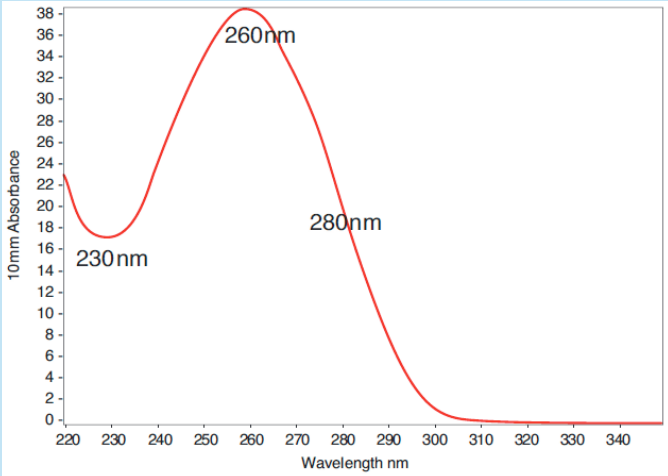
To measure using a custom factor

1. From the Home screen, select the Nucleic Acids tab and tap Custom Factor.
2. Enter the factor to be used for the calculations and specify a baseline correction if desired.
3. Pipette 1–2 µL blanking solution 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.
4. Tap 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.)
5. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.
6. Pipette 1-2 µL sample solution onto the pedestal and lower the arm, or insert the sample cuvette into the cuvette holder.
7. Start the sample measurement:
   - Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is off, lower arm and tap Measure.
   - Cuvette: Tap Measure.

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).
8. When you are finished measuring samples, tap End Experiment.
9. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.



Typical nucleic acid spectrum

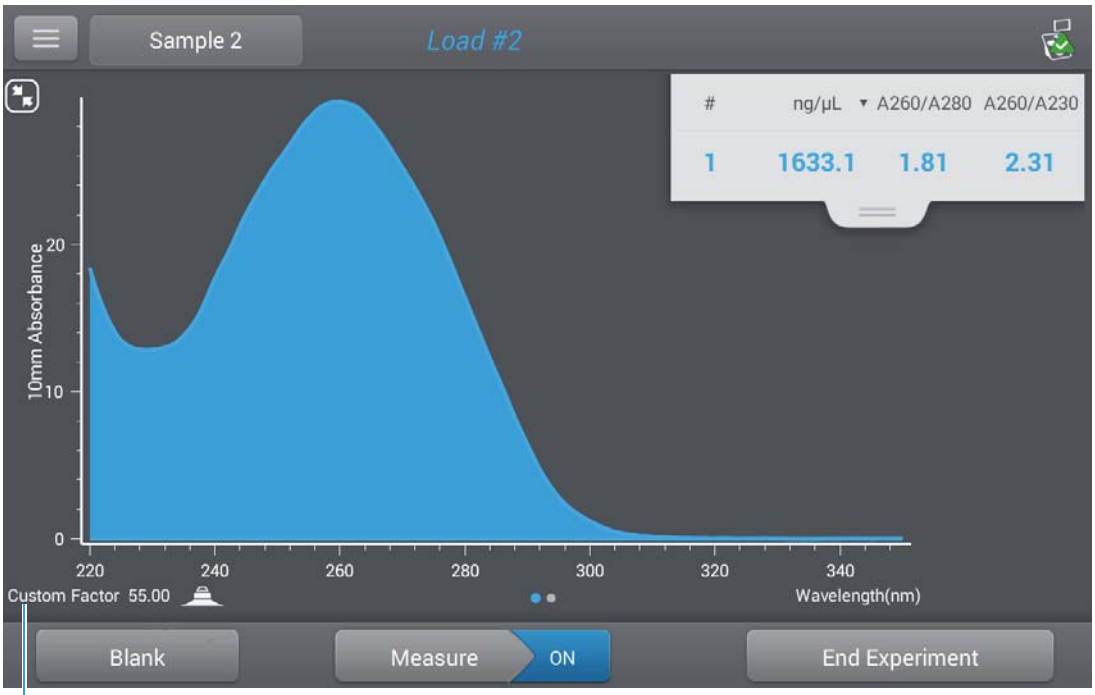
Related Topics

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

Custom Factor Reported Results

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

Note The Custom Factor measurement screen is identical to the measurement screen for the other nucleic acid applications except the Custom Factor is reported in the lower left corner (see image below).



Custom factor used to calculate nucleic acid concentration

Related Topics

- Basic Instrument Operations
- Nucleic Acid Reported Results
- Nucleic Acid Calculations

Settings for Nucleic Acid Measurements using a Custom Factor

To show the Custom Factor settings, tap ☰ > Custom Factor Setup.

Setting	Available Options	Description
Custom Factor	Enter an integer value between 15 ng-cm/µL and 150 ng-cm/µL	Constant used to calculate nucleic acid concentration in modified Beer's Law equation. Based on extinction coefficient and pathlength: $f = 1/(\varepsilon_{260} * b)$ where: f = factor ε_{260} = molar extinction coefficient at 260 nm in ng-cm/µL b = sample pathlength in cm (1 cm for nucleic acids measured with the NanoDrop One instruments)
Baseline Correction	On or off Enter baseline correction wavelength in nm or use default value (340 nm)	Optional user-defined baseline correction. Can be used to correct for any offset caused by light scattering particulates by subtracting measured absorbance 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.

Related Topics

- Instrument Settings

Detection Limits for Nucleic Acid Measurements using a Custom Factor

The lower detection limits and reproducibility specifications for nucleic acids are provided here. The upper detection limits are dependent on the upper absorbance limit of the instrument and the user-defined extinction coefficients.

To calculate upper detection limits for nucleic acid samples

To calculate upper detection limits in ng/µL, use the following equation:

$$(\text{upper absorbance limit}_{\text{instrument}} * \text{extinction coefficient}_{\text{sample}})$$

For example, for a sample measurement using an extinction coefficient of 55, the equation looks like this:

(550 AU * 55 ng-cm/µL) = 30,250 ng/µL

Note For measurements with 10 mm pathlength cuvettes, the upper absorbance limit is 1.5 AU, which is approximately 75 ng/µL for dsDNA.

Related Topics

- Detection Limits for All Applications

Measure Oligo DNA or Oligo RNA

Measures the concentration of purified ssDNA or RNA oligonucleotides that absorb at 260 nm.

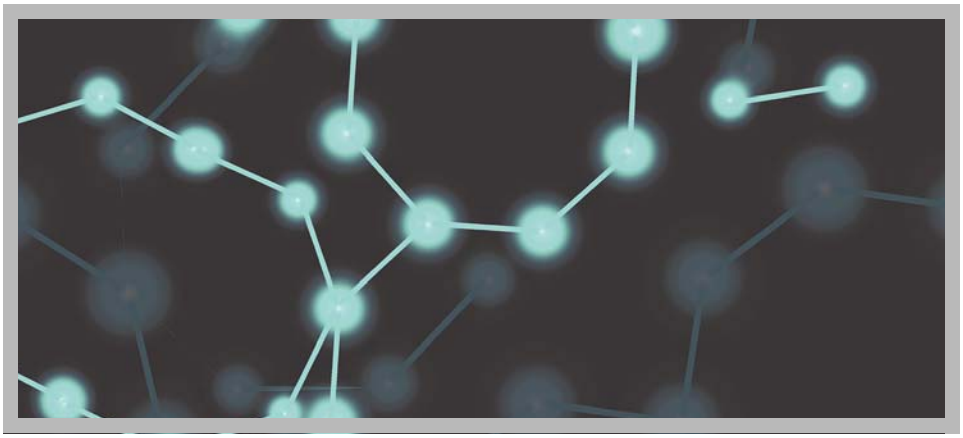
Measure Oligo DNA or RNA

Reported Results

Settings

Detection Limits

Calculations



Measure Oligo DNA or Oligo RNA

Use the Oligo DNA and Oligo RNA applications to quantify oligonucleotides that absorb at 260 nm. Molar extinction coefficients are calculated automatically based on the user-defined base sequence of the sample. These applications report nucleic acid concentration and two absorbance ratios (A260/A280 and A260/A230). A single-point baseline correction can also be used.

Note If the oligonucleotide has been modified, for example with a fluorophore dye, check with the oligo manufacturer to determine if the modification contributes absorbance at 260 nm. If it does, we recommend using the Microarray application to quantify nucleic acid concentration. The Microarray application includes a correction to remove any absorbance contribution due to the dye from the oligo quantification result.

To measure Oligo DNA or Oligo RNA samples

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.

Before you begin...

Before taking pedestal measurements with the NanoDrop One 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.

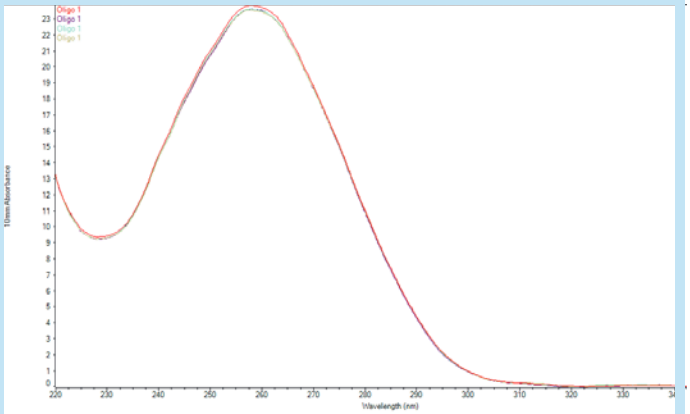
To measure an oligonucleotide sample

1. From the Home screen, select the Nucleic Acids tab and tap either Oligo DNA or Oligo RNA, as needed.
2. Specify the Oligo base sequence and a baseline correction if desired.
3. Pipette 1–2 µL blanking solution 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.
4. Tap 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.)
5. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.
6. Pipette 1-2 µL sample solution onto the pedestal and lower the arm, or insert the sample cuvette into the cuvette holder.
7. Start the sample measurement:
   - Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is off, lower arm and tap Measure.
   - Cuvette: Tap Measure.

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).
8. When you are finished measuring samples, tap End Experiment.
9. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.



Example Oligo DNA spectrum

Related Topics

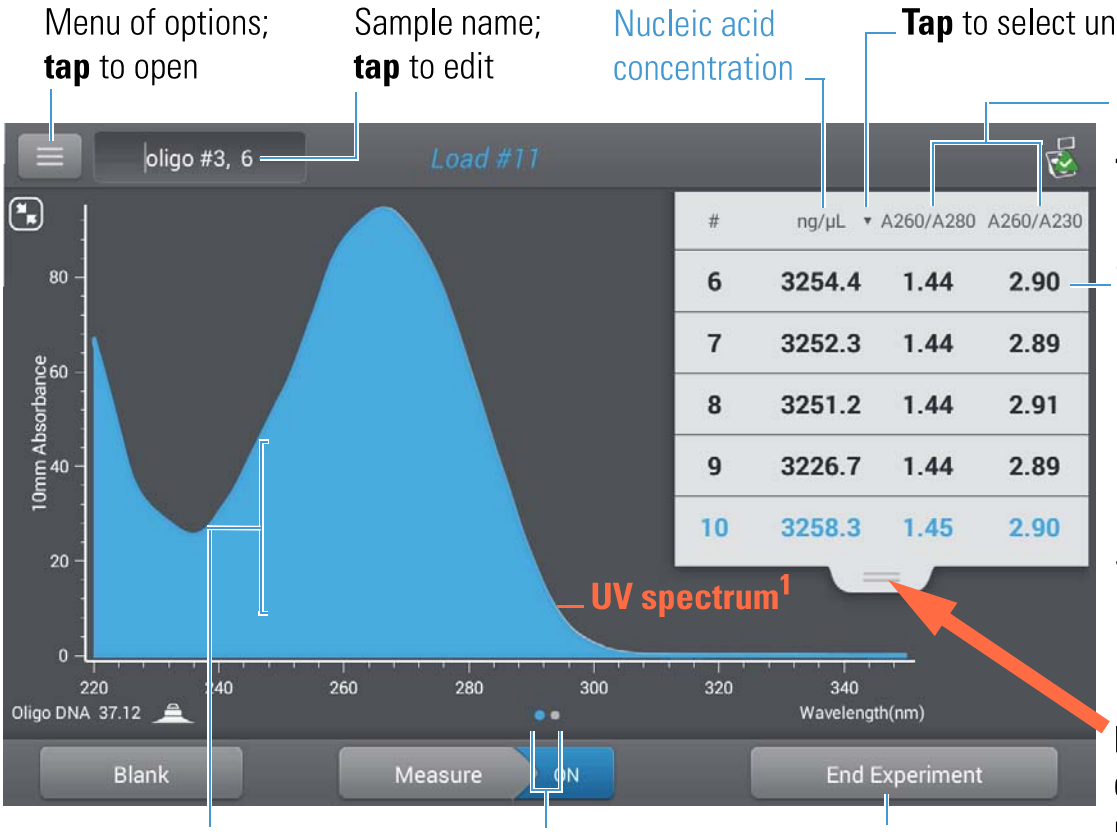
- Best Practices for Nucleic Acid Measurements

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

Oligo Reported Results

Oligo DNA measurement screen

For each measured sample, the Oligo DNA and Oligo RNA applications show the UV absorbance spectrum and a summary of the results. Here is an example:



[1]Measured oligo: TTT TTT TTT TTT TTT TTT TTT TTT

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

Oligo DNA and Oligo RNA 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:

- sample details (application and sampling method used, i.e., pedestal or cuvette)
- sample name
- created on (date sample measurement was taken)
- nucleic acid concentration
- A260/A280
- A260/A230
- A260
- A280
- factor
- oligo sequence
- baseline correction
- stirrer status

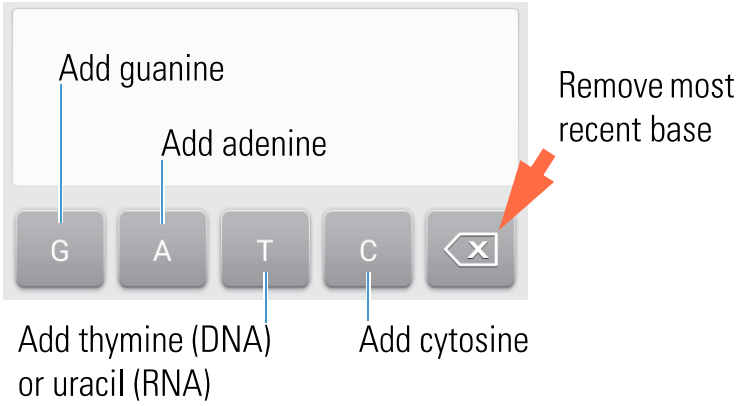
Note The five nucleotides that comprise DNA and RNA exhibit widely varying A260/A280 ratios. See Oligo Purity Ratios for more information.

Related Topics

- Basic Instrument Operations
- Oligo Calculations

Settings for Oligo DNA and Oligo RNA Measurements

The Oligo setup screen appears after you select the Oligo DNA or Oligo RNA application from the Nucleic Acids tab on the Home screen. To show the Oligo settings from the Oligo DNA or Oligo RNA measurement screen, tap ☰ > Oligo DNA Setup (or Oligo RNA Setup).

Setting	Available Options	Description
Oligo Base Sequence	for DNA: Use the G, A, T and C keys to specify the DNA base sequence for RNA: Use the G, A, U and C keys to specify the RNA base sequence	Specify your DNA or RNA base sequence by tapping the corresponding keys:  Each time a base is added to the sequence, the software calculates the following: • Factor. Constant used to calculate oligonucleotide concentration in modified Beer's Law equation. Based on extinction coefficient and pathlength: f = 1/(ε_{260} * b) where: f = factor ε = molar extinction coefficient at 260 nm in ng-cm/µL b = sample pathlength in cm (0.1 cm for nucleic acids measured with the NanoDrop One instrument)

Setting	Available Options	Description
		• Molecular Weight of oligo calculated from user-defined base sequence. • Number of Bases entered. • Molar Ext. Coefficient (260 nm). Molar extinction coefficient of oligo (in ng-cm/µL) at 260 nm calculated from entered base sequence. • %GC. Percentage of guanine and cytosine residues in total number of bases entered.
Baseline Correction	On or off Enter baseline correction wavelength in nm or use default value (340 nm)	Corrects for any offset caused by light scattering particulates by subtracting measured absorbance 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. Tip: If the sample has a modification that absorbs light at 340 nm, select a different correction wavelength or turn off Baseline Correction.

Related Topics

- Instrument Settings

Detection Limits for Oligo DNA and Oligo RNA Measurements

The lower detection limits and reproducibility specifications for the oligonucleotide sample types (ssDNA and RNA) are provided here. The upper detection limits are dependent on the upper absorbance limit of the instrument and the extinction coefficients for the user-defined base sequences.

To calculate upper detection limits for nucleic acid samples

To calculate upper detection limits in ng/µL, use the following equation:

$$(\text{upper absorbance limit}_{\text{instrument}} * \text{extinction coefficient}_{\text{sample}})$$

For example, for a sample measurement using an extinction coefficient of 55, the equation looks like this:

(550 AU * 55 ng-cm/µL) = 30,250 ng/µL

Note For measurements with 10 mm pathlength cuvettes, the upper absorbance limit is 1.5 AU, which is approximately 75 ng/µL for dsDNA.

Related Topics

- Detection Limits for All Applications

Calculations for Oligo DNA and Oligo RNA Measurements

As with the other nucleic acid applications, the Oligo applications use the Beer-Lambert equation to correlate absorbance with concentration based on the sample's extinction coefficient and pathlength. Because oligonucleotides are short, single-stranded molecules (or longer molecules of repeating sequences), their spectrum and extinction coefficient (ε) are closely dependent on base composition and sequence.

(The generally accepted extinction coefficients and factors for single-stranded DNA and RNA provide a reasonable estimate for natural, essentially randomized, sequences but not for short, synthetic oligo sequences.) To ensure the most accurate results, we use the exact value of ε_{260} to calculate oligonucleotide concentration.

The NanoDrop software allows you to specify the base sequence of an oligonucleotide before it is measured. For any entered base sequence, the software uses the equation at the right to calculate the extinction coefficient.

Tip: The extinction coefficient is wavelength specific for each oligonucleotide and can be affected by buffer type, ionic strength and pH.

Extinction Coefficients for Oligonucleotides

The software uses the nearest neighbor method and the following formula to calculate molar extinction coefficients for specific oligonucleotide base sequences:

$$\varepsilon_{260} = \sum_{1}^{N-1} \varepsilon_{1} - \sum_{2}^{N-1} \varepsilon_{2} + \sum_{1}^{N} \varepsilon_{3}$$

where:

ε = molar extinction coefficient in L/mole-cm

1 = nearest neighbor

2 = individual bases

3 = modifications, such as fluorescent dyes

Calculated nucleic acid concentrations are based on the absorbance value at 260 nm, the factor used and the sample pathlength. A single-point baseline correction (or analysis correction) may also be applied.

Concentration is reported in mass units. Calculators are available on the Internet to convert concentration from mass to molar units based on sample sequence.

Absorbance values at 260 nm, 280 nm and sometimes 230 nm are used to calculate purity ratios for the measured nucleic acid samples. Purity ratios are sensitive to the presence of contaminants in the sample, such as residual solvents and reagents typically used during sample purification.

Measured Values

A260 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.

- Nucleic acid absorbance values are measured at 260 nm using the normalized spectrum. This is the reported A260 value if Baseline Correction is not selected.
- If Baseline Correction is selected, the absorbance value at the correction wavelength is subtracted from the sample absorbance at 260 nm. The corrected absorbance at 260 nm is reported and used to calculate nucleic acid concentration.

A230, A280 absorbance

- Normalized absorbance values at 230 nm, 260 nm and 280 nm are used to calculate A260/A230 and A260/A280 ratios.

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, pathlength is determined by the cuvette Pathlength setting in the software (see General Settings).
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.
-

The five nucleotides that comprise DNA and RNA exhibit widely varying A260/A280 ratios. Estimated A260/A280 ratios for each independently measured nucleotide are provided below:

Guanine: 1.15
Adenine: 4.50
Cytosine: 1.51
Uracil: 4.00
Thymine: 1.47

The A260/A280 ratio for a specific nucleic acid sequence is approximately equal to the weighted average of the A260/A280 ratios for the four nucleotides present.

Note: RNA will typically have a higher 260/280 ratio due to the higher ratio of Uracil compared to that of Thymine.

Reported Values

- **Nucleic acid concentration.** Reported in selected unit (i.e., ng/µL, µg/uL or µg/mL). Calculations are based on modified Beer's Law equation using corrected nucleic acid absorbance value.
- **A260/A280 purity ratio.** Ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm.
- **A260/A230 purity ratio.** Ratio of corrected absorbance at 260 nm to corrected absorbance at 230 nm.

Note: The traditional purity ratios (A260/A280 and A260/A230), which are used as indicators of the presence of various contaminants in nucleic acid samples, do not apply for oligonucleotides because the shapes of their spectra are highly dependent on their base compositions. See side bar for more information.

Related Topics

- Calculations for Nucleic Acid Measurements

Measure Protein A280

Measures the concentration of purified protein populations that absorb at 280 nm.

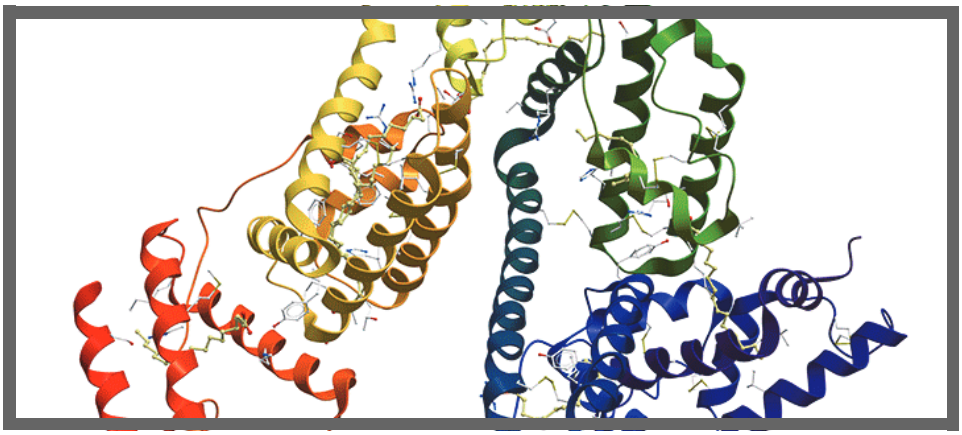
Measure A280 Proteins

Reported Results

Settings

Detection Limits

Calculations



Measure Protein Concentration at A280

Use the Protein A280 application to quantify purified protein populations that contain amino acids such as tryptophan or tyrosine, or cys-cys disulfide bonds, which exhibit absorbance at 280 nm. This application reports protein concentration measured at 280 nm and one absorbance ratio (A260/A280). A single-point baseline correction can also be used. This application does not require a standard curve.

Note If your samples contain mainly peptide bonds and little or no amino acids, use the Protein A205 application instead of Protein A280.

To measure Protein A280 samples

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.

Before you begin...

Before taking pedestal measurements with the NanoDrop One 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.

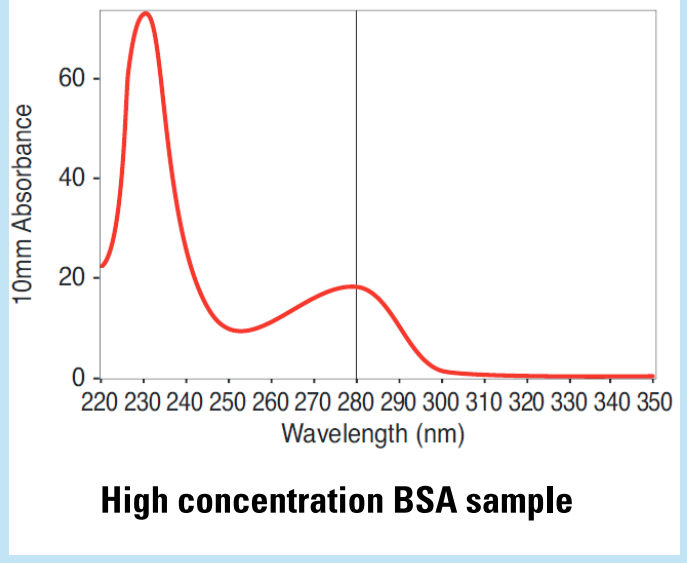
To measure a Protein A280 sample

1. From the Home screen, select the Proteins tab and tap Protein A280.
2. Specify a sample type and baseline correction if desired.
3. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder.

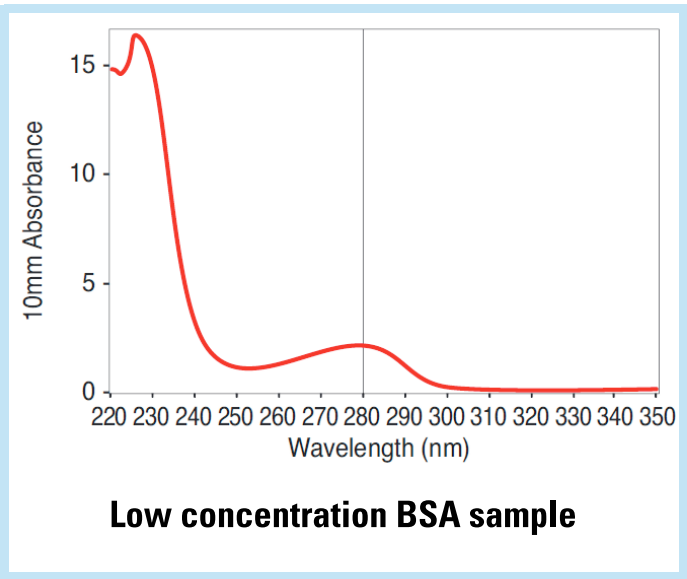
   Tip: If using a cuvette, make sure to align the cuvette light path with the instrument light path.
4. Tap Blank and wait for the measurement to complete.

   Tip: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements.)
5. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.
6. Pipette 2 µL sample solution onto the pedestal and lower the arm, or insert the sample cuvette into the cuvette holder.
7. Start the sample measurement:
   - Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is off, lower arm and tap Measure.
   - Cuvette: Tap Measure

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).
8. When you are finished measuring samples, tap End Experiment.
9. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.



High concentration BSA sample



Low concentration BSA sample

Best practices for protein measurements

- Isolate and purify protein samples before measurement to remove impurities. Depending on the sample, impurities could include DNA, RNA and some buffer components. See Preparing Samples for more information.

  Note Extraction reagents that contribute absorbance between 200 nm and 280 nm will affect measurement results if present in samples (even residual amounts).

- Ensure the sample absorbance is within the instrument's absorbance detection limits.
- Choosing a blank:
  - For the Protein A280, Protein A205, and Proteins & Labels applications, blank with the same buffer solution used to resuspend the analyte of interest. The blanking solution should be a similar pH and ionic strength as the analyte solution.
  - For the Protein BCA, Protein Bradford, and Protein Lowry applications, blank with deionized water (DI H_2O).
  - For the Protein Pierce 660 application, blank with the reference solution used to make the standard curve (reference solution should contain none of the standard protein stock). For more information, see Working with standard curves.
- Run a blanking cycle to assess the absorbance contribution of your buffer solution. If the buffer exhibits strong absorbance at or near the analysis wavelength (typically 280 nm or 205 nm), you may need to choose a different buffer or application, such as a colorimetric assay (for example, BCA or Pierce 660). See Choosing and Measuring a Blank for more information.

  Note Buffers such as Triton X, RIPA, and NDSB contribute significant absorbance and are not compatible with direct A280 or A205 measurements.

- For micro-volume measurements:
  - Ensure pedestal surfaces are properly cleaned and conditioned. (Proteins tend to stick to pedestal surfaces.)
  - Gently (but thoroughly) vortex samples before taking a measurement. Avoid introducing bubbles when mixing and pipetting.
  - Follow best practices for micro-volume measurements.
  - Use a 2 µL sample volume. See Recommended Sample Volumes for more information.
- For cuvette measurements (NanoDrop One^C instruments only), use compatible cuvettes and follow best practices for cuvette measurements.

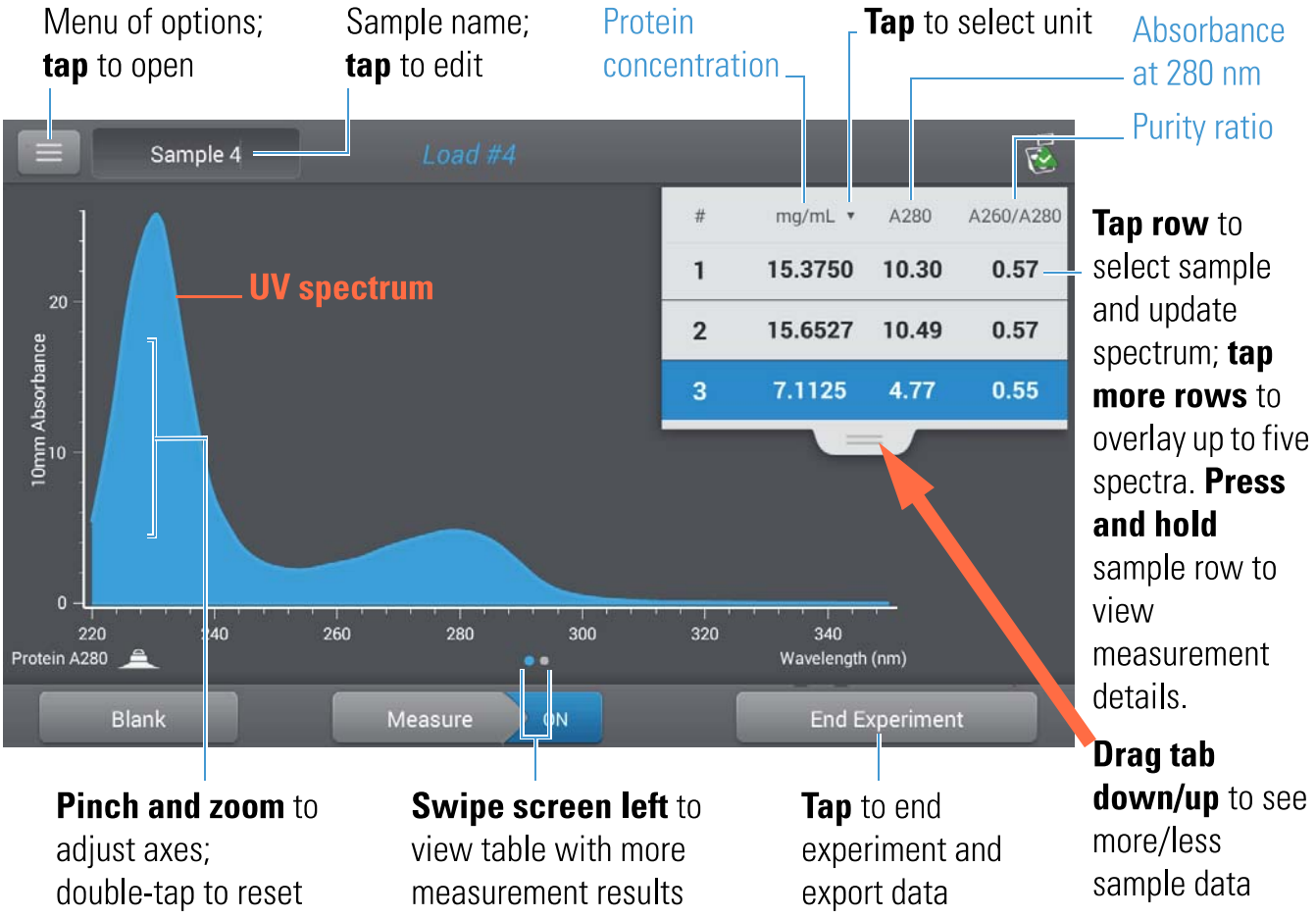
Related Topics

- Best practices for protein measurements
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette
- Prepare Samples and Blanks
- Basic Instrument Operations

Protein A280 Reported Results

Protein A280 measurement screen

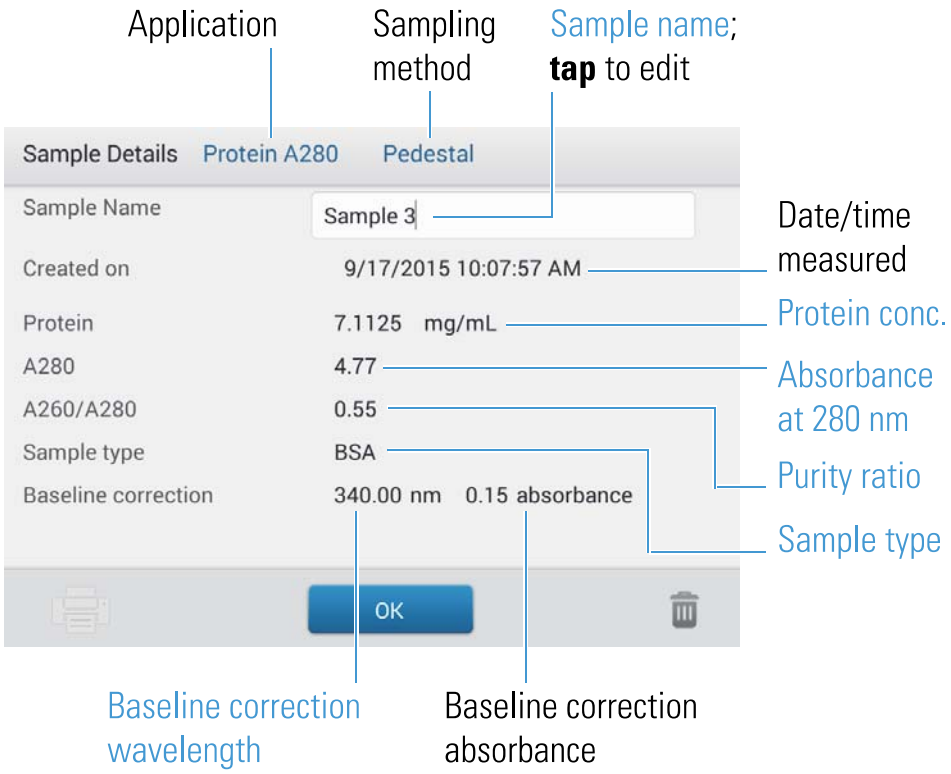
For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example:



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

Protein A280 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:



Related Topics

- Basic Instrument Operations
- Protein A280 Calculations

Settings for Protein A280 Measurements

To show the Protein A280 settings, from the Protein A280 measurement screen, tap ☰ > Protein A280 Setup.

Protein A280 settings

The Protein A280 application provides a variety of sample type options for purified protein analysis.

Each sample type applies a unique extinction coefficient to the protein calculations. If the extinction coefficient of the sample is known, choose the MW (molar) or % (mass) option and enter the value. Otherwise, calculate the extinction coefficient or choose the option that best matches the sample solution. If you only need a rough estimate of protein concentration and the sample extinction coefficient is unknown, select the 1 Abs=1 mg/mL sample type option.

Tip Ideally, the extinction coefficient should be determined empirically using a solution of the study protein at a known concentration using the same buffer.

Setting	Available Options	Mass Ext. Coefficient (L/gm-cm)	Description
Sample type[a]	1 Abs = 1 mg/mL	General reference	Recommended when extinction coefficient is unknown and rough estimate of protein concentration is acceptable for a solution with no other interfering substances. Assumes 0.1% (1 mg/mL) protein solution produces 1.0A at 280 nm (where pathlength is 10 mm), i.e., % = 10.
	BSA	6.67	Calculates BSA (Bovine Serum Albumin) protein concentration using mass extinction coefficient ( ) of 6.67 L/gm-cm at 280 nm for 1% (i.e., 10 mg/mL) BSA solution. Assuming MW is 66,400 daltons (Da), molar extinction coefficient at 280 nm for BSA is approximately 43,824 $M^{-1}cm^{-1}$.
	IgG	13.7	Suitable for most mammalian antibodies (i.e., immunoglobulin G or IgG). Calculates protein concentration using mass extinction coefficient ( ) of 13.7 L/gm-cm at 280 nm for 1% (i.e., 10 mg/mL) IgG solution. Assuming MW is 150,000 Da, molar extinction coefficient at 280 nm for IgG is approximately 210,000 $M^{-1}cm^{-1}$.
	Lysozyme	26.4	Calculates lysozyme protein concentration using mass extinction coefficient ( ) of 26.4 L/gm-cm at 280 nm for 1% (i.e., 10 mg/mL) lysozyme solution. Assumes molar extinction coefficient for egg white lysozyme ranges between 36,000 $M^{-1}cm^{-1}$ and 39,000 $M^{-1}cm^{-1}$.

Setting	Available Options	Mass Ext. Coefficient (L/gm-cm)	Description
	Other protein (molar MW)	User entered molar extinction coefficient and molecular weight	Assumes protein has known molar extinction coefficient (molar) and molecular weight (MW), where: $(\text{molar})*10=(\text{percent})*(MW_{protein})$ Enter MW in kiloDaltons (kDa) and molar extinction coefficient (molar) in $M^{-1}cm^{-1}$ divided by 1000 (i.e., percent/1000). For example, for protein with molar extinction coefficient of 210,000 $M^{-1}cm^{-1}$, enter 210.
	Other protein (percent)[a]	User entered mass extinction coefficient	Assumes protein has known mass extinction coefficient (percent). Enter mass extinction coefficient in L/gm-cm for 10 mg/mL (1%) protein solution.
Baseline Correction	On or off Enter baseline correction wavelength in nm or use default value (340 nm)	N/A	Corrects for any offset caused by light scattering particulates by subtracting measured absorbance 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. Tip: If the sample has a modification that absorbs light at 340 nm, select a different correction wavelength or turn off Baseline Correction.

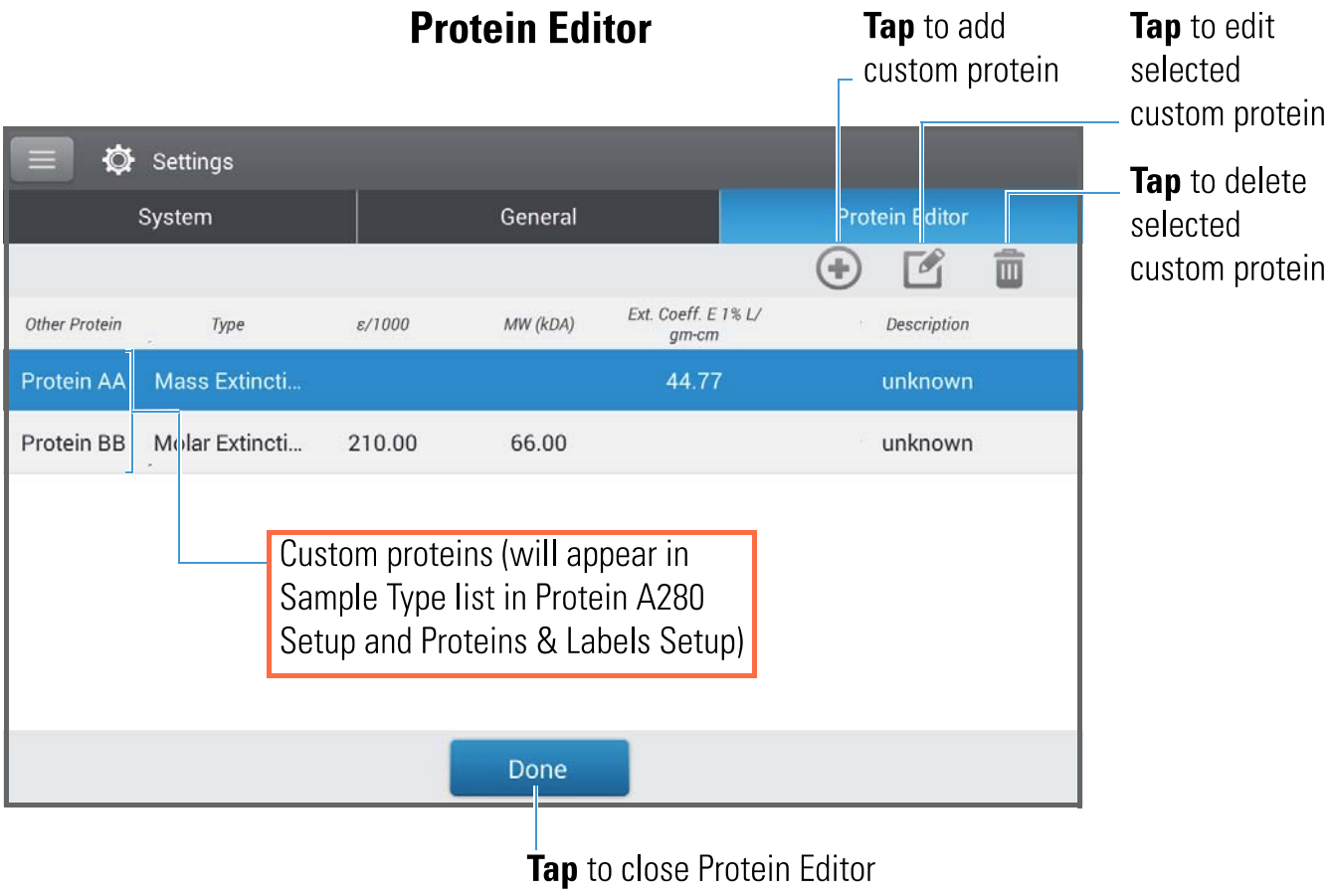
[a] To add or edit a custom protein, use Protein Editor.

Protein editor

Use the Protein Editor to add a custom protein to the list of available protein sample types in Protein A280 Setup and Proteins & Labels Setup.

To access the Protein Editor:

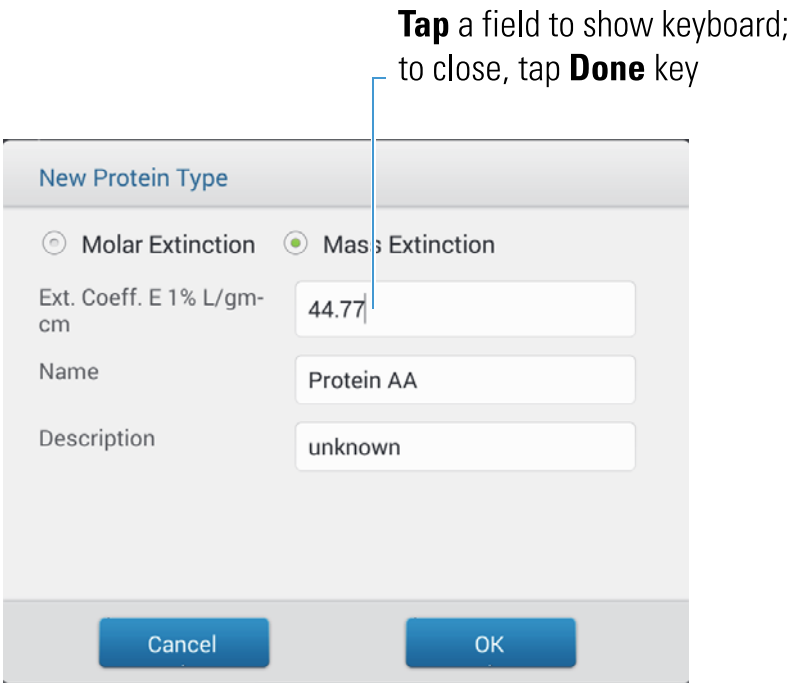
- from the Home screen, tap > Protein Editor
- from the Protein A280 or Proteins & Labels measurement screen, tap > Settings > Protein Editor



These operations are available from the Protein Editor:

Add custom protein

- in Protein Editor, tap ⊕ to show New Protein Type box
- enter unique Name for new protein (tap field to display keyboard, tap Done key to close keyboard)
- enter Description for new protein
- specify whether to enter Molar Extinction coefficient or Mass Extinction coefficient for custom protein
  - if Mass Extinction coefficient is selected
    - enter mass extinction coefficient in L/gm-cm for 10 mg/mL (F0 F2) protein solution



  - if Molar Extinction is selected
    - enter molar extinction coefficient (in $M^{-1}cm^{-1}$ divided by 1000 (i.e., 000). For example, for protein with molar extinction coefficient of 210,000 $M^{-1}cm^{-1}$, enter 210
    - enter molecular weight (MW) in kiloDaltons (kDa)
  - tap OK to close New Protein Type box

After you choose OK, the new custom protein appears in the Type list in Protein A280 Setup and Proteins & Labels Setup.

Edit custom protein

- in Protein Editor, tap to select custom protein
- tap to show Edit Protein Type box
- edit any entries or settings
- tap OK

Delete custom protein

- in Protein Editor, tap to select custom protein
- tap 

Note Deleting a custom protein permanently removes the protein and all associated information from the software.

Related Topics

- Instrument Settings

Detection Limits for Protein A280 Measurements

Detection limits and reproducibility specifications for purified BSA proteins are provided here. The BSA lower detection limit and reproducibility values apply to any protein sample type. The upper detection limits are dependent on the upper absorbance limit of the instrument and the sample's extinction coefficient.

To calculate upper detection limits for other (non-BSA) protein sample types

To calculate upper detection limits in ng/µL for proteins, use the following equation:

$$(\text{upper absorbance limit}_{\text{instrument}} / \text{mass extinction coefficient}_{\text{sample}}) * 10$$

For example, if the sample's mass extinction coefficient at 280 nm is 6.67 for a 1% (10 mg/mL) solution, the equation looks like this:

(550 / 6.67) * 10 = 824.6 (or ~825)

Related Topics

- Detection Limits for All Applications

Calculations for Protein A280 Measurements

The Protein A280 application uses the Beer-Lambert equation to correlate absorbance with concentration. Solving Beer's law for concentration yields the equation at the right.

Beer-Lambert Equation (solved for concentration)

$$c = A / (\varepsilon * b)$$

where:

A = UV absorbance in absorbance units (AU)

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)

Note: Dividing the measured absorbance of a sample solution by its molar extinction coefficient yields the molar concentration of the sample. See Published Extinction Coefficients for more information regarding molar vs. mass concentration values.

The extinction coefficient of a peptide or protein is related to its tryptophan (W), tyrosin (Y) and cysteine (C) amino acid composition.

Tip: The extinction coefficient is wavelength specific for each protein and can be affected by buffer type, ionic strength and pH.

Extinction Coefficients for Proteins

At 280 nm, the extinction coefficient is approximated by the weighted sum of the 280 nm molar extinction coefficients of the three constituent amino acids, as described in this equation:

$$\varepsilon = (nW * 5500) + (nY * 1490) + (nC * 125)$$

where:
ε = molar extinction coefficient
n = number of each amino acid residue
5500, 1490 and 125 = amino acid molar absorptivities at 280 nm

This application offers six options (shown at right) for selecting an appropriate extinction coefficient for each measured sample, to be used in conjunction with Beer's Law to calculate sample concentration.

If the extinction coefficient of the sample is known, choose the MW (molar) or (mass) option and enter the value. Otherwise, calculate the extinction coefficient or choose the option that best matches the sample solution.

Tip: Ideally, the extinction coefficient should be determined empirically using a solution of the study protein at a known concentration using the same buffer.

Available Options for Extinction Coefficient

- 1 Abs = 1 mg/mL, where sample type and/or ext. coefficient is unknown (produces rough estimate of protein concentration)
- BSA (Bovine Serum Albumin, 6.67 L/gm-cm)
- IgG (any mammalian antibody, 13.7 L/gm-cm)
- Lysozyme (egg white lysozyme, 26.4 L/gm-cm)
- Other protein (MW), user-specified molar ext. coefficient
- Other protein ( ), user-specified mass ext. coefficient
-

Note: See Sample Type for details.

Most sources report extinction coefficients for proteins measured at or near 280 nm in phosphate or other physiologic buffer. These values provide sufficient accuracy for routine assessments of protein concentration.

The equation at the right shows the relationship between molar extinction coefficient ( ) and percent extinction coefficient ( ).

Published Extinction Coefficients

Published extinction coefficients for proteins may be reported as:

- wavelength-dependent molar absorptivity (or extinction) coefficient ( ) with units of $M^{-1}cm^{-1}$
- percent solution extinction coefficient ( ) with units of $(g/100\ mL)^{-1}cm^{-1}$ (i.e., 1% or 1 g/100 mL solution measured in a 1 cm cuvette)
- protein absorbance values for 0.1% (i.e., 1 mg/mL) solutions

Tip: Assess published values carefully to ensure unit of measure is applied correctly.

Conversions Between and

$$(\text{molar}) * 10 = (\text{1\%}) * (MW_{protein})$$

Example: To determine percent solution extinction coefficient ( ) for a protein that has a molar extinction coefficient of 43,824 $M^{-1}cm^{-1}$ and a molecular weight (MW) of 66,400 daltons (Da), rearrange and solve the above equation as follows:

$$\text{1\%} = (\text{molar} * 10) / (MW_{protein})$$

$$\text{1\%} = (43{,}824 * 10) / 66{,}400\ Da)$$

$$\text{1\%} = 6.6\ g/100\ mL$$

To determine concentration (c) of a sample in mg/mL, use the equation at the right and a conversion factor of 10.

Tip: The NanoDrop One software includes the conversion factor when reporting protein concentrations.

Conversions Between g/100 mL and mg/mL

$$C_{protein} \text{ in mg/mL} = (A / \text{F0 1\%}) * 10$$

Example: If measured absorbance for a protein sample at 280 nm relative to the reference is 5.8 A, protein concentration can be calculated as:

$$C_{protein} = (A / \text{F0 1\%}) * 10$$

$$C_{protein} = (5.8/6.6 \text{ g/100 mL}) * 10$$

$$C_{protein} = 8.79 \text{ mg/mL}$$

Calculated protein concentrations are based on the absorbance value at 280 nm, the selected (or entered) extinction coefficient and the sample pathlength. A single-point baseline correction (or analysis correction) may be applied.

Concentration is reported in mass units. Calculators are available on the Internet to convert concentration from mass to molar units based on sample sequence.

Absorbance values at 260 nm and 280 nm are used to calculate purity ratios for the measured protein samples.

Purity ratios are sensitive to the presence of contaminants in the sample, such as residual solvents and reagents typically used during sample purification.

Measured Values

A280 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.

- Protein absorbance values are measured at 280 nm using the normalized spectrum. If Baseline Correction is not selected, this is the reported A280 value and the value used to calculate protein concentration.
- If Baseline Correction is selected, the normalized and baseline-corrected absorbance value at 280 nm is reported and used to calculate protein concentration.

A260 absorbance

- Normalized and baseline-corrected (if selected) absorbance value at 260 nm 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, pathlength is determined by the cuvette Pathlength setting in the software (see General Settings).
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.
-

Reported Values

- **Protein concentration.** Reported in selected unit (mg/mL or µg/mL). Calculations are based on Beer-Lambert equation using corrected protein absorbance value.
- **A260/A280 purity ratio.** Ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm. An A260/A280 purity ratio of ~0.57 is generally accepted as "pure" for proteins.

Note: Although purity ratios are important indicators of sample quality, the best indicator of protein quality is functionality in the downstream application of interest (e.g., real-time PCR).

Measure Proteins and Labels

Measures the concentration of purified proteins that have been labeled with up to two fluorescent dyes.

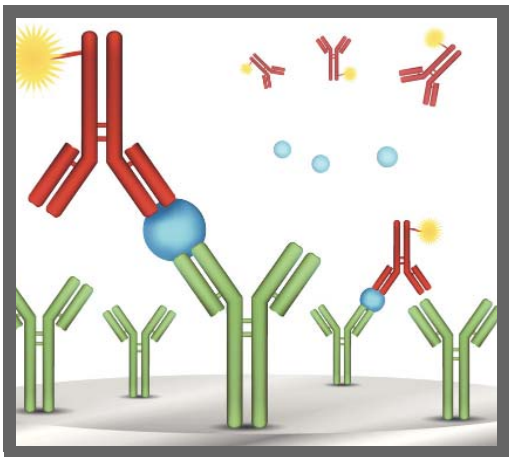
Measure Labeled Proteins

Reported Results

Settings

Detection Limits

Calculations



Measure Labeled Protein Samples

Use the Proteins and Labels application to quantify proteins and fluorescent dyes for protein array conjugates, as well as metalloproteins such as hemoglobin, using wavelength ratios. This application reports protein concentration measured at 280 nm, an A269/A280 absorbance ratio, and the concentrations and measured absorbance values of the dyes, allowing detection of dye concentrations as low as 0.2 picomole per microliter. This information is useful for evaluating protein/dye conjugation (degree of labeling) for use in downstream applications.

To measure labeled protein samples

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.

Before you begin...

Before taking pedestal measurements with the NanoDrop One 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.

To measure a labeled protein sample

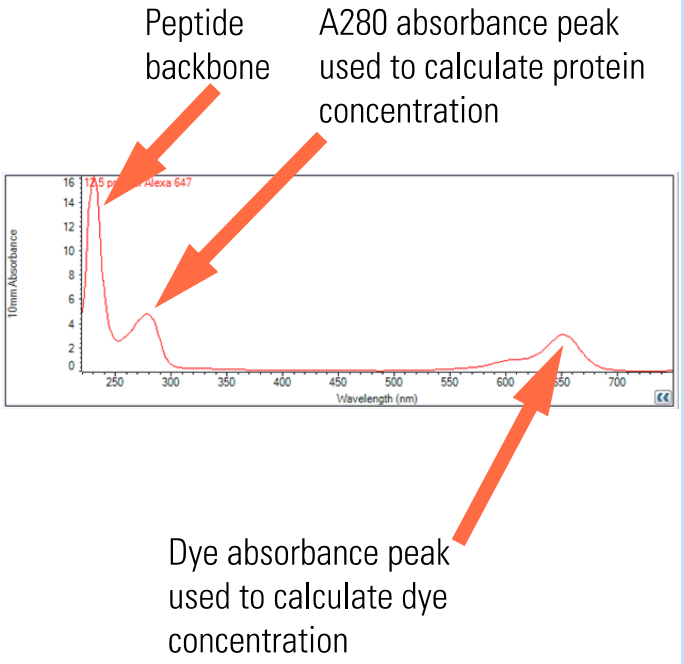
1. From the Home screen, select the Proteins tab and then tap Protein & Labels.
2. Specify the sample type and the type of dye(s) used.

   Tip: Select a dye from the pre-defined list or add a custom dye using the Dye/Chromophore Editor.
3. Pipette 1–2 µL of the blanking solution 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.
4. Tap 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.)
5. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.
6. Pipette 2 µL sample solution onto the pedestal and lower the arm, or insert the sample cuvette into the cuvette holder.
7. Start the sample measurement:
   - Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is off, lower arm and tap Measure.
   - Cuvette: Tap Measure

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).
8. When you are finished measuring samples, tap End Experiment.
9. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.



Typical sample spectrum measured with Proteins & Labels application

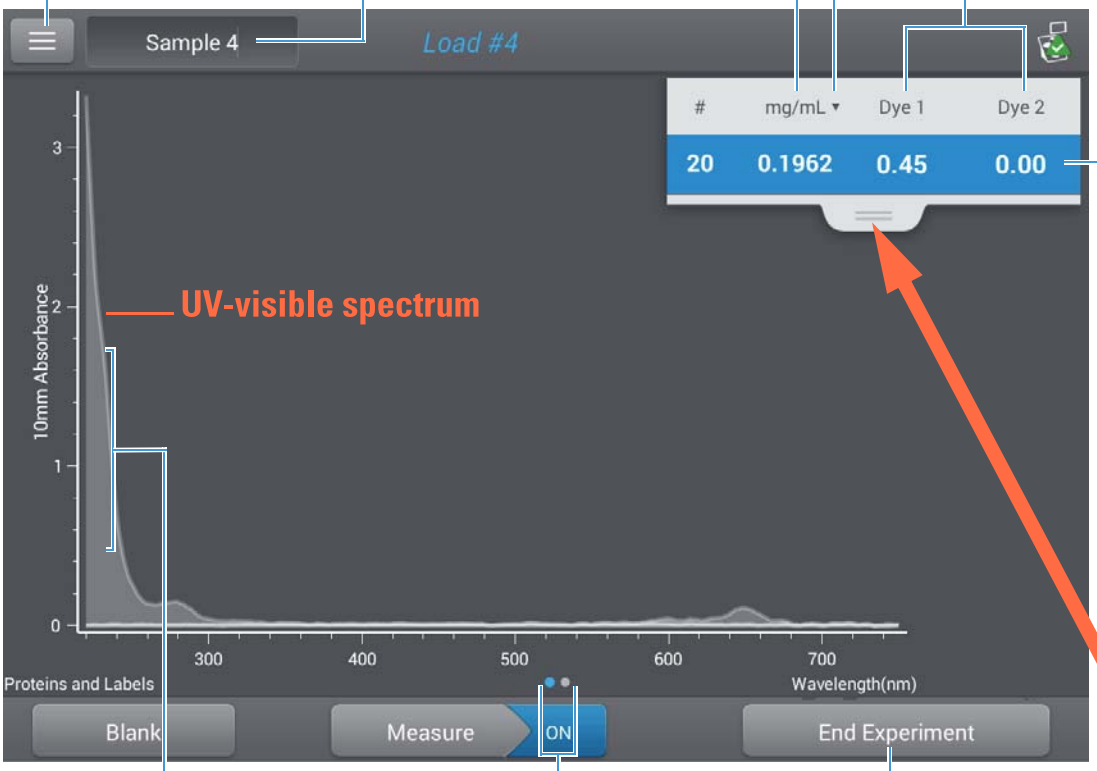
Related Topics

- Best practices for protein measurements
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette
- Prepare Samples and Blanks
- Basic Instrument Operations

Proteins & Labels Reported Results

Proteins & Labels measurement screen

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



Note

- A baseline correction is performed at 750 nm (absorbance value at 750 nm is subtracted from absorbance values at all wavelengths in sample spectrum).
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

Proteins & Labels 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:

Reported values for Proteins & Labels application

- Sample details (application and sampling method used, i.e., pedestal or cuvette)
- Sample Name
- Creation date
- Protein
- A280
- Sample Type
- Dye 1/Dye 2
- Sloping Dye Correction
- Analysis Correction

Related Topics

- Basic Instrument Operations
- Proteins & Labels calculations

Settings for Proteins and Labels Measurements

To show the Proteins & Labels settings, from the Proteins & Labels measurement screen, tap  > Proteins & Labels Setup.

Setting	Available Options	Mass Ext. Coefficient (L/gm-cm)	Description
Sample type[a]	1 Abs = 1 mg/mL	General reference	Tap here for detailed description of each available setting.
	BSA	6.67	Each sample type applies a unique extinction coefficient to the protein calculations. If the extinction coefficient of the sample is known, choose the ε + MW (molar) or ε1% (mass) option and enter the value. Otherwise, calculate the extinction coefficient or choose the option that best matches the sample solution. If you only need a rough estimate of protein concentration and the sample extinction coefficient is unknown, select the 1 Abs=1 mg/mL sample type option.
	IgG	13.7	
	Lysozyme	26.4	
	Other protein (ε + MW)	user-entered molar extinction coefficient/molecular weight	
	Other protein (ε1%)	User entered mass extinction coefficient	Tip: Ideally, the extinction coefficient should be determined empirically using a solution of the study protein at a known concentration using the same buffer.
Analysis Correction[b]	On or off Enter analysis correction wavelength in nm or use default value (340 nm)	N/A	Corrects sample absorbance measurement for any offset caused by light scattering particulates by subtracting absorbance value at specified analysis correction wavelength from absorbance value at analysis wavelength. Corrected value is used to calculate sample concentration. Tip: If the sample has a modification that absorbs light at 340 nm, select a different correction wavelength or turn off Analysis Correction.
Dye 1/Dye 2 Type[c]	Cy3, 5, 3.5, or 5.5, Alexa Fluor 488, 546, 555, 594, 647, or 660	See Dye/Chromophore Editor for specific values for each dye	Select pre-defined dye used to label sample material, or one that has been added using Dye/Chrom. Editor.
Dye 1/Dye 2 Unit	picomoles/microliter (pmol/uL), micromoles (uM), or millimoles (mM)	not applicable	Select unit for reporting dye concentrations.
Sloping Dye Correction[d]	On or off		Corrects dye absorbance measurements for any offset caused by light scattering particulates by subtracting absorbance value of a sloping baseline from 400 nm to 750 nm from absorbance value at dye's analysis wavelength.

[a] To add or edit a custom protein, use Protein Editor.

[b] Analysis Correction affects calculation for protein concentration only.

[c] To add custom dye or edit list of available dyes, use Dye/Chromophore Editor.

[d] Sloping Dye Correction affects calculations for dye concentration only.

Related Topics

- Instrument Settings
- Protein Editor
- Dye/Chromophore Editor

Detection Limits for Proteins and Labels Measurements

Detection limits and reproducibility specifications for purified BSA proteins and dyes that are pre-defined in the software are provided here. The BSA lower detection limit and reproducibility values apply to any protein sample type. The upper detection limits are dependent on the upper absorbance limit of the instrument and the sample's extinction coefficient.

To calculate upper detection limits for other (non-BSA) protein sample types

To calculate upper detection limits in ng/µL for proteins, use the following equation:

$$(\text{upper absorbance limit}_{\text{instrument}} / \text{mass extinction coefficient}_{\text{sample}}) * 10$$

For example, if the sample's mass extinction coefficient at 280 nm is 6.67 for a 1% (10 mg/mL) solution, the equation looks like this:

(550 / 6.67) * 10 = 824.6 (or ~825)

Related Topics

- Detection Limits for All Applications

Calculations for Proteins and Labels Measurements

As with the other protein applications, Proteins & Labels uses the Beer-Lambert equation to correlate absorbance with concentration based on the sample's extinction coefficient and pathlength.

This application offers six options (shown at right) for selecting an appropriate extinction coefficient for each measured sample, to be used in conjunction with Beer's Law to calculate sample concentration.

If the extinction coefficient of the sample is known, choose the  (molar) or  (mass) option and enter the value. Otherwise, calculate the extinction coefficient or choose the option that best matches the sample solution.

Tip: Ideally, the extinction coefficient should be determined empirically using a solution of the study protein at a known concentration using the same buffer.

Calculated protein concentrations are based on the absorbance value at 280 nm, the selected (or entered) extinction coefficient and the sample pathlength. A single-point baseline correction (or analysis correction) may be applied.

Concentration is reported in mass units. Calculators are available on the Internet to convert concentration from mass to molar units based on sample sequence.

Available Options for Extinction Coefficient

- 1 Abs = 1 mg/mL, where sample type and/or ext. coefficient is unknown (produces rough estimate of protein concentration)
- BSA (Bovine Serum Albumin, 6.67 L/gm-cm)
- IgG (any mammalian antibody, 13.7 L/gm-cm)
- Lysozyme (egg white lysozyme, 26.4 L/gm-cm)
- Other protein ( MW), user-specified molar ext. coefficient
- Other protein (), user-specified mass ext. coefficient

Note: See Sample Type for details.

Measured Values

A280 absorbance

Note: The absorbance value at 750 nm is subtracted from all wavelengths in the spectrum. As a result, the absorbance at 750 nm is zero in the displayed spectra. Also, 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.

- Protein absorbance values are measured at 280 nm using the 750 nm-corrected and normalized spectrum. If Analysis Correction and Dye Correction are not selected, this is the reported A280 value and the value used to calculate protein concentration.
- If Analysis Correction is selected, the 750-corrected, normalized and analysis-corrected absorbance value at 280 nm is reported and used to calculate protein concentration.
- If a Dye is used, the 750-corrected, normalized, analysis-corrected and dye-corrected absorbance value at 280 nm is reported and used to calculate protein concentration.

Dye concentrations are calculated from the absorbance value at the dye's analysis wavelength, the dye's extinction coefficient, and the sample pathlength. A sloped-line dye correction may also be used.

Dye absorbance

- Dye absorbance values are measured at specific wavelengths. See Dye/Chromophore Editor for analysis wavelengths used.
- If Sloping Dye Correction is selected, a linear baseline is drawn between 400 nm and 750 nm and, for each dye, the absorbance value of the sloping baseline is subtracted from the absorbance value at each dye's analysis wavelength. Baseline-corrected dye absorbance values are reported and used to calculate dye concentrations.

Dye correction

- Pre-defined dyes have known correction values for A260 and A280. See Dye/Chromophore Editor for correction values used.
- A280 dye correction is subtracted from A280 absorbance value used to calculate protein concentration.

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, pathlength is determined by the cuvette Pathlength setting in the software (see General Settings).
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.

Reported Values

- Protein concentration. Reported in selected unit (mg/mL or µg/mL). Calculations are based on Beer-Lambert equation using corrected protein absorbance value.
- Dye1/Dye2 concentration. Reported in pmol/µL. Calculations are based on Beer's Law equation using (sloping) baseline-corrected dye absorbance value(s).

Related Topics

- Beer-Lambert Equation
- Protein A280 Calculations

Measure Protein A205

Measures the concentration of purified protein populations that absorb at 205 nm.

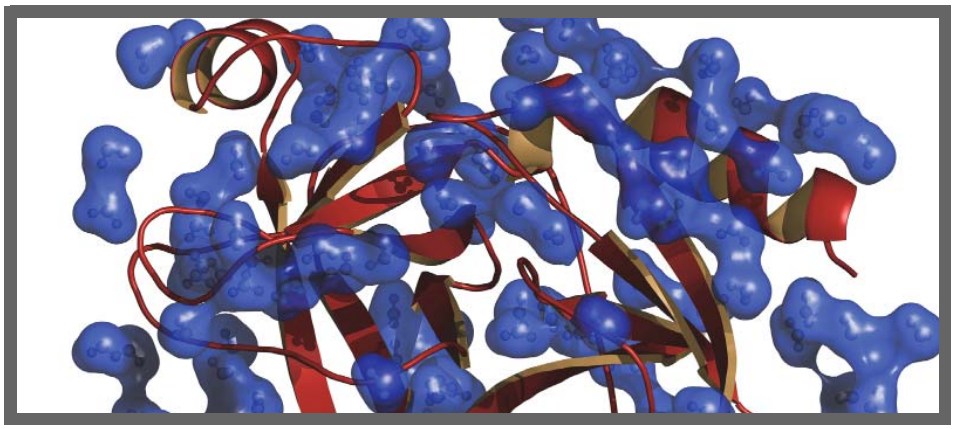
Measure A205 Proteins

Reported Results

Settings

Detection Limits

Calculations



Measure Protein Concentration at A205

Use the Protein A205 application to quantify purified peptides and other proteins that contain peptide bonds, which exhibit absorbance at 205 nm. This application reports protein concentration and two absorbance values (A205 and A280). A single-point baseline correction can also be used. This application does not require a standard curve.

Note If your samples contain mainly amino acids such as tryptophan or tyrosine, or cys-cys disulfide bonds, use the Protein A280 application instead of Protein A205.

To measure Protein A205 samples

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.

Before you begin...

Before taking pedestal measurements with the NanoDrop One 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.

To measure a Protein A205 sample

1. From the Home screen, select the Proteins tab and then tap Protein A205.
2. Specify a sample type and baseline correction if desired.
3. Pipette 1–2 µL of the blanking solution 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.
4. Tap 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.)
5. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.
6. Pipette 2 µL sample solution onto the pedestal and lower the arm, or insert the sample cuvette into the cuvette holder.
7. Start the sample measurement:
   - Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is off, lower arm and tap Measure.
   - Cuvette: Tap Measure

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).
8. When you are finished measuring samples, tap End Experiment.
9. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.

Related Topics

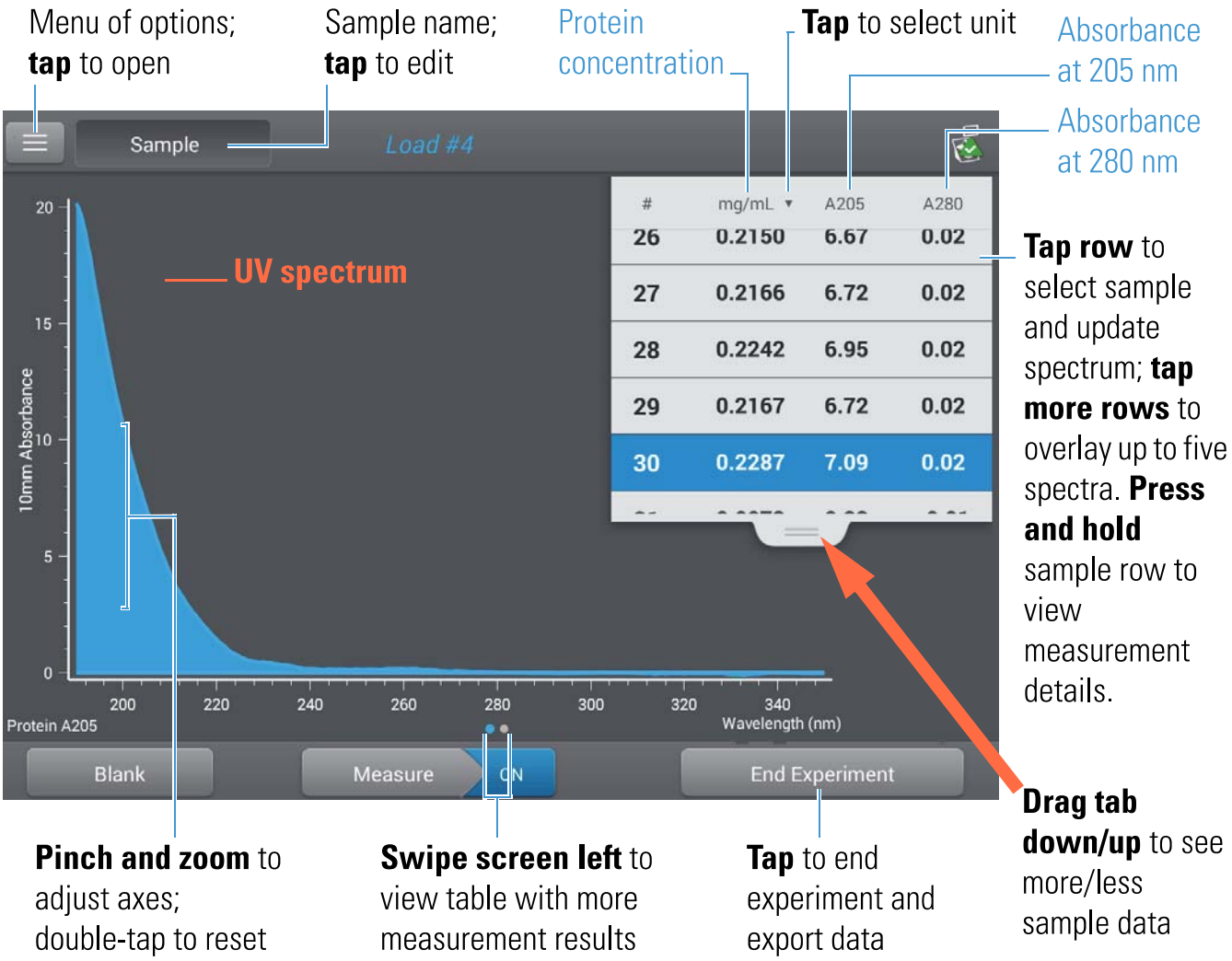
- Best Practices for Protein Measurements
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette

- Prepare Samples and Blanks
- Basic Instrument Operations

Protein A205 Reported Results

Protein A205 measurement screen

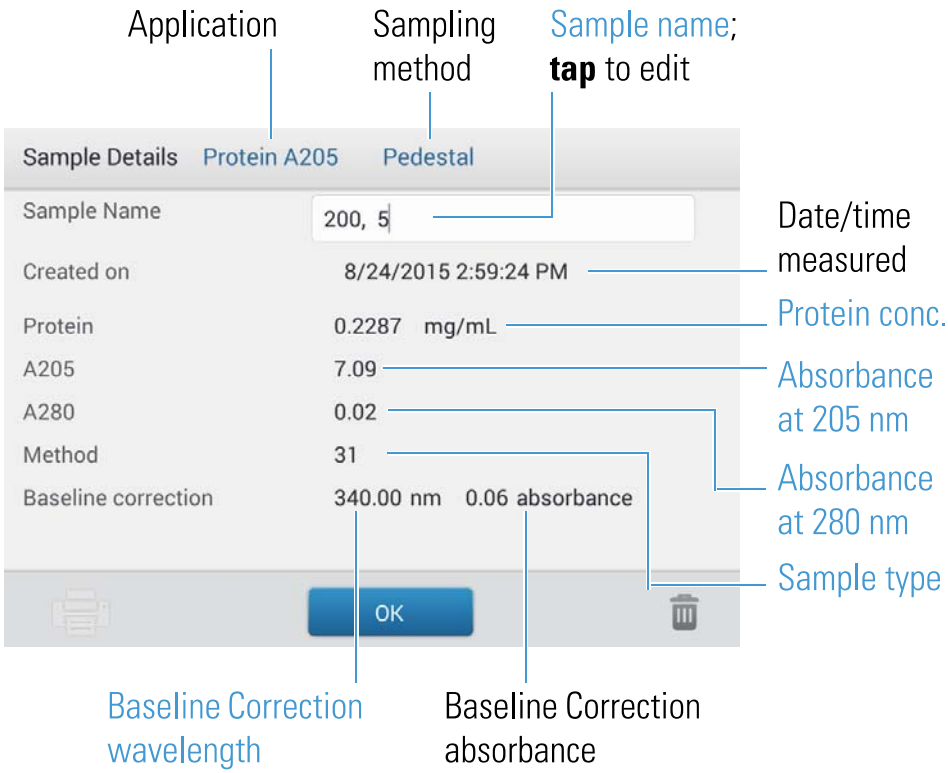
For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example:



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

Protein A205 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:



Related Topics

- Basic Instrument Operations
- Protein A205 Calculations

Settings for Protein A205 Measurements

To show the Protein A205 settings, from the Protein A205 measurement screen, tap ☰ > Protein A205 Setup.

Setting	Available Options	Mass Ext. Coefficient (L/gm-cm)	Description
Sample type	31	31	Assumes 0.1% (1 mg/mL) at 205 nm = 31
	Scopes	27 + 120 * (A280/A205)	Assumes 0.1% (1 mg/mL) at 205 nm = 27 + 120 * (A280/A205)
	Other protein (0.1%)	User entered mass extinction coefficient	Assumes protein has known mass extinction coefficient (0.1%). Enter mass extinction coefficient in L/gm-cm for 1 mg/mL (0.1%) protein solution.
Baseline Correction	On or off Enter baseline correction wavelength in nm or use default value (340 nm)	N/A	Corrects for any offset caused by light scattering particulates by subtracting measured absorbance 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. Tip: If the sample has a modification that absorbs light at 340 nm, select a different correction wavelength or turn off Baseline Correction.

Related Topics

- Instrument Settings

Calculations for Protein A205 Measurements

As with the other protein applications, Proteins A205 uses the Beer-Lambert equation to correlate absorbance with concentration based on the sample's extinction coefficient and pathlength.

This application offers three options (shown at right) for selecting an appropriate extinction coefficient for each measured sample, to be used in conjunction with Beer's Law to calculate sample concentration.

If the extinction coefficient of the sample is known, choose the  (mass) option and enter the value. Otherwise, calculate the extinction coefficient or choose the option that best matches the sample solution.

Tip: Ideally, the extinction coefficient should be determined empirically using a solution of the study protein at a known concentration using the same buffer.

Calculated protein concentrations are based on the absorbance value at 205 nm, the selected (or entered) extinction coefficient and the sample pathlength. A single-point baseline correction may also be applied.

Concentration is reported in mass units. Calculators are available on the Internet to convert concentration from mass to molar units based on the sample sequence.

Available Options for Extinction Coefficient

- 31, assumes  % (1 mg/mL) at 205 nm = 31
- Scopes, assumes  % (1 mg/mL) at 205 nm = 27 + 120 * (A280/A205)
- Other protein, enter mass extinction coefficient in L/gm-cm for 1 mg/mL ( %) protein solution

Note: See Sample Type for details.

Measured Values

A205 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.

- Protein absorbance values are measured at 205 nm using the normalized spectrum. If Baseline Correction is not selected, this is the reported A205 value and the value used to calculate protein concentration.
- If Baseline Correction is selected, the normalized and baseline-corrected absorbance value at 205 nm is reported and used to calculate protein concentration.

A280 absorbance

- Normalized and baseline-corrected (if selected) absorbance value at 280 nm 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, pathlength is determined by the cuvette Pathlength setting in the software (see General Settings).
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent.

Reported Values

- **Protein concentration.** Reported in selected unit (mg/mL or µg/mL). Calculations are based on Beer-Lambert equation using corrected protein absorbance value.

Related Topics

- Beer-Lambert Equation
- Protein A280 Calculations

Measure Protein BCA

Measures total protein concentration of unpurified protein samples using a bicinchoninic acid colorimetric detection reagent.

Measure Total Protein

Reported Results

Settings

Detection Limits

Measure Total Protein Concentration

The Protein BCA assay uses bicinchoninic acid as a colorimetric detection reagent to determine total protein concentration in unpurified protein samples. This application is useful for measuring dilute protein solutions or proteins in the presence of components that exhibit significant absorbance between 200 nm and 280 nm, which rules out direct protein measurements at 280 nm or 205 nm. This application measures absorbance at 562 nm and uses a standard curve to calculate protein concentration. A single-point baseline correction is applied.

Theory of Protein BCA assay

The Protein BCA assay uses bicinchoninic acid (BCA) as the detection reagent for Cu^{+1}, which is formed when Cu^{+2} is reduced by certain proteins in an alkaline environment. A purple reaction product is formed by the chelation of two molecules of BCA with one cuprous ion (Cu^{+1}). The resulting Cu-BCA chelate formed in the presence of protein is measured at 562 nm and baseline-corrected using the absorbance value at 750 nm. Pre-formulated kits of BCA reagent and $CuSO_4$ are available from us or a local distributor.

Protein assay kits and protocols

Please refer to the NanoDrop website for up-to-date kits and protocols for the NanoDrop One instruments. Follow the assay kit manufacturer's recommendations for all standards and samples (unknowns). Ensure each is subjected to the same timing and temperature throughout the assay.

Protein standards for generating a standard curve may also be provided by the kit manufacturer. Since the NanoDrop One pedestals can measure higher protein concentrations than traditional cuvette-based spectrophotometers, you may need to supply your own protein standards at higher concentrations than provided by the manufacturer. For example, additional standards may be required to ensure the standard curve covers the dynamic range of the assay and the expected range of the unknown samples.

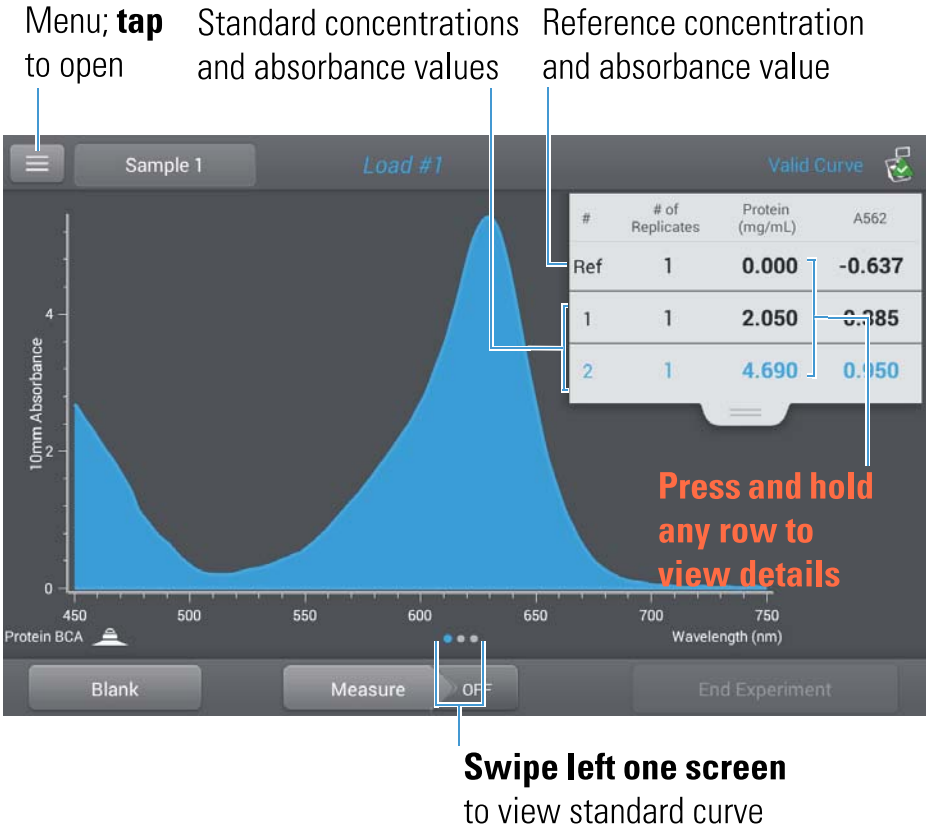
Working with standard curves

A standard curve is required for colorimetric protein analysis.

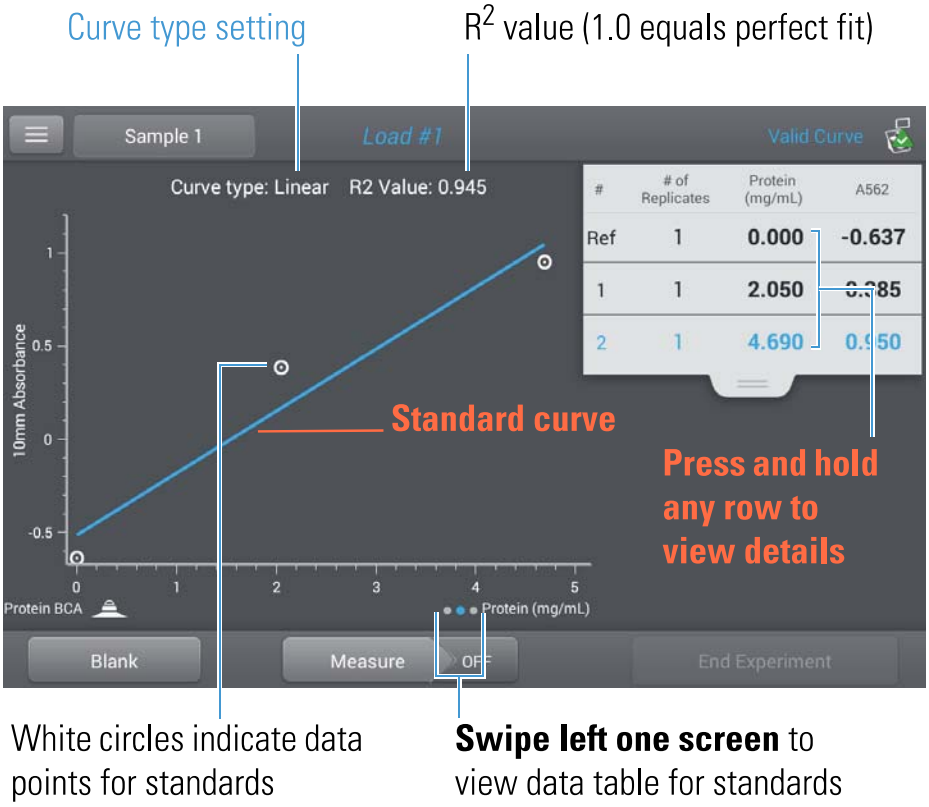
- Each experiment requires a new standard curve.
- Prepare standards and unknown samples the same way. See the kit manufacturer's guidelines and recommendations.
  - **All reference and standards solutions** should be the same buffer used to resuspend the samples plus the same volume of reagent added to the samples.
  - **First standard** is 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. This application requires both.)
  - **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.
- Use the application setup screen to enter concentration values for the standards and to specify how standards and samples will be measured (number of replicates, etc.).
  - Depending on the Curve Type setting, a standard curve can be generated using two or more standards.
  - The software **requires one reference measurement** and allows **up to 7 standards**.
  - **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.

- For all colorimetric assays except Protein Pierce 660, **blank the instrument** with DI H_2O (deionized water). For Protein Pierce 660, blank with the reference solution (see below).
- **Measure the reference and all standards** before you start analyzing samples. (After the first sample has been measured, no additional changes are allowed to the standard curve.)

  As you measure the standards, a measurement screen appears, similar to the measurement screens for samples.

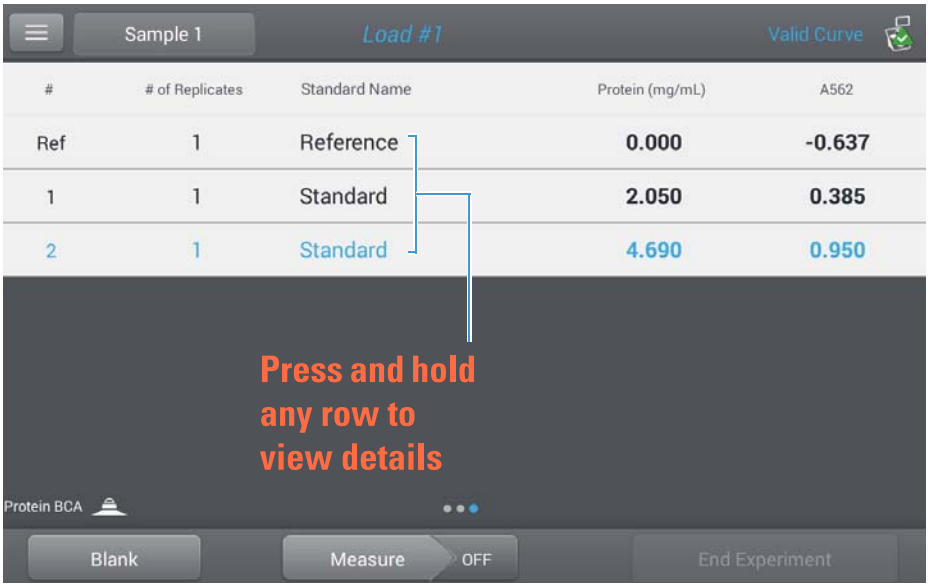


Swipe left one screen to see the standard curve as you build it. Here is an example:

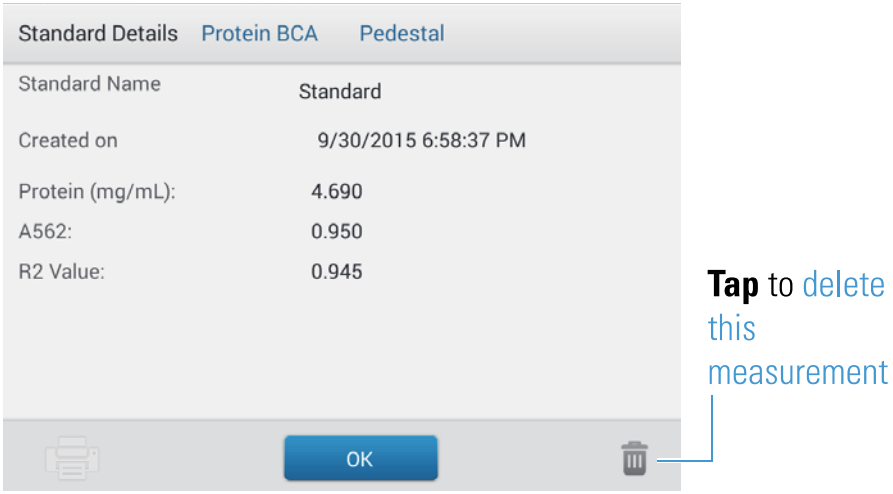


The R^2 value indicates how well the standard curve fits the standard data points (1.0 is a perfect fit; all points lie exactly on the curve).

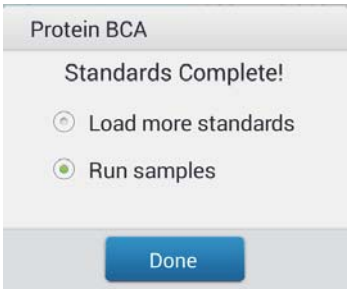
Swipe left one screen to see the data table for the standards. Here is an example:



Press and hold a row in any of the previous screens to view details about an individual standard. Here is an example:



After the minimum number of standards has been measured for the selected curve type, a message similar to the following appears:



Load more standards: returns to the setup screen where you can add or edit the concentration value for any standard and then measure the standard.

Run samples: continues to sample measurement screen, after which standards can no longer be edited.

- You can add, edit or delete a standard any time before the first sample measurement.

Add standard

– from standards measurement screen, tap ☰ > [application name] Setup

– tap the next empty Concentration field and enter the concentration value for the new standard

– tap Done

Edit standard

– from standards measurement screen, tap ☰ > [application name] Setup

– tap the Concentration field and edit the concentration value

– tap Done

Delete standard

– from standards measurement screen, standard curve screen, or standards data table, press and hold the row to show Standard Details box

– tap 🗑

The standard no longer appears in the table on the measurement screen and its concentration value no longer appears on the setup screen.

Note You can use this method to delete the reference measurement; however, a new reference must be measured immediately afterwards.

- After the minimum number of standards has been measured for the selected curve type, the message “Invalid Curve” changes to “Valid Curve.” (This occurs even when additional standards have been defined but not yet measured.) If the “Invalid Curve” message remains after all entered standards have been measured, try:
  - selecting a different curve type
  - remeasuring standards using the correct standard material

  Valid Curve indicator: This is only an indicator that the required minimum number of points has been established for the selected curve type. It does not validate the integrity of the curve. For example, additional standards may be required to cover the expected assay concentration range.

To measure Protein BCA standards and samples

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.

Before you begin...

Before taking pedestal measurements with the NanoDrop One 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.

To measure Protein BCA standards and samples

1. From the Home screen, select the Proteins tab and tap Protein BCA.
2. Specify a curve type and number of replicates for each standard and enter the concentration of each standard.

   Tip: For this assay, we recommend setting Curve Type to “Linear”.
3. Measure blank:
   - pipette 2 µL DI H_2O onto lower pedestal and lower arm, or insert DI H_2O blanking cuvette into cuvette holder

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

- tap Blank and wait for measurement to complete
- lift arm and clean both pedestals with new laboratory wipe, or remove cuvette

4. Measure reference standard:
   - pipette 2 µL reference solution onto pedestal, or insert reference cuvette (reference solution should contain none of the standard protein stock, see Working With Standard Curves for details)
   - lower arm to start measurement (or tap Measure if Auto-Measure is off)
   - lift arm and clean both pedestals with new wipe, or remove cuvette
   - if Replicates setting is greater than 1, repeat measurement
5. Measure remaining standards:
   - pipette 2 µL standard 1 onto pedestal, or insert standard 1 cuvette
   - lower arm to start measurement (or tap Measure if Auto-Measure is off)
   - lift arm and clean both pedestals with new wipe, or remove cuvette
   - if Replicates setting is greater than 1, repeat measurement
   - repeat substeps above for each additional standard (when specified number of standards and replicates have been measured, a message asks whether to load more standards or begin measuring samples)
   - if finished measuring standards, tap Done (swipe left to view standard curve)
6. Measure samples:
   - pipette 2 µL sample 1 onto pedestal, or insert sample 1 cuvette
   - lower arm to start measurement (or tap Measure if Auto-Measure is off)
   - lift arm and clean both pedestals with new wipe, or remove cuvette
   - if Replicates setting is greater than 1, repeat measurement
7. When you are finished measuring samples, tap End Experiment.
8. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.

Related Topics

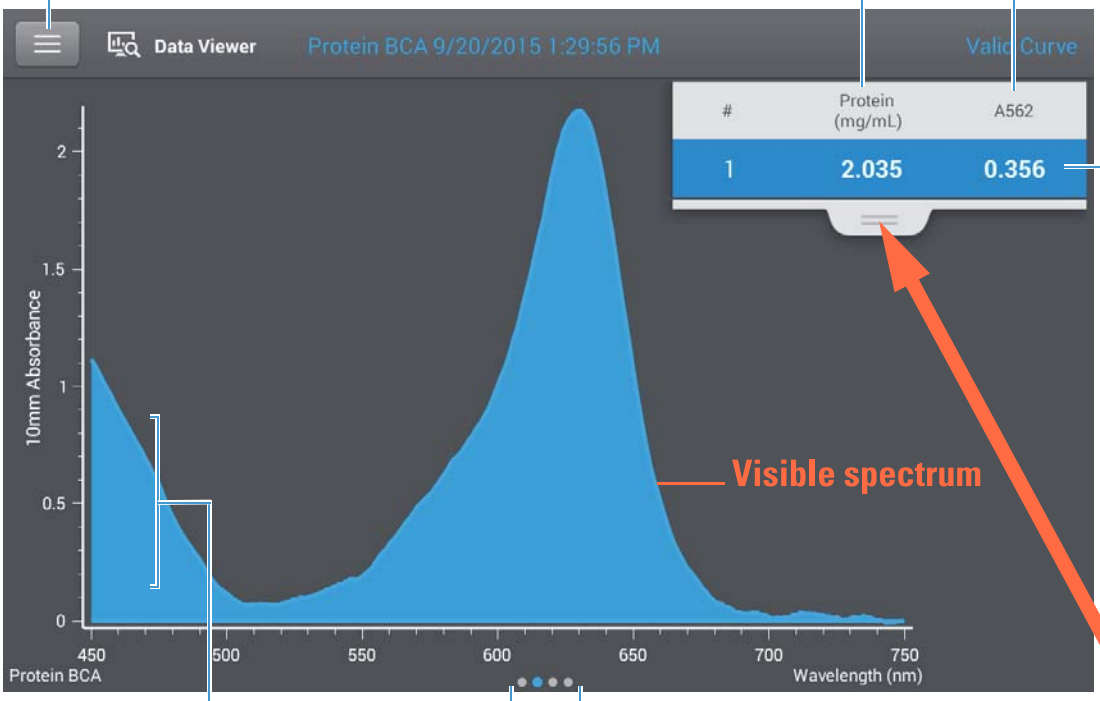
- Best practices for protein measurements
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette

- Prepare Samples and Blanks
- Basic Instrument Operations

Protein BCA Reported Results

Protein BCA measurement screen (shown from Data Viewer)

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. The standard curve is also available by swiping left from the measurement screen (or in the Data Viewer as shown below).



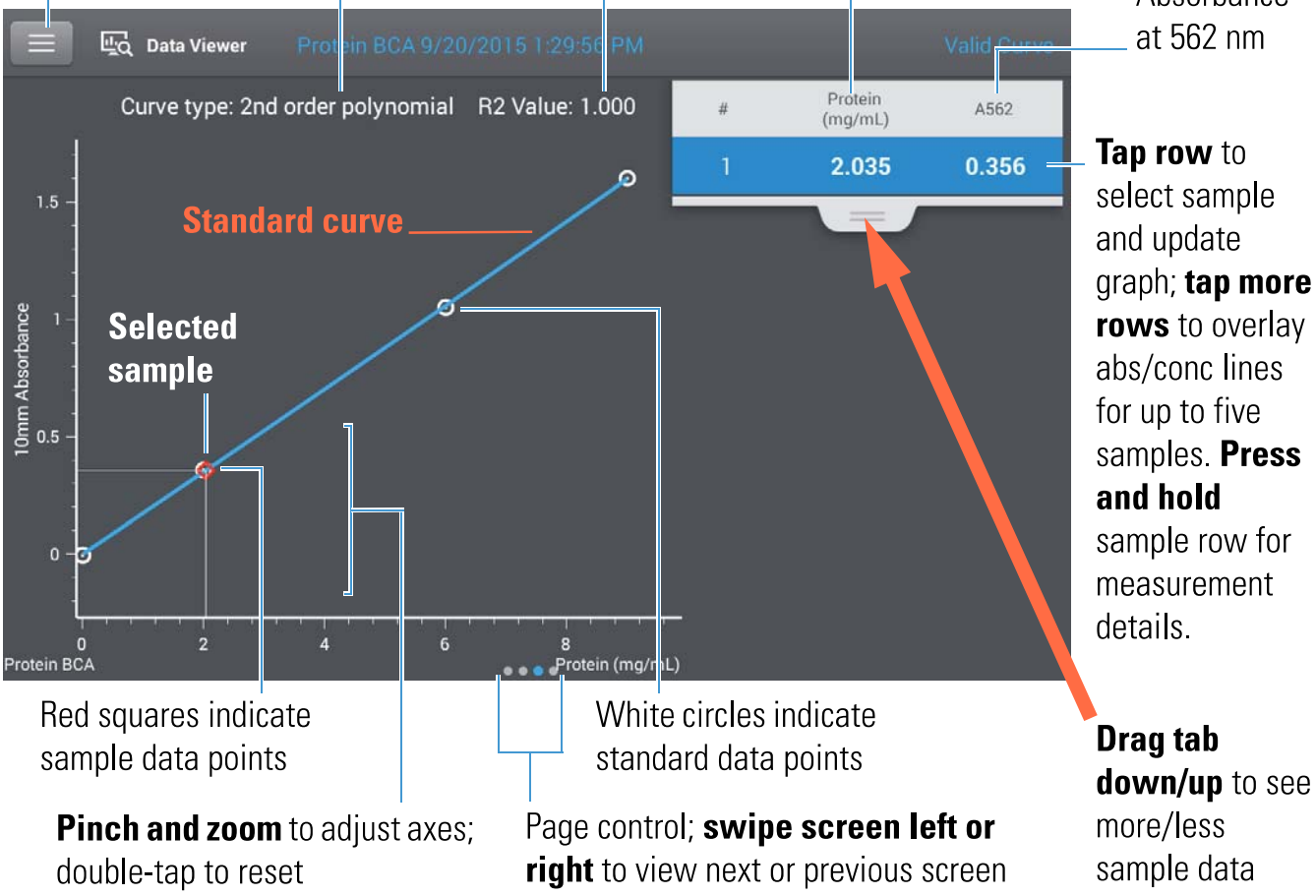
Note

- A baseline correction is performed at 750 nm (absorbance value at 750 nm is subtracted from absorbance values at all wavelengths in sample spectrum).
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

Protein BCA standard curve screen

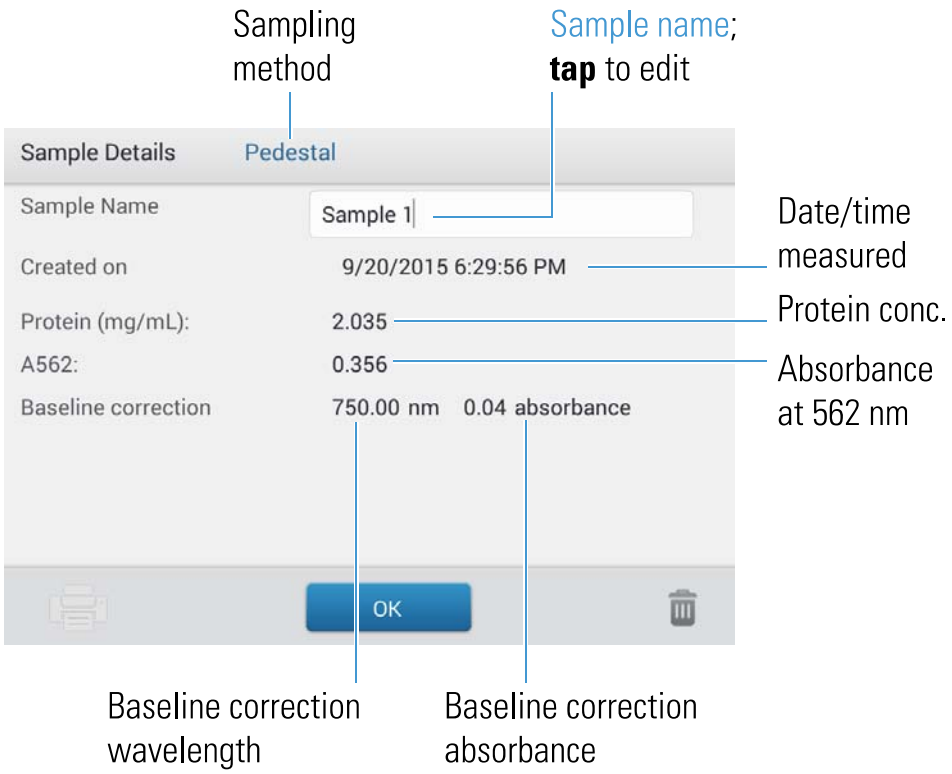
The standard curve screen shows graphically the relationship between the measured standards, the calculated standard curve, and the measured absorbance and calculated concentration for a selected sample. A horizontal line connects the sample absorbance value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis.

The R^2 value indicates how well the standard curve fits the standard data points (1.0 is a perfect fit; that is, all points lie exactly on the curve).



Protein BCA reported values

The initial screen that appears after each measurement and the standards screen (see previous image) show 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
- Protein A280 Calculations

Settings for Protein BCA Measurements

To show the Protein BCA settings, from the Protein BCA measurement screen, tap > Protein BCA Setup.

Note You can edit the Curve Type setting when measuring standards by changing the list box at the top of the application measurement screen. You can edit the concentration value for a standard from the application setup screen. After the first sample measurement, these settings cannot be changed.

Setting	Description
Curve 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) – Interpolation: Draws a series of straight lines to connect all measured standards (requires reference measurement and at least one standard) – 2nd order polynomial: Draws the 2nd order least squares polynomial using all measured standards (requires reference measurement and at least two standards) – 3rd order polynomial: Draws the 3rd order least squares polynomial using all measured standards (requires reference measurement and at least three standards)
Replicates	Enter number of measurements of the reference or the same standard or sample that are averaged together to produce its associated concentration value. Note: Replicates setting cannot be changed after the first standard has been measured.
Standards	Enter actual concentration value of each standard. Note: Concentration values can be entered in any order but the standards must be measured in the order they were entered.

Related Topics

- Instrument Settings

Measure Protein Bradford

Measures total protein concentration of unpurified protein samples using a Coomassie Blue dye colorimetric detection reagent.

Measure Total Protein

Reported Results

Settings

Detection Limits

Measure Total Protein Concentration

The Protein Bradford assay uses Coomassie Blue dye as a colorimetric detection reagent to determine total protein concentration in unpurified protein samples. This application is useful for measuring dilute protein solutions that require lower detection sensitivity or proteins in the presence of components that exhibit significant absorbance between 200 nm and 280 nm, which rules out direct protein measurements at 280 nm or 205 nm. This application measures absorbance at 595 nm and uses a standard curve to calculate protein concentration. See Working with Standard Curves for more information. A single-point baseline correction is applied.

Theory of Protein Bradford assay

The Protein Bradford assay uses the protein-induced absorbance shift of Coomassie Blue dye to determine total protein concentration. The bound protein-dye complex is measured at 595 nm and baseline-corrected using the absorbance value at 750 nm. Pre-formulated kits of stabilized reagent mixture containing Coomassie Blue dye, alcohol, and surfactant are available from us or a local distributor.

To maximize reliability with the Protein Bradford assay:

- Work quickly and do not allow prepared standards or samples to sit longer than necessary. Coomassie dye-dye and Coomassie dye-protein aggregates can form particulates with increasing development time, resulting in significant fluctuations in absorbance readings.
- Measure standards and samples in triplicate using a new aliquot for each measurement. For pedestal measurements, the total analyte (protein-dye) signal at 595 nm is limited to ~0-0.150A due to the pedestal's 1.0 mm pathlength, the Coomassie dye concentration, and the acidic pH.

Note If you have a NanoDrop One^{C} model instrument, using the cuvette option will result in a higher absorbance signal.

Protein assay kits and protocols

Please refer to the NanoDrop website for up-to-date kits and protocols for the NanoDrop One instruments. Follow the assay kit manufacturer's recommendations for all standards and samples (unknowns). Ensure each is subjected to the same timing and temperature throughout the assay.

Protein standards for generating a standard curve may also be provided by the kit manufacturer. Since the NanoDrop One pedestals can measure higher protein concentrations than traditional cuvette-based spectrophotometers, you may need to supply your own protein standards at higher concentrations than provided by the manufacturer. For example, additional standards may be required to ensure the standard curve covers the dynamic range of the assay and the expected range of the unknown samples.

To measure Protein Bradford standards and samples

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.

Before you begin...

Before taking pedestal measurements with the NanoDrop One 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.

To measure Protein Bradford standards and samples

1. From the Home screen, select the Proteins tab and tap Protein Bradford.
2. Specify a curve type and number of replicates for each standard and enter the concentration of each standard.

   Tip: For this assay, set Curve Type to "2nd Order Polynomial" and Replicates to 3.
3. Measure blank:
   - pipette 2 µL DI H_2O onto lower pedestal and lower arm, or insert DI H_2O blanking cuvette into cuvette holder

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

- tap Blank and wait for measurement to complete
- lift arm and clean both pedestals with new laboratory wipe, or remove cuvette

4. Measure reference standard:
   - pipette 2 µL reference solution onto pedestal, or insert reference cuvette (reference solution should contain none of the standard protein stock, see Working With Standard Curves for details)
   - lower arm to start measurement (or tap Measure if Auto-Measure is off)
   - lift arm and clean both pedestals with new wipe, or remove cuvette
   - if Replicates setting is greater than 1, repeat measurement
5. Measure remaining standards:
   - pipette 2 µL standard 1 onto pedestal, or insert standard 1 cuvette
   - lower arm to start measurement (or tap Measure if Auto-Measure is off)
   - lift arm and clean both pedestals with new wipe, or remove cuvette
   - if Replicates setting is greater than 1, repeat measurement
   - repeat substeps above for each additional standard (when specified number of standards and replicates have been measured, a message asks whether to load more standards or begin measuring samples)
   - if finished measuring standards, tap Done (swipe left to view standard curve)
6. Measure samples:
   - pipette 2 µL sample 1 onto pedestal, or insert sample 1 cuvette
   - lower arm to start measurement (or tap Measure if Auto-Measure is off)
   - lift arm and clean both pedestals with new wipe, or remove cuvette
   - if Replicates setting is greater than 1, repeat measurement
7. When you are finished measuring samples, tap End Experiment.
8. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.

Related Topics

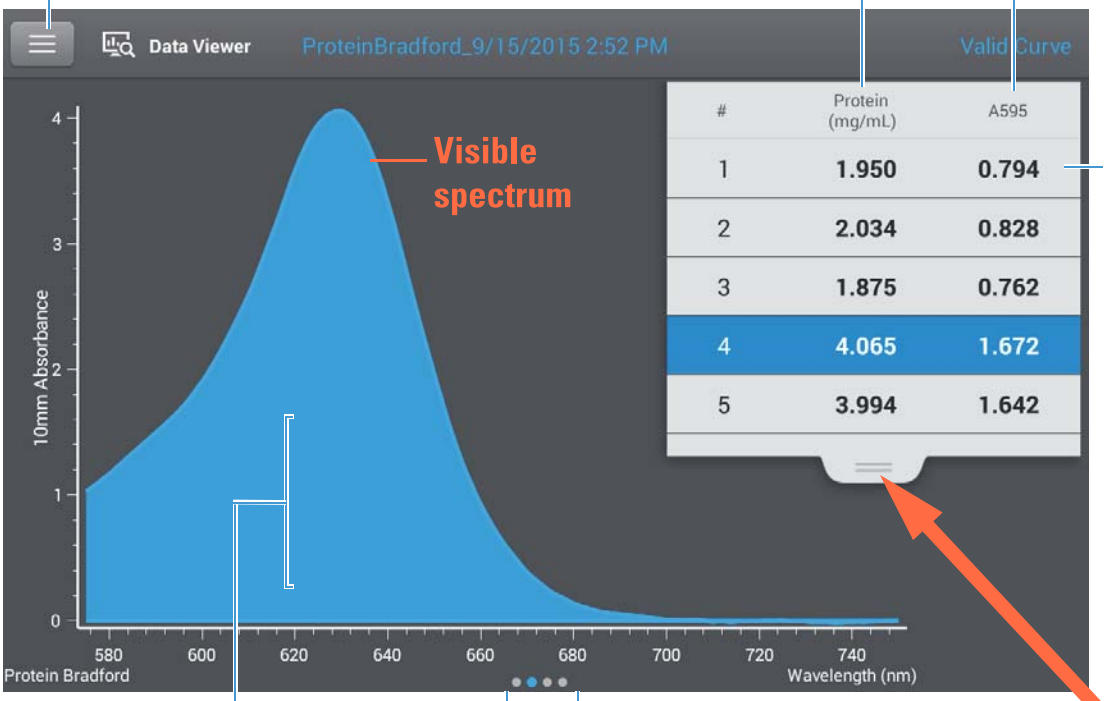
- Working with standard curves
- Best practices for protein measurements
- Measure a Micro-Volume Sample

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

Protein Bradford Reported Results

Protein Bradford measurement screen (shown from Data Viewer)

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. The standard curve is also available by swiping left from the measurement screen (or in the Data Viewer as shown below).



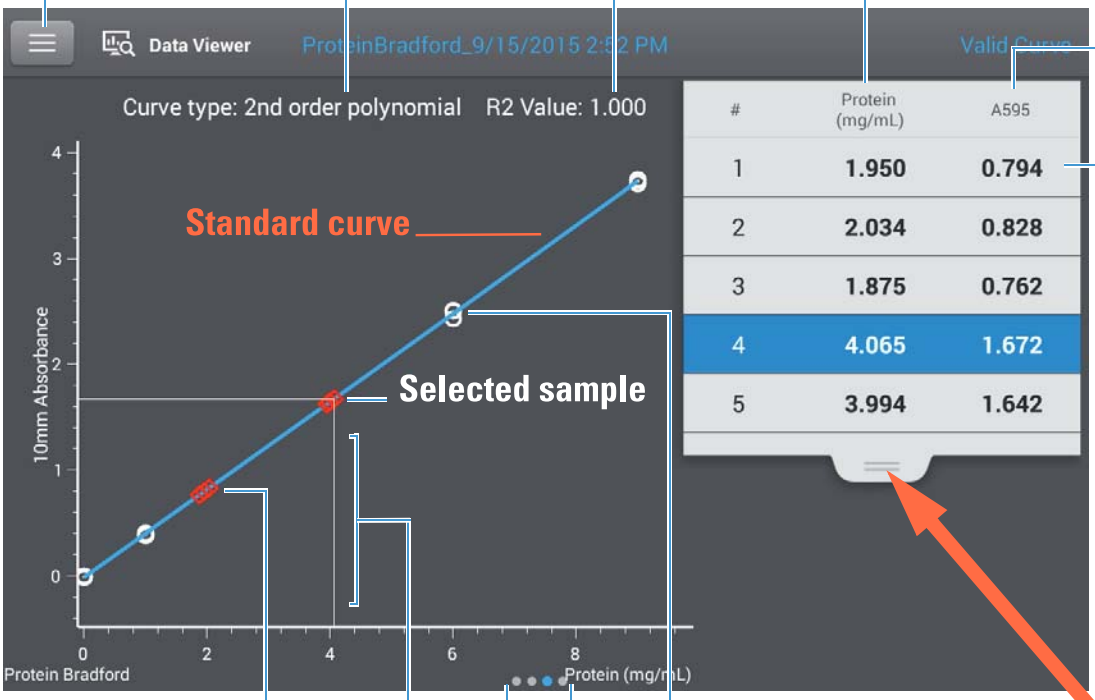
Note

- A baseline correction is performed at 750 nm (absorbance value at 750 nm is subtracted from absorbance values at all wavelengths in sample spectrum).
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

Protein Bradford standard curve screen

The standard curve screen shows graphically the relationship between the measured standards, the calculated standard curve, and the measured absorbance and calculated concentration for a selected sample. A horizontal line connects the sample absorbance value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis.

The R^2 value indicates how well the standard curve fits the standard data points (1.0 is a perfect fit; that is, all points lie exactly on the curve).



Menu of options; **tap** to open

Curve type

R^2 value (1.0 equals perfect fit)

Protein concentration for selected sample

Absorbance at 595 nm

Tap row to select sample and update graph; **tap more rows** to overlay abs/conc lines for up to five samples. **Press and hold** sample row for measurement details.

Red squares indicate sample data points

White circles indicate standard data points

Drag tab down/up to see more/less sample data

Pinch and zoom to adjust axes; double-tap to reset

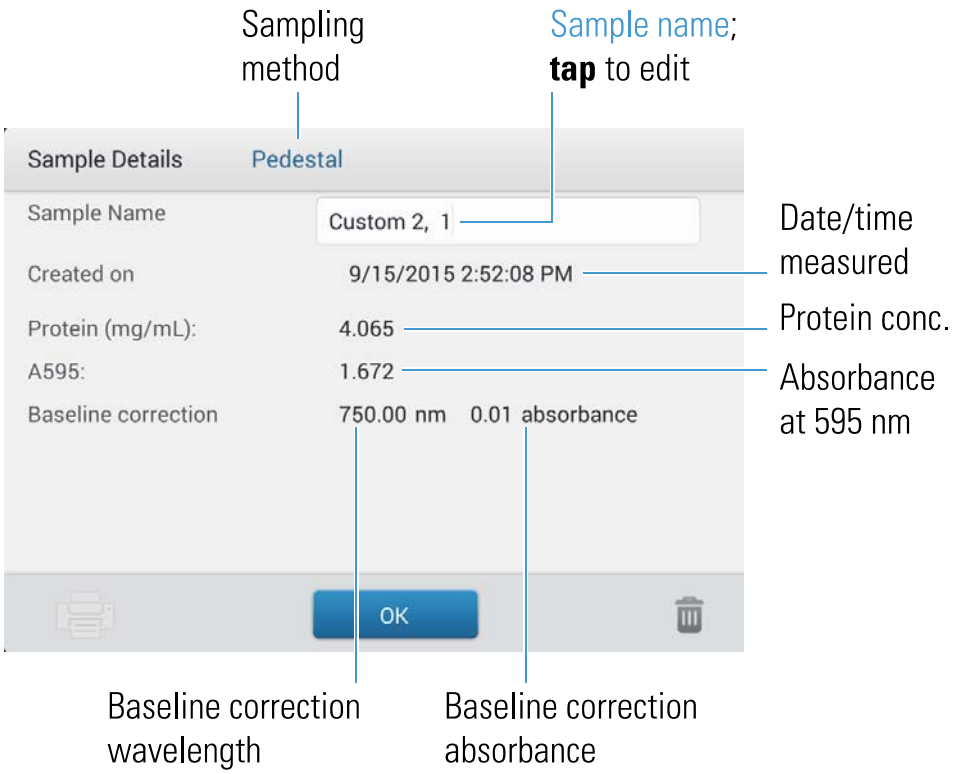
Page control; **swipe screen left or right** to view next or previous screen

Note

- A baseline correction is performed at 750 nm (absorbance value at 750 nm is subtracted from absorbance values at all wavelengths in sample spectrum).
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

Protein Bradford reported values

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



Related Topics

- Example standard curve
- Basic Instrument Operations
- Protein A280 Calculations

Settings for Protein Bradford Measurements

To show the Protein Bradford settings, from the Protein Bradford measurement screen, tap > Protein Bradford Setup.

Note You can edit the Curve Type setting when measuring standards by changing the list box at the top of the application measurement screen. You can edit the concentration value for a standard from the application setup screen. After the first sample measurement, these settings cannot be changed.

Setting	Description
Curve 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) – Interpolation: Draws a series of straight lines to connect all measured standards (requires reference measurement and at least one standard) – 2nd order polynomial: Draws the 2nd order least squares polynomial using all measured standards (requires reference measurement and at least two standards) – 3rd order polynomial: Draws the 3rd order least squares polynomial using all measured standards (requires reference measurement and at least three standards)
Replicates	Enter number of measurements of the reference or the same standard or sample that are averaged together to produce its associated concentration value. Note: Replicates setting cannot be changed after the first standard has been measured.
Standards	Enter actual concentration value of each standard. Note: Concentration values can be entered in any order but the standards must be measured in the order they were entered.

Related Topics

- Instrument Settings

Measure Protein Lowry

Measures total protein concentration of unpurified protein samples using a Folin-Ciocalteu colorimetric detection reagent.

Measure Total Protein

Reported Results

Settings

Detection Limits

Measure Total Protein Concentration

The Protein Lowry assay uses Folin-Ciocalteu as a colorimetric detection reagent to determine total protein concentration in unpurified protein samples. This application is an alternative to the other colorimetric applications for measuring dilute protein solutions or proteins in the presence of components that exhibit significant absorbance between 200 nm and 280 nm. This application measures absorbance at 650 nm and uses a standard curve to calculate protein concentration. See Working with Standard Curves for more information. A single-point baseline correction is applied.

Theory of Protein Lowry assay

The Protein Lowry assay involves the reaction of protein with cupric sulfate in alkaline solution, resulting in the formation of tetradentate copper-protein complexes. The Folin-Ciocalteu reagent is effectively reduced in proportion to the chelated copper-complexes. The water-soluble blue reaction product is measured at 650 nm and baseline-corrected using the absorbance value at 405 nm. Pre-formulated kits of Folin-Ciocalteu reagent and $CuSO_4$ are available from us or a local distributor.

Protein assay kits and protocols

Follow the assay kit manufacturer's recommendations for all standards and samples (unknowns). Ensure each is subjected to the same timing and temperature throughout the assay.

To measure Protein Lowry standards and samples

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.

Before you begin...

Before taking pedestal measurements with the NanoDrop One 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.

To measure Protein Lowry standards and samples

1. From the Home screen, select the Proteins tab and tap Protein Lowry.
2. Specify a curve type and number of replicates for each standard and enter the concentration of each standard.

   Tip: For this assay, we recommend setting Curve Type to "2nd Order Polynomial."
3. Measure blank:
   - pipette 2 µL DI H_2O onto lower pedestal and lower arm, or insert DI H_2O blanking cuvette into cuvette holder

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

- tap Blank and wait for measurement to complete
- lift arm and clean both pedestals with new laboratory wipe, or remove cuvette

4. Measure reference standard:
   - pipette 2 µL reference solution onto pedestal, or insert reference cuvette (reference solution should contain none of the standard protein stock, see Working With Standard Curves for details)
   - lower arm to start measurement (or tap Measure if Auto-Measure is off)
   - lift arm and clean both pedestals with new wipe, or remove cuvette
   - if Replicates setting is greater than 1, repeat measurement
5. Measure remaining standards:
   - pipette 2 µL standard 1 onto pedestal, or insert standard 1 cuvette
   - lower arm to start measurement (or tap Measure if Auto-Measure is off)
   - lift arm and clean both pedestals with new wipe, or remove cuvette
   - if Replicates setting is greater than 1, repeat measurement
   - repeat substeps above for each additional standard (when specified number of standards and replicates have been measured, a message asks whether to load more standards or begin measuring samples)
   - if finished measuring standards, tap Done (swipe left to view standard curve)
6. Measure samples:
   - pipette 2 µL sample 1 onto pedestal, or insert sample 1 cuvette
   - lower arm to start measurement (or tap Measure if Auto-Measure is off)
   - lift arm and clean both pedestals with new wipe, or remove cuvette
   - if Replicates setting is greater than 1, repeat measurement
7. When you are finished measuring samples, tap End Experiment.
8. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.

Related Topics

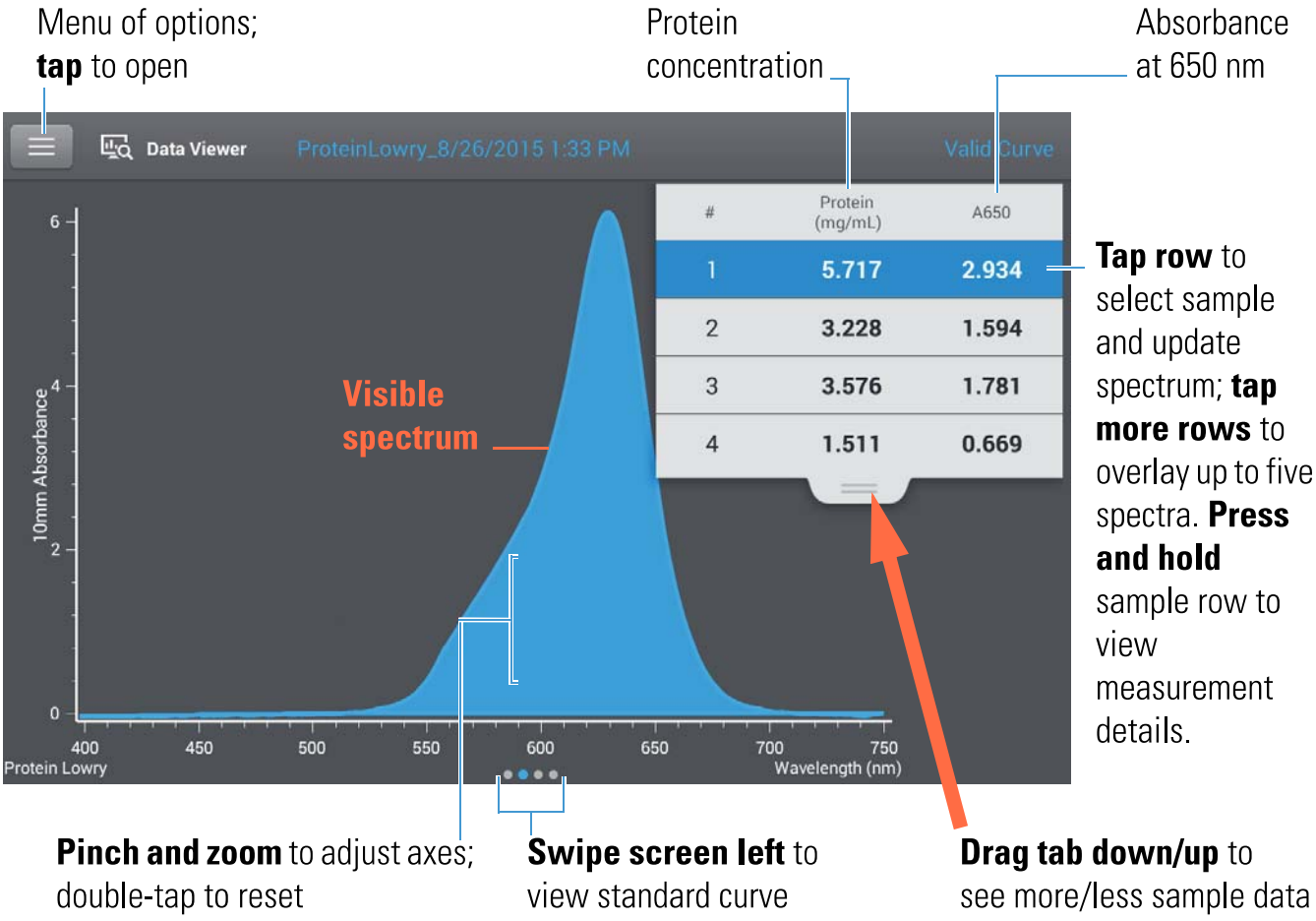
- Working with standard curves
- Best practices for protein measurements
- Measure a Micro-Volume Sample

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

Protein Lowry Reported Results

Protein Lowry measurement screen (shown from Data Viewer)

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. The standard curve is also available by swiping left from the measurement screen (or in the Data Viewer as shown below).



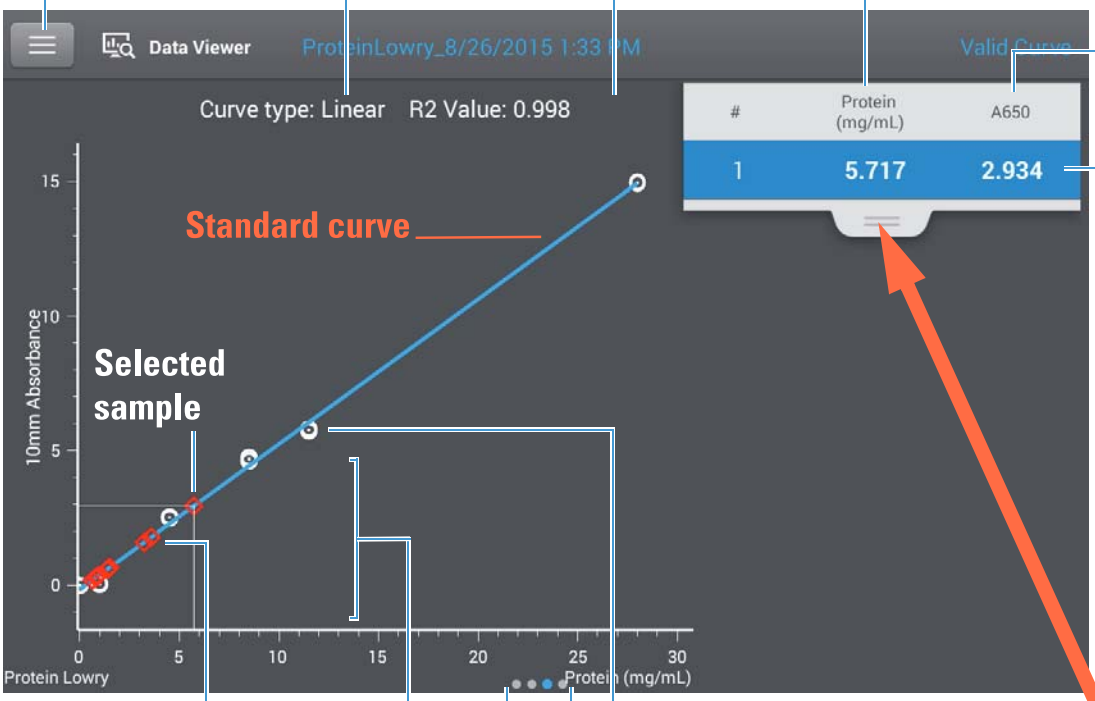
Note

- A baseline correction is performed at 405 nm (absorbance value at 405 nm is subtracted from absorbance values at all wavelengths in sample spectrum).
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

Protein Lowry standard curve screen

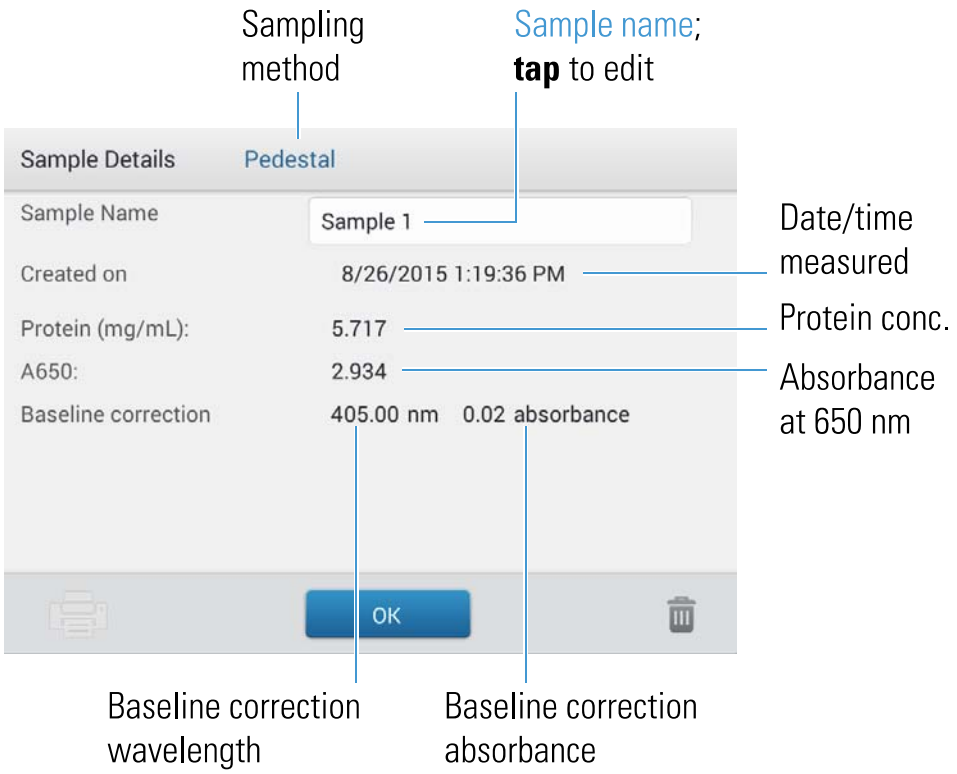
The standard curve screen shows graphically the relationship between the measured standards, the calculated standard curve, and the measured absorbance and calculated concentration for a selected sample. A horizontal line connects the sample absorbance value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis.

The R^2 value indicates how well the standard curve fits the standard data points (1.0 is a perfect fit; that is, all points lie exactly on the curve).


Menu of options; **tap** to open
Curve type
R^2 value (1.0 equals perfect fit)
Protein concentration for selected sample
Absorbance at 650 nm
Data Viewer
ProteinLowry_8/26/2015 1:33 PM
Valid Curve
Curve type: Linear R2 Value: 0.998
Protein (mg/mL) A650
1 5.717 2.934
Standard curve
Selected sample
10mm Absorbance
Protein Lowry
Protein (mg/mL)
Tap row to select sample and update graph; **tap more rows** to overlay abs/conc lines for up to five samples. **Press and hold** sample row for measurement details.
Red squares indicate sample data points
White circles indicate standard data points
Drag tab down/up to see more/less sample data
Pinch and zoom to adjust axes; double-tap to reset
Page control; **swipe screen left or right** to view next or previous screen

Protein Lowry reported values

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



Related Topics

- Example standard curve
- Basic Instrument Operations

Settings for Protein Lowry Measurements

To show the Protein Lowry settings, from the Protein Lowry measurement screen, tap > Protein Lowry Setup.

Note You can edit the Curve Type setting when measuring standards by changing the list box at the top of the application measurement screen. You can edit the concentration value for a standard from the application setup screen. After the first sample measurement, these settings cannot be changed.

Setting	Description
Curve 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) – Interpolation: Draws a series of straight lines to connect all measured standards (requires reference measurement and at least one standard) – 2nd order polynomial: Draws the 2nd order least squares polynomial using all measured standards (requires reference measurement and at least two standards) – 3rd order polynomial: Draws the 3rd order least squares polynomial using all measured standards (requires reference measurement and at least three standards)
Replicates	Enter number of measurements of the reference or the same standard or sample that are averaged together to produce its associated concentration value. Note: Replicates setting cannot be changed after the first standard has been measured.
Standards	Enter actual concentration value of each standard. Note: Concentration values can be entered in any order but the standards must be measured in the order they were entered.

Related Topics

- Instrument Settings

Measure Protein Pierce 660

Measures total protein concentration of unpurified protein samples using a proprietary colorimetric detection reagent.

Measure Total Protein

Reported Results

Settings

Detection Limits

Measure Total Protein Concentration

The Protein Pierce 660 assay uses a proprietary protein binding material as a colorimetric detection reagent to determine total protein concentration in unpurified protein samples. This application is suitable for protein solutions that contain high concentrations of detergents, reducing agents and other commonly used reagents. The Pierce 660 application measures absorbance at 660 nm and uses a standard curve to calculate protein concentration (see Working with Standard Curves for more information). A single-point baseline correction is applied.

Theory of Protein Pierce 660 assay

The Protein Pierce 660 assay is based on the binding of a proprietary dye-metal complex to protein in acidic conditions that causes a shift in the dye's absorption maximum, which is measured at 660 nm. The dye-metal complex is reddish-brown and changes to green upon protein binding. The color change is produced by deprotonation of the dye at low pH facilitated by interactions with positively charged amino acid groups in proteins. The dye interacts mainly with basic residues in proteins such as histidine, arginine and lysine and to a lesser extent tyrosine, tryptophan and phenylalanine. The reaction product is measured at 660 nm and baseline-corrected using the absorbance value at 750 nm.

The color produced in the assay is stable and increases in proportion to a broad range of increasing protein concentrations. An optional Ionic Detergent Compatibility Reagent (IDCR) may be added to the assay reagent to increase compatibility with high amounts of ionic detergents, including Laemmli SDS sample buffer with bromophenol blue. The IDCR dissolves completely by thorough mixing and has no effect on the assay. Pre-formulated kits of the protein binding material are available from us or a local distributor. For information about IDCR, refer to the kit manufacturer.

Protein assay kits and protocols

Please refer to the NanoDrop website for up-to-date kits and protocols for the NanoDrop One instruments. Follow the assay kit manufacturer's recommendations for all standards and samples (unknowns). Ensure each is subjected to the same timing and temperature throughout the assay.

Protein standards for generating a standard curve may also be provided by the kit manufacturer. Since the NanoDrop One pedestals can measure higher protein concentrations than traditional cuvette-based spectrophotometers, you may need to supply your own protein standards at higher concentrations than provided by the manufacturer. For example, additional standards may be required to ensure the standard curve covers the dynamic range of the assay and the expected range of the unknown samples.

To measure Protein Pierce 660 standards and samples

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.

Before you begin...

Before taking pedestal measurements with the NanoDrop One 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.

To measure Protein Pierce 660 standards and samples

1. From the Home screen, select the Proteins tab and then tap Protein Pierce 660.
2. Specify a curve type and number of replicates for each standard and enter the concentration of each standard.

   Tip: For this assay, we recommend setting Curve Type to “Linear”.
3. Measure blank:
   - Pipette 2 µL reference solution onto lower pedestal and lower arm, or insert reference solution blanking cuvette into cuvette holder (reference solution should contain none of the standard protein stock; see Working With Standard Curves for details)

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

- tap Blank and wait for measurement to complete
- lift arm and clean both pedestals with new laboratory wipe, or remove cuvette

4. Measure reference standard:
   - pipette 2 µL reference solution onto pedestal, or insert reference cuvette (reference solution should contain none of the standard protein stock, see Working With Standard Curves for details)
   - lower arm to start measurement (or tap Measure if Auto-Measure is off)
   - lift arm and clean both pedestals with new wipe, or remove cuvette
   - if Replicates setting is greater than 1, repeat measurement
5. Measure remaining standards:
   - pipette 2 µL standard 1 onto pedestal, or insert standard 1 cuvette
   - lower arm to start measurement (or tap Measure if Auto-Measure is off)
   - lift arm and clean both pedestals with new wipe, or remove cuvette
   - if Replicates setting is greater than 1, repeat measurement
   - repeat substeps above for each additional standard (when specified number of standards and replicates have been measured, a message asks whether to load more standards or begin measuring samples)
   - if finished measuring standards, tap Done (swipe left to view standard curve)
6. Measure samples:
   - pipette 2 µL sample 1 onto pedestal, or insert sample 1 cuvette
   - lower arm to start measurement (or tap Measure if Auto-Measure is off)
   - lift arm and clean both pedestals with new wipe, or remove cuvette
   - if Replicates setting is greater than 1, repeat measurement
7. When you are finished measuring samples, tap End Experiment.
8. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.

Related Topics

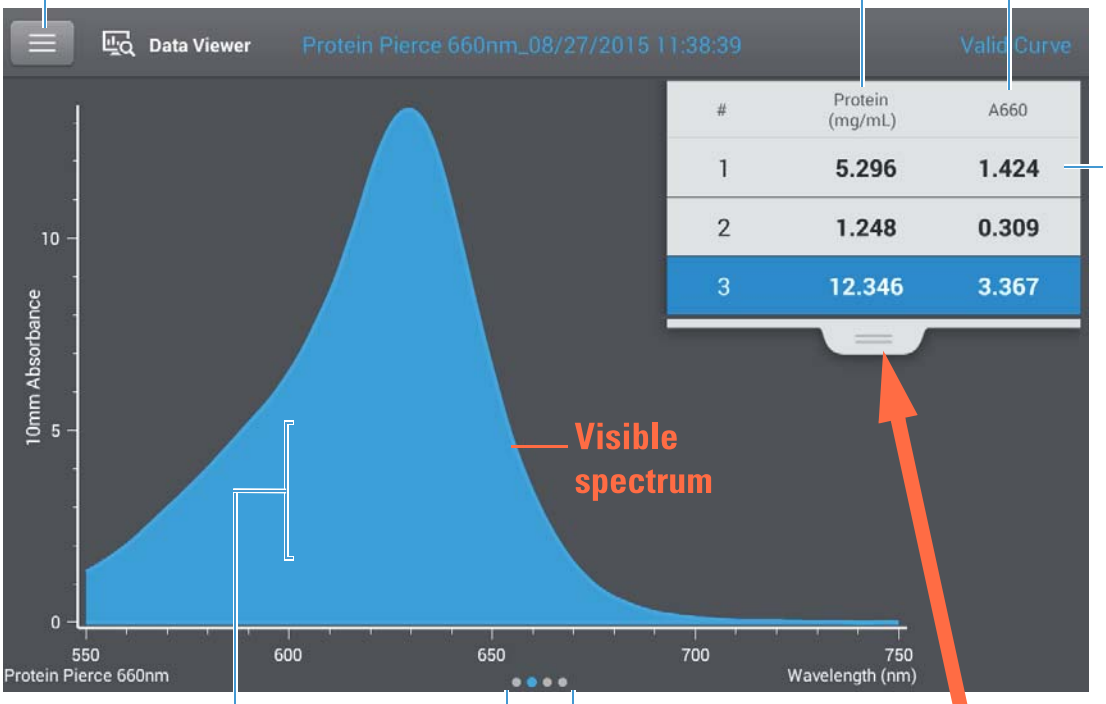
- Working with standard curves
- Best practices for protein measurements
- Measure a Micro-Volume Sample

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

Protein Pierce 660 Reported Results

Protein Pierce 660 measurement screen (shown from Data Viewer)

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. The standard curve is also available by swiping left from the measurement screen (or in the Data Viewer as shown below).



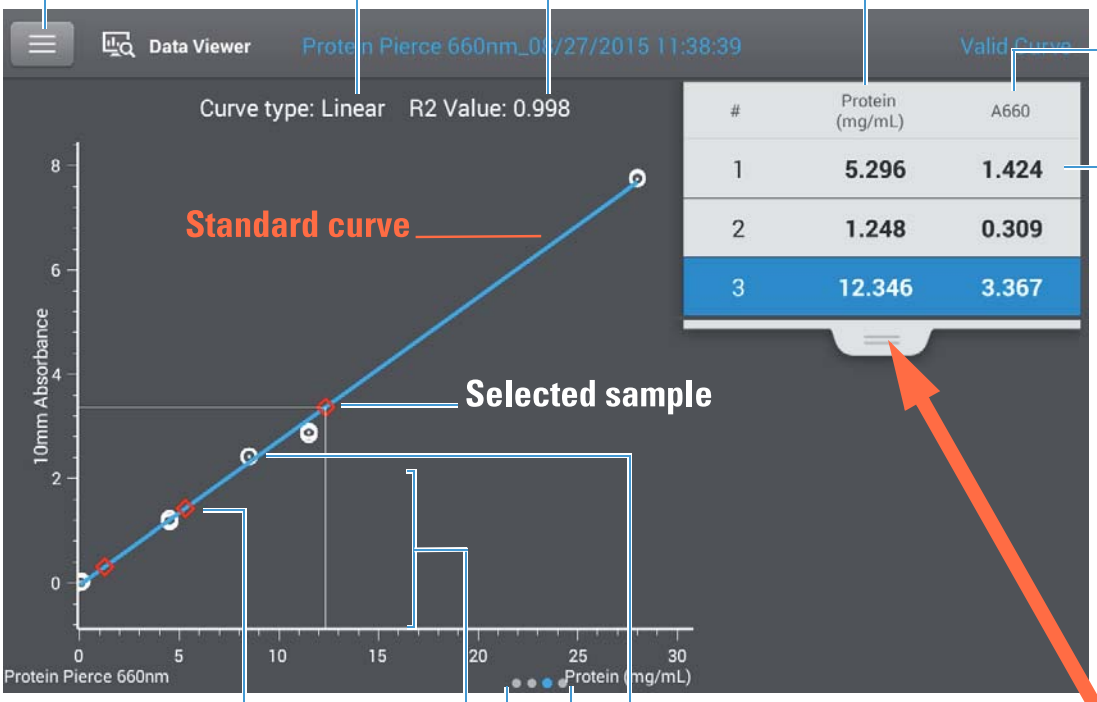
Note

- A baseline correction is performed at 750 nm (absorbance value at 750 nm is subtracted from absorbance values at all wavelengths in sample spectrum).
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

Protein Pierce 660 standard curve screen

The standard curve screen shows graphically the relationship between the measured standards, the calculated standard curve, and the measured absorbance and calculated concentration for a selected sample. A horizontal line connects the sample absorbance value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis.

The R^2 value indicates how well the standard curve fits the standard data points (1.0 is a perfect fit; that is, all points lie exactly on the curve).



Menu of options; **tap** to open

Curve type

R^2 value (1.0 equals perfect fit)

Protein concentration for selected sample

Absorbance at 660 nm

Tap row to select sample and update graph; **tap more rows** to overlay abs/conc lines for up to five samples. **Press and hold** sample row for measurement details.

Red squares indicate sample data points

White circles indicate standard data points

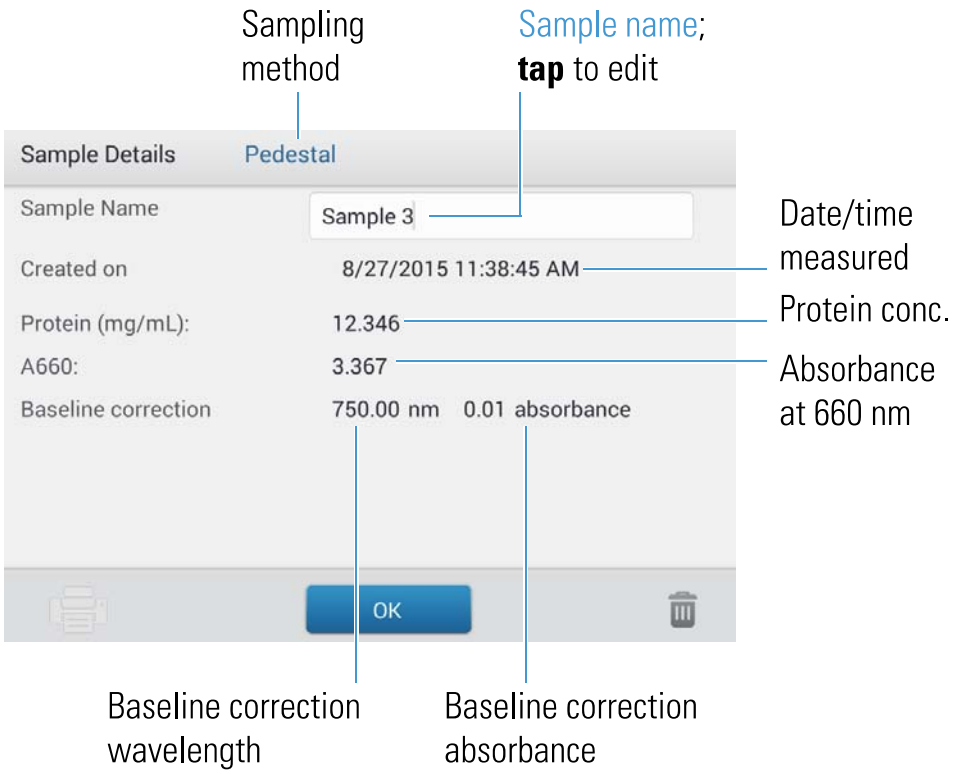
Drag tab down/up to see more/less sample data

Pinch and zoom to adjust axes; double-tap to reset

Page control; **swipe screen left or right** to view next or previous screen

Protein Pierce 660 reported values

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



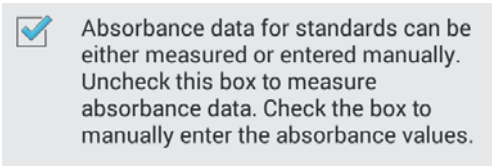
Related Topics

- Example standard curve
- Basic Instrument Operations

Settings for Protein Pierce 660 Measurements

To show the Protein Pierce 660 settings, from the Protein Pierce 660 measurement screen, tap > Protein Pierce 660 Setup.

Note You can edit the Curve Type setting when measuring standards by changing the list box at the top of the application measurement screen. You can edit the concentration value for a standard from the application setup screen. After the first sample measurement, these settings cannot be changed.

Setting	Description
Curve 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) – Interpolation: Draws a series of straight lines to connect all measured standards (requires reference measurement and at least one standard) – 2nd order polynomial: Draws the 2nd order least squares polynomial using all measured standards (requires reference measurement and at least two standards) – 3rd order polynomial: Draws the 3rd order least squares polynomial using all measured standards (requires reference measurement and at least three standards)
Replicates	Enter number of measurements of the reference or the same standard or sample that are averaged together to produce its associated concentration value. Note: Replicates setting cannot be changed after the first standard has been measured.
Standards	Enter actual concentration value of each standard. Note: Concentration values can be entered in any order but the standards must be measured in the order in which they were entered. If you also want to enter previously measured absorbance values for the standards, select this check box:  and then enter absorbance values for all the standards.

Related Topics

- Instrument Settings

Measure OD600

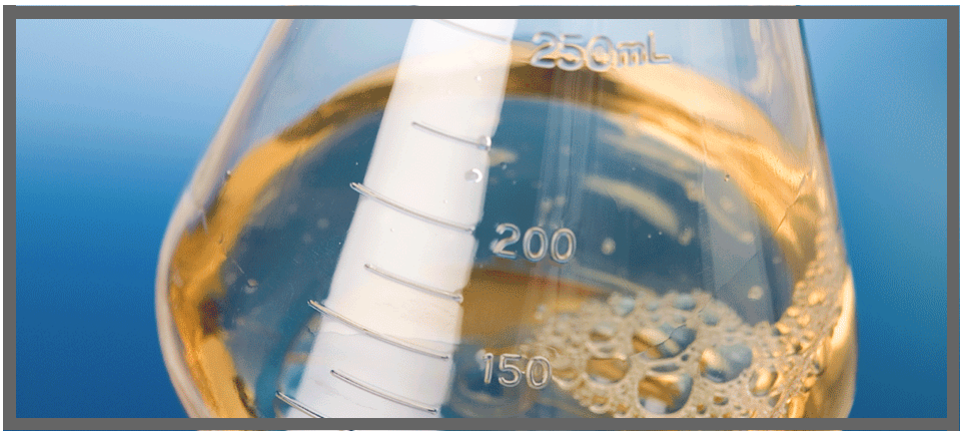
Measures the concentration of microbial cell cultures in solution by measuring scattered light at 600 nm.

Measure OD600

Reported Results

Settings

Calculations



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.

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

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 One instrument, 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 One^{C} 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.

To measure OD600 samples

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.

Before you begin...

Before taking pedestal measurements with the NanoDrop One 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.

To measure an OD600 sample

1. From the Home screen, select the OD600 tab and tap OD600.
2. Specify the cell number conversion factor and a second monitored wavelength or absorbance correction if desired.
3. 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.
4. Tap 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.)
5. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.
6. Pipette 2 µL sample solution onto the pedestal and lower the arm, or insert the sample cuvette into the cuvette holder.
7. Start the sample measurement:
   - Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is off, lower arm and tap Measure.
   - Cuvette: Tap Measure

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).
8. When you are finished measuring samples, tap End Experiment.
9. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.

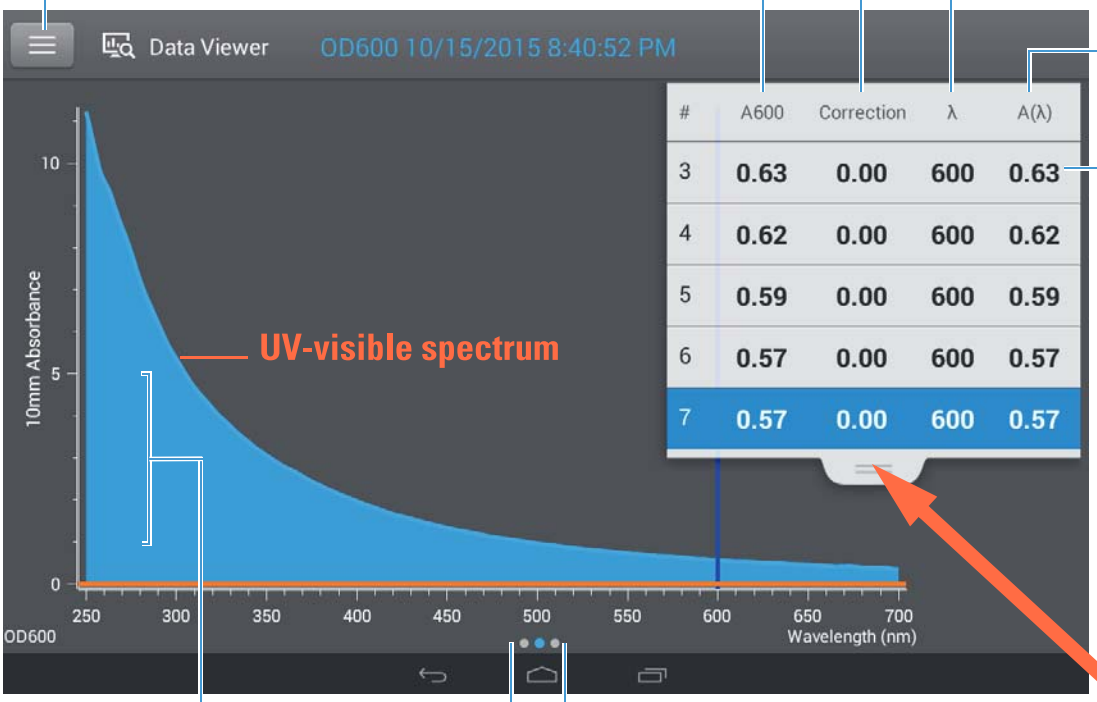
Related Topics

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

OD600 Reported Results

OD600 measurement screen (shown from Data Viewer)

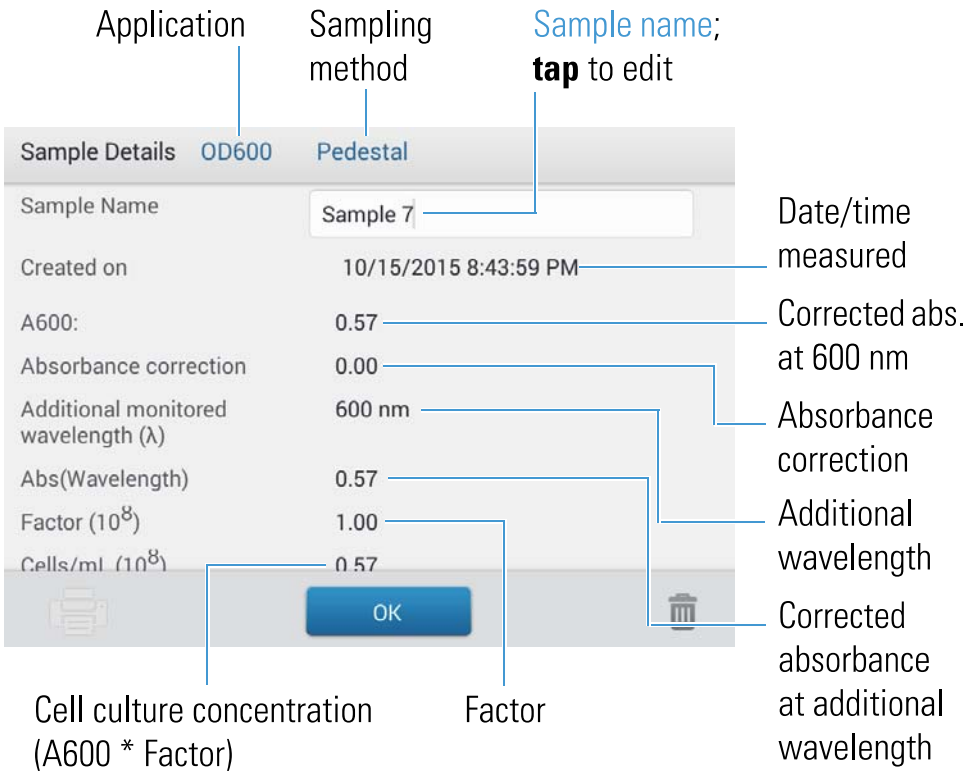
For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example:



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

OD600 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:



Related Topics

- Basic Instrument Operations
- OD600 Calculations

Settings for OD600 Measurements

To show the OD600 settings, from the OD600 measurement screen, tap ☰ > OD600 Setup.

Setting	Available Options	Description
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 ()	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()* factor()** where: **c** = analyte concentration in cells/mL **A**() = UV-visible absorbance at specified wavelength in absorbance units (A) factor() = 1/( * b) in mL/cell-cm where:  = molar absorption coefficient (or extinction coefficient) at specified wavelength **b** = pathlength in cm (1.0 cm for the NanoDrop One 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 for OD600 Measurements

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( ) absorbance

- Normalized and (absorbance) corrected (if used) absorbance value at any specified Additional Monitored Wavelength ( ) 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, pathlength is determined by the cuvette Pathlength setting in the software (see General 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.

Measure Custom

Runs a custom measurement method created using NanoDrop One Viewer software.

Measure Custom Method

Delete Custom Method

Reported Results

Measure using a Custom Method

Use the Custom application to run a user-defined method created using the NanoDrop One Viewer software running on a personal computer. For more information, see Create Custom Method.

To load a custom method

Custom methods can only be created on a personal computer running the NanoDrop One Viewer software. If you want to run a custom method and store the measurement results on the instrument, the method must also reside on the instrument. (This is the only way to run a custom method if your instrument is not connected to the computer with an Ethernet cable or through a wireless network.)

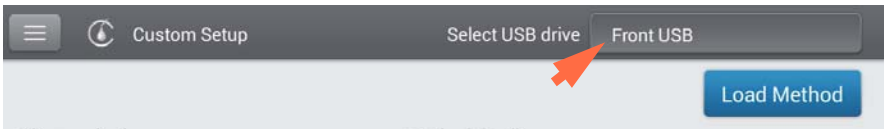
Note If the computer is connected to the instrument with an Ethernet cable or through a wireless network, custom methods can reside on the computer and the measurement results will be stored in that computer's database. For more information, see "Set Up Ethernet Connection" or "Set Up Wi-Fi Connections" in Set Up the Instrument.

Load custom methods onto the instrument

1. Export the method from the personal computer and copy the method file to the root of a portable USB device such as a memory stick.

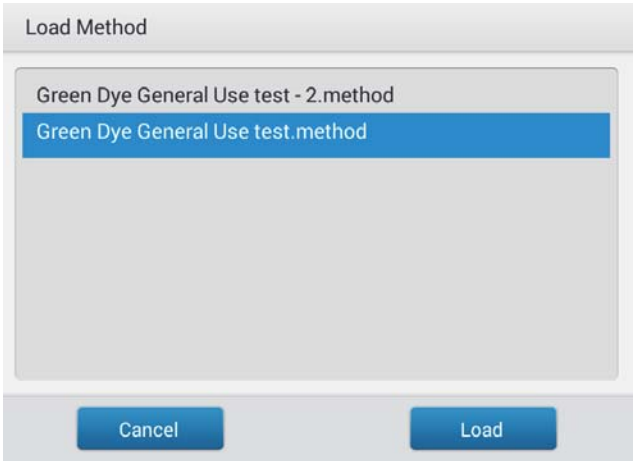
   Method files have a ".method" filename extension.

2. Connect the USB device to one of the USB ports on the instrument.
3. From the Home screen, select the Custom tab and tap Custom.
4. Use the list box at the top of the screen to indicate the USB port used.



5. Tap Load Method.

   A message box shows the NanoDrop One methods available on the selected USB device.



6. Tap one or more method names in the Load Method box to select the methods to load.
7. Tap Load.

To measure using a custom method

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.

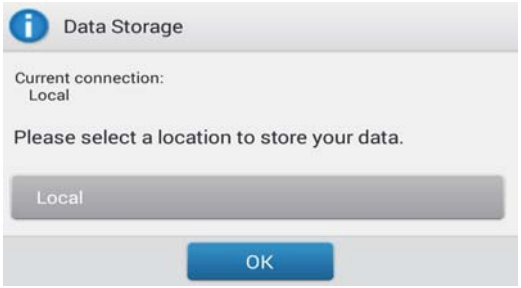
Before you begin...

Before taking pedestal measurements with the NanoDrop One 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.

To measure a sample using a custom method

1. Make sure the method resides in same location as the database where you want to store the measurement results (see To Load a Custom Method for details).
2. From the Home screen, select the **Custom** tab and tap **Custom**.

   If the instrument has a working Ethernet or wireless connection to a remote personal computer (PC), the Data Storage message box appears.

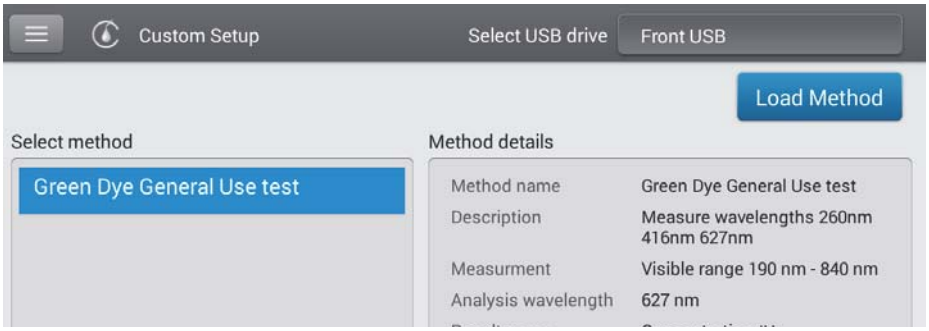


   – To run a custom method that is loaded on the instrument and store all subsequently acquired measurement results in the database on the instrument, set Data Storage to **Local** (see example above).

   – To run a custom method that resides on a personal computer connected to the instrument with an Ethernet cable and store all subsequently acquired measurement results in the database on that computer, set Data Storage to **Direct-Connect PC** (see "Set Up Ethernet Connection" in Set Up the Instrument for more information).

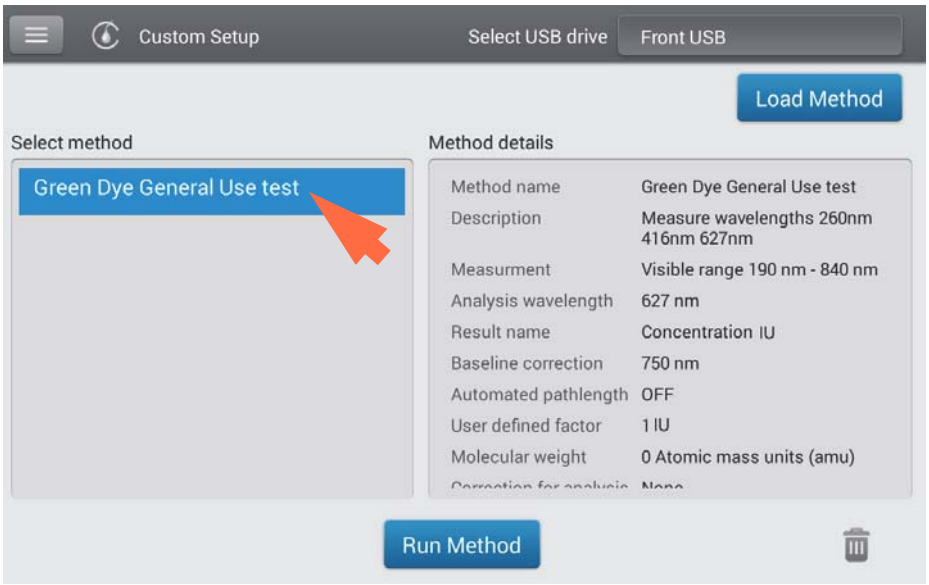
   – To run a custom method that resides on a personal computer connected to the instrument through a wireless network and store all subsequently acquired measurement results in the database on that computer, set Data Storage to the **computer's assigned name** (see "Set Up Wi-Fi Connections" in Set Up the Instrument for more information).

After you tap OK, the Custom Setup box (local or remote) is displayed.



- If Data Storage is set to **Local** (see the previous step), Custom Setup shows only custom methods that reside on the instrument (see To Load a Custom Method for more information).
- If Data Storage is set to **Direct-Connect PC** (Ethernet) or a specific **computer name** (wireless), Custom Setup shows only custom methods that reside on the wired (Ethernet) or specified (wireless) computer (see Create Custom Method for more information).

3. In the Select Method box, tap to select the method to run.



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

4. Tap **Run Method**.
5. Follow the on-screen instructions to measure a sample.

Related Topics

- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette
- Create Custom Method
- Set Up the Instrument
- Export Custom Method

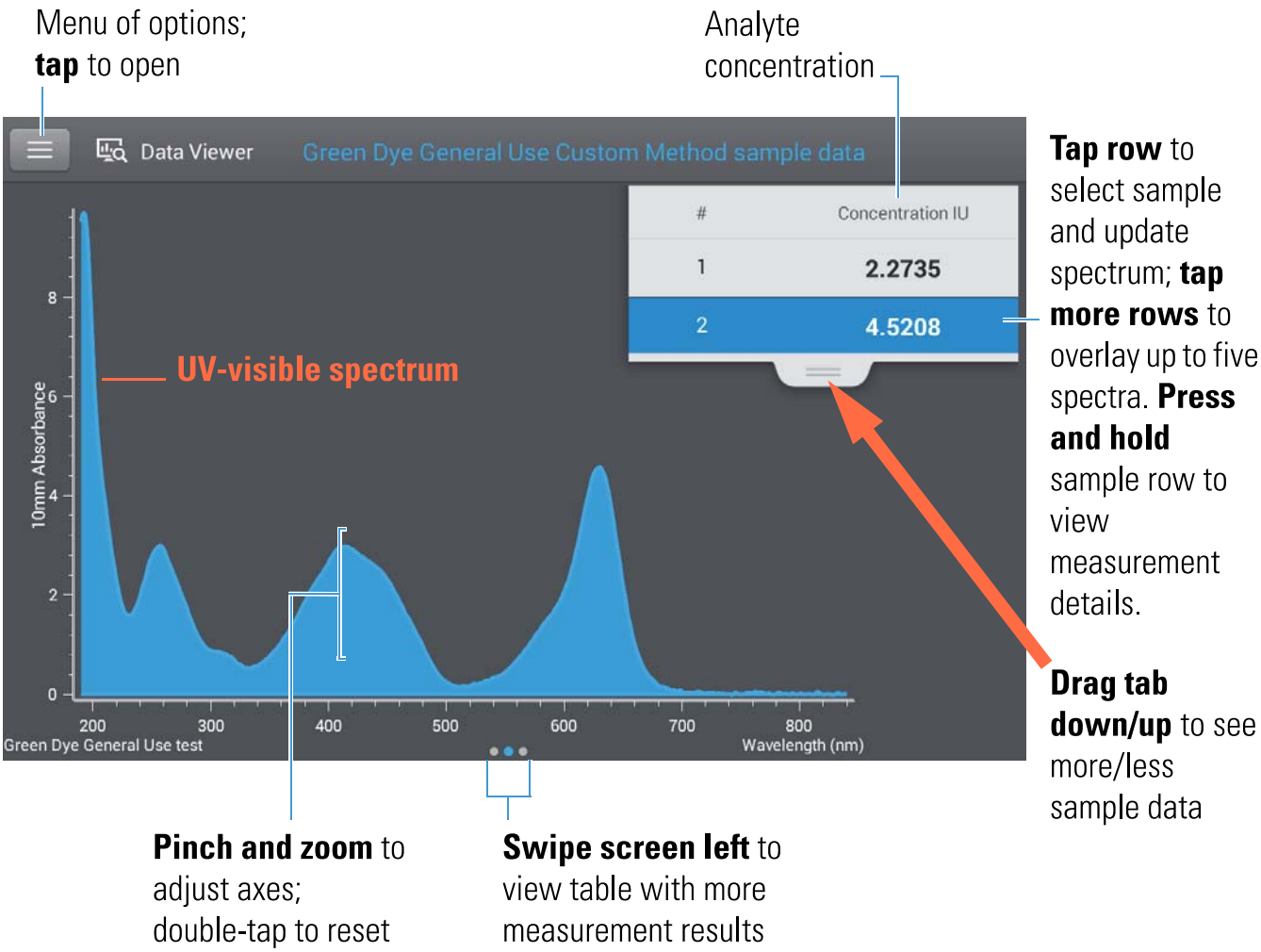
Delete Custom Method

– From Home screen, select Custom tab and tap Custom.
– In Select Method box, tap to select method to delete
– tap 

Custom Method Reported Results

Custom method measurement screen (shown from Data Viewer)

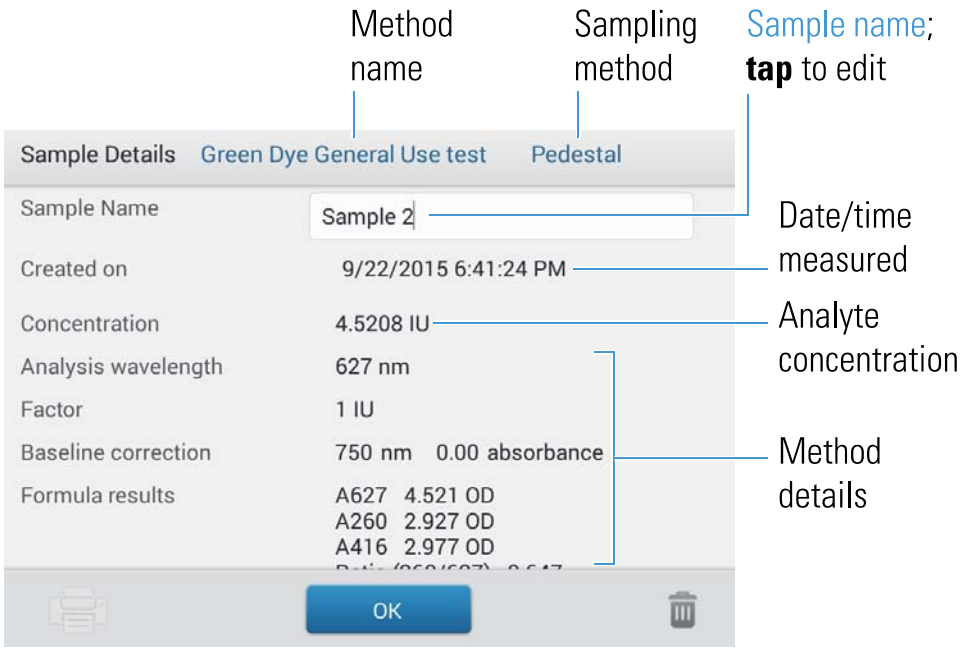
For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example:



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

Custom method 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:



Related Topics

- Basic Instrument Operations

Measure UV-Vis

Measures the absorbance of any sample at up to 40 wavelengths across the ultra-violet (UV) and visible regions of the spectrum.

Measure UV-Vis

Reported Results

Settings

Detection Limits

Measure UV-Vis

The UV-Vis application allows the instrument to function as a conventional spectrophotometer. Sample absorbance is displayed on the screen from 190 nm to 850 nm. Up to 40 wavelengths can be designated for absorbance monitoring and inclusion in the report. Automatic pathlength adjustment and a single-point baseline correction can also be used.

To make UV-Vis measurements

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.

Before you begin...

Before taking pedestal measurements with the NanoDrop One 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.

To measure a sample using the UV-Vis application

1. From the Home screen, select the Custom tab and tap UV-Vis.
2. Specify up to 40 wavelengths to monitor (or you can specify them later if desired) and whether automated pathlength adjustment and baseline correction will be used.
3. Pipette 1–2 µL blanking solution 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.
4. Tap 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.)
5. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.
6. Pipette 1-2 µL sample solution onto the pedestal and lower the arm, or insert the sample cuvette into the cuvette holder.
7. Start the sample measurement:
   - Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is off, lower arm and tap Measure.
   - Cuvette: Tap Measure.

   When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).
8. When you are finished measuring samples, tap End Experiment.
9. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.

Best practices for UV-Vis measurements

- Ensure the sample absorbance is within the instrument's absorbance detection limits.
- Blank with the same buffer solution used to resuspend the analyte of interest. The blanking solution should be a similar pH and ionic strength as the analyte solution.
- Run a blanking cycle to assess the absorbance contribution of your buffer solution. If the buffer exhibits strong absorbance at or near an analysis wavelength, you may need to choose a different buffer or application. See Choosing and Measuring a Blank for more information.
- For micro-volume measurements:
  - Ensure pedestal surfaces are properly cleaned and conditioned.
  - Ensure samples are homogeneous before taking a measurement. Avoid introducing bubbles when mixing and pipetting.
  - Follow best practices for micro-volume measurements.
  - Use a 1-2 µL sample volume. See Recommended Sample Volumes for more information.
- For cuvette measurements (NanoDrop One^{C} instruments only), use compatible cuvettes and follow best practices for cuvette measurements.

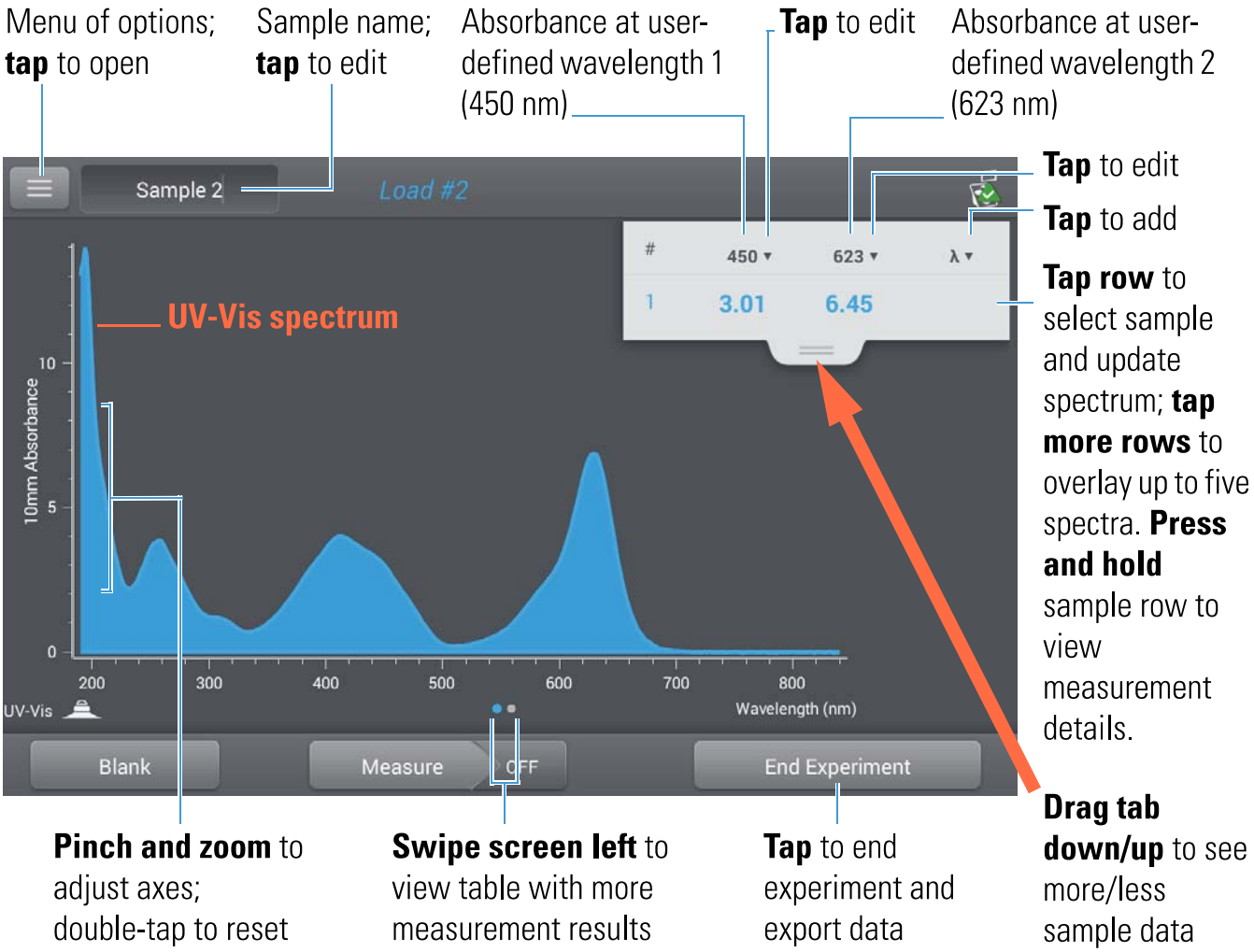
Related Topics

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

UV-Vis Reported Results

UV-Vis measurement screen

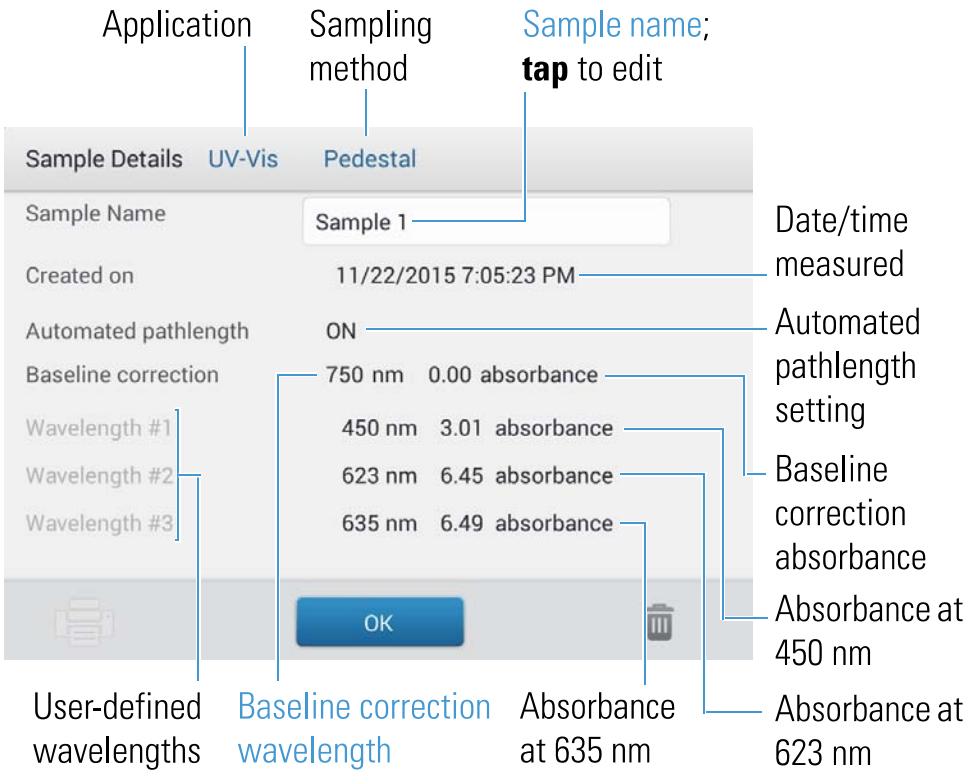
For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example:



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

UV-Vis 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:



Note Scroll up to display absorbance values for any additional user-defined wavelengths.

Related Topics

- Basic Instrument Operations

Settings for UV-Vis Measurements

To show the UV-Vis settings, from the UV-Vis measurement screen, tap > UV-Vis Setup.

Setting	Available Options	Description
Monitored wavelengths	Enter up to 40 wavelengths between 190 nm and 850 nm	User-defined wavelengths to be measured and reported at run time. Absorbance values for the first three entered wavelengths are displayed in the measurement screen. To see absorbance values for 8 monitored wavelengths, swipe left in the measurement screen to show the Data table. To see all monitored wavelengths, press and hold a sample row to show the Sample Details screen (scroll up to display absorbance values for any additional user-defined wavelengths). Note: If Baseline Correction is selected, all displayed absorbance values are the corrected values.
Automated Pathlength	On or Off (affects pedestal measurements only)	Optional automated pathlength selection. Allows the software to use the optimal (shorter) pedestal pathlength for high concentration samples to help prevent detector saturation (see Detection Limits for details). • When selected, the shorter pathlength is used when any wavelength between 220 nm and 850 nm has 10 mm equivalent absorbance value of 12.5 or higher. For wavelengths between 190 nm and 219 nm the change to the shorter pathlength occurs when any wavelength in this range has a 10 mm equivalent absorbance value of 10 or higher. • When deselected, the pedestal pathlength is restricted to 10 mm across all wavelengths. Note: In either case, displayed absorbance values have been normalized to a 10 mm pathlength equivalent.
Baseline Correction	On or off Enter baseline correction wavelength in nm or use default value (750 nm)	Optional user-defined baseline correction. Can be used to correct for any offset caused by light scattering particulates by subtracting measured absorbance 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.

Related Topics

- Instrument Settings

Measure Kinetics

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

Measure Kinetics

Create Kinetics Method

Edit Kinetics Method

Reported Results

Settings

Detection Limits

Measure Kinetics

The NanoDrop One^{C} model instrument 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

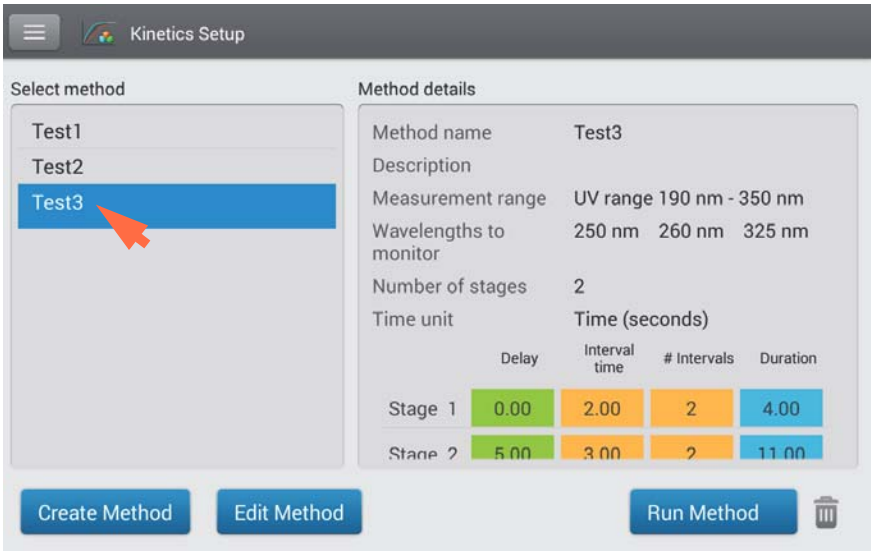
NOTICE

- 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.

To measure a sample using the Kinetics application

1. From the instrument Home screen, select the Kinetics tab and tap the Kinetics icon.

   The Kinetics Setup screen is displayed. If one or more kinetic methods exist in the currently selected Data Storage Location, they will be listed in the Select Method box. A description of the selected method appears in the Method Details box.



2. Select a method:

   - select an existing method by tapping the method name in the Select Method box
   - create a new method by tapping Create Method, specifying the method settings and choosing Save Method
   - edit an existing method by tapping the method name and choosing Edit Method

3. Specify any cuvette options such as heating or stirring by tapping ☰ > Settings (see General settings for details).

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

4. Tap Run Method.

5. 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

   – Tap Blank

If Heat Cuvette to 37 °C is selected in General 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.

6. 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
   - Tap Measure

     If Heat Cuvette to 37 °C is selected in General 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 button at the bottom of the measurement screen to pause the experiment (if you need to end the experiment early, tap 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.

7. When you are finished reviewing the data, tap 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

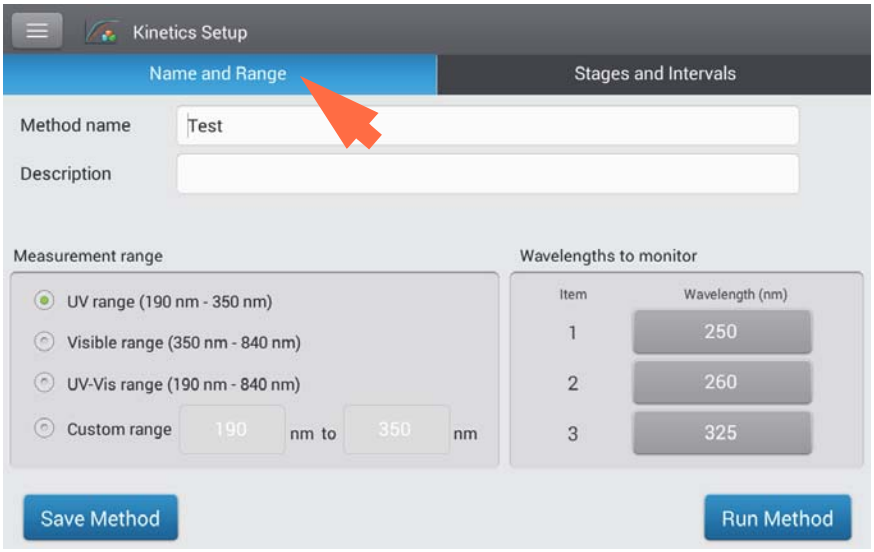
- Best Practices for Cuvette Measurements
- Prepare Samples and Blanks
- Basic Instrument Operations

Create Kinetics Method

Kinetics methods can be created and run only on the NanoDrop One instrument. However, once the method is created, it can be saved in the NanoDrop One database on the local instrument, or in the NanoDrop Viewer database on a connected PC. To create a new kinetics method:

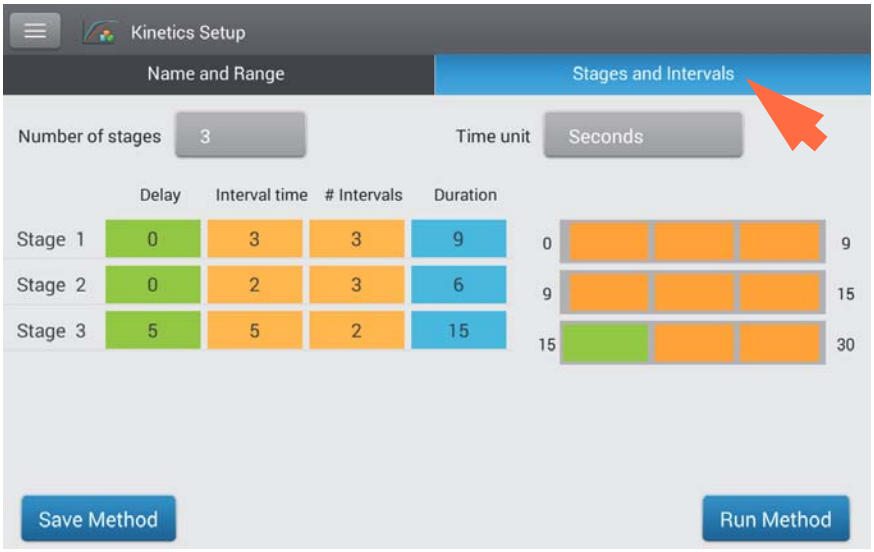
– from Home screen, tap Kinetics tab > Kinetics application

– tap Create Method (the method settings are displayed with Name and Range tab selected)



– enter Method Name and Description (if desired), select Measurement range and specify up to three Wavelengths to monitor

– tap Stages and Intervals tab (the stages and intervals settings are displayed)



– select Number of stages and Time unit (minutes or seconds)

– 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.

– to save the method and return to the Kinetics menu, tap Save Method

> **Note** The method is saved in the currently selected Data Storage Location (local instrument or a connected PC).

– to run the method, tap Run Method

Related Topics

- Edit Kinetics Method

Edit Kinetics Method

Kinetics methods can be edited only on the NanoDrop One instrument. To edit an existing kinetics method:

– if the instrument has a connected PC (Ethernet or Wi-Fi), make sure Data Storage is set to the correct location for the kinetics method you want to edit

- from Home screen, tap **Kinetics** tab > **Kinetics** application
- select a method by tapping the method name in the Select Method box
- tap **Edit Method**
- edit method settings as desired
- tap **Save Method** to save your changes
- tap **Run Method** to run the updated method

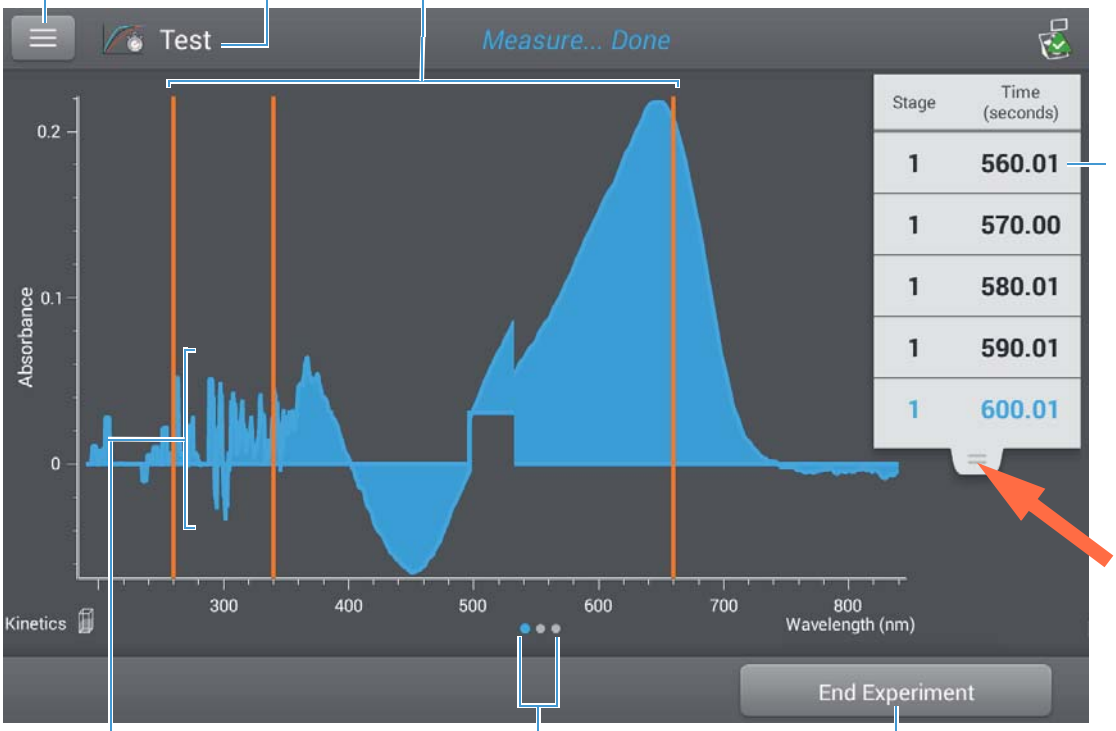
Related Topics

- Create Kinetic Method

Kinetics Reported Results

Absorbance measurement screen

The absorbance measurement screen appears immediately after you tap 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 to see more entries). Each item in the list at the right has a corresponding absorbance spectrum at the left. The image below highlights the available features.

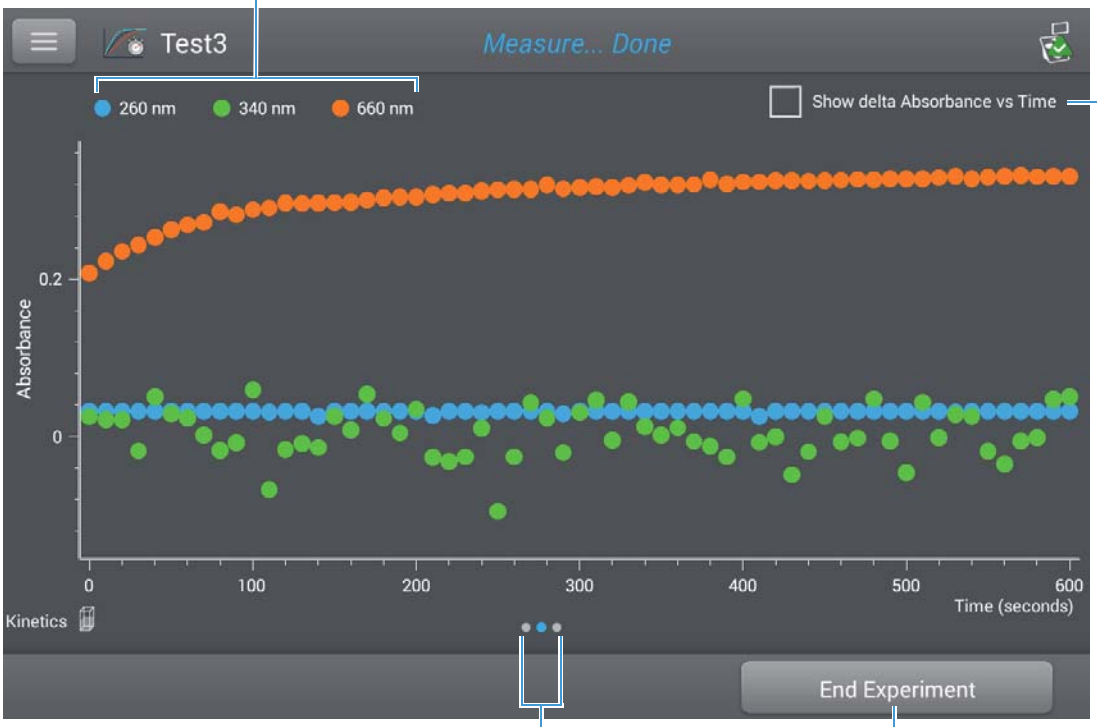


Note For measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent.

Rate measurement screen

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.

User-defined wavelengths



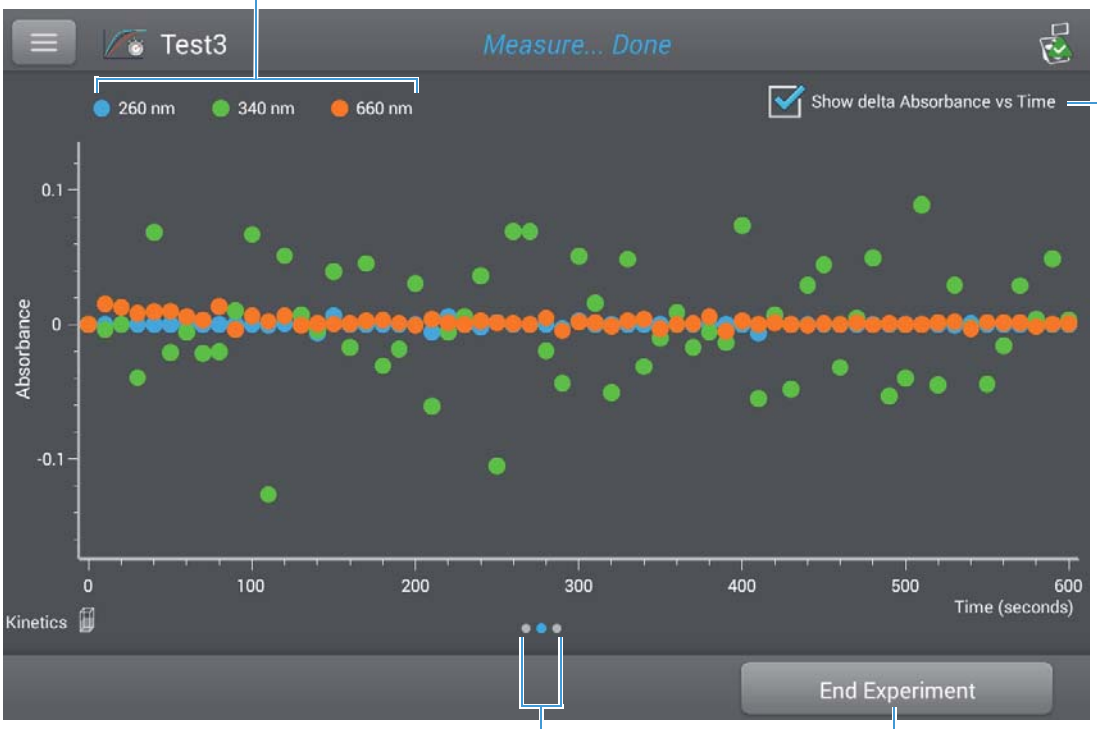
Tap to update plot to show change in measured absorbance over time (see example below)

Swipe screen left to view data table; **swipe right** to return to absorbance measurement screen

Tap to end experiment and export data

Tap **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.

User-defined wavelengths



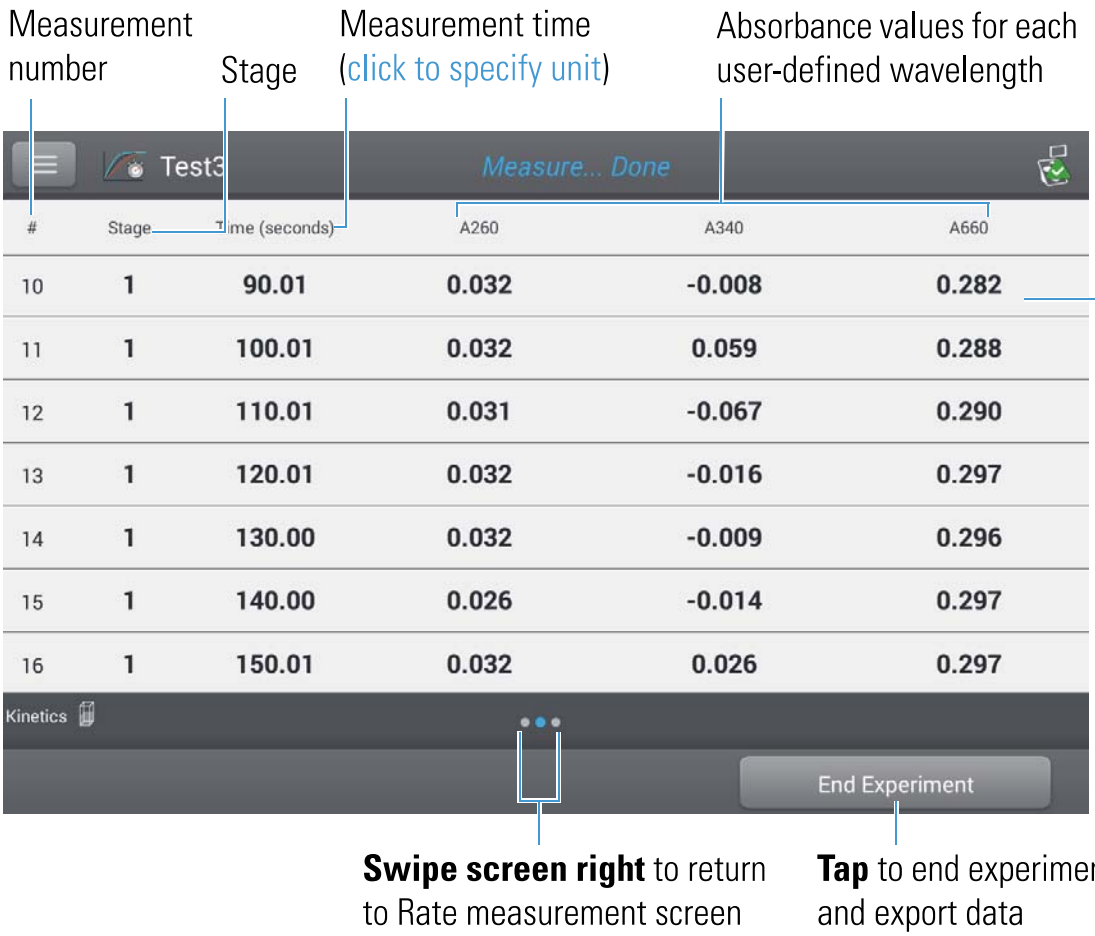
Tap to update plot to show measured absorbance over time (see example above)

Swipe screen left to view data table; **swipe right** to return to absorbance measurement screen

Tap to end experiment and export data

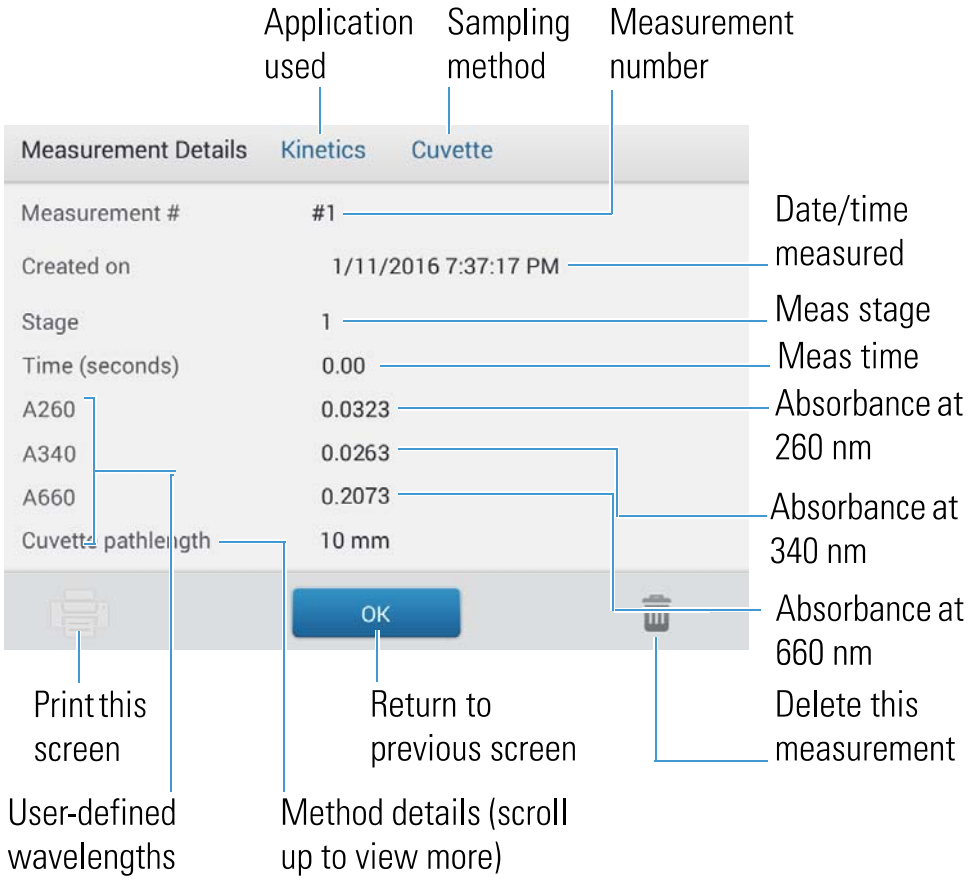
Data Table

To see the data table, swipe the rate measurement screen (see above) 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.



Measurement Details

To view details for a measurement, from the absorbance measurement screen or data table, press and hold the measurement row. Here is an example:



Related Topics

- Basic Instrument Operations

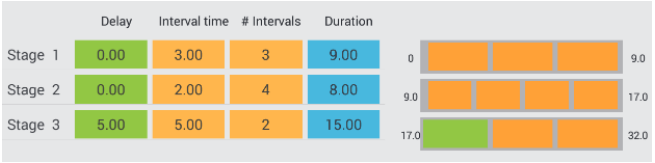
Settings for Kinetic Measurements

To show the Kinetics settings, from the instrument Home screen, tap Kinetics (tab) > Kinetics (Method), and either tap Create Method or select a method and tap Edit Method. You can also display the settings from any Kinetics measurement screen, by tapping ☰ > Kinetics Setup.

Note If the instrument has a connected PC (Ethernet or Wi-Fi), kinetics methods can be located in the NanoDrop One database on the local instrument, or in the NanoDrop Viewer database on a connected PC. Use the Data Storage box to select which database is active and then follow the steps to display the Kinetics methods that are stored in that location and their associated settings.

The settings appear on two tabs: "Name and Range" and "Stages and Intervals." See the table below for details.

Tab	Setting	Description
Name and Range	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 (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.
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.

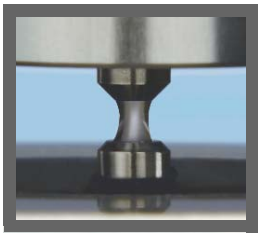
Tab	Setting	Description
	Time Unit	Select the unit for time-based measurements (seconds or minutes).
	Stage 1, 2, etc.	Specify the available settings for each stage: • Delay. Specify a delay before a stage starts. • Interval Time. Specify the length of time between measurements taken during this stage (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.
		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:

Tab	Setting	Description
		• 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.

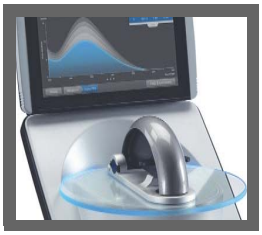
Related Topics

- Instrument Settings

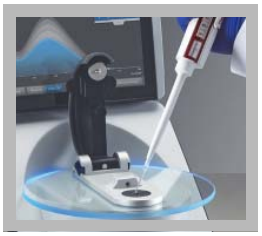
Learning Center



How the Instrument Works

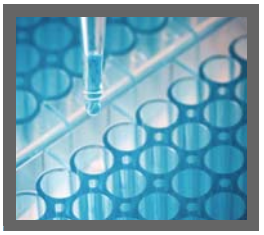


Set Up the Instrument



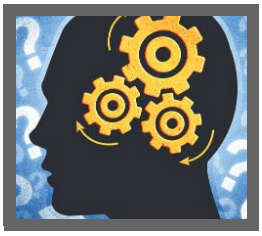
Measure a Micro-Volume Sample

Measure using a Cuvette

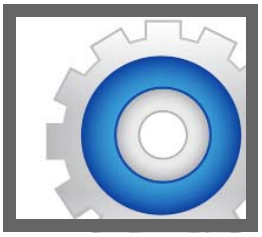


Prepare Samples and Blanks

Basic Instrument Operations



Acclaro Sample Intelligence



Instrument Settings

NanoDrop One Viewer

Multimedia

Micro-Volume Sampling—How it Works

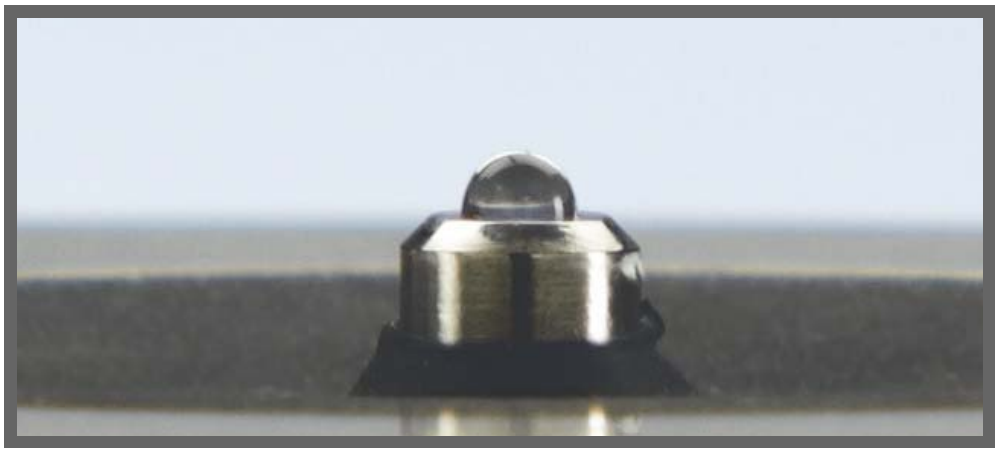
Surface Tension

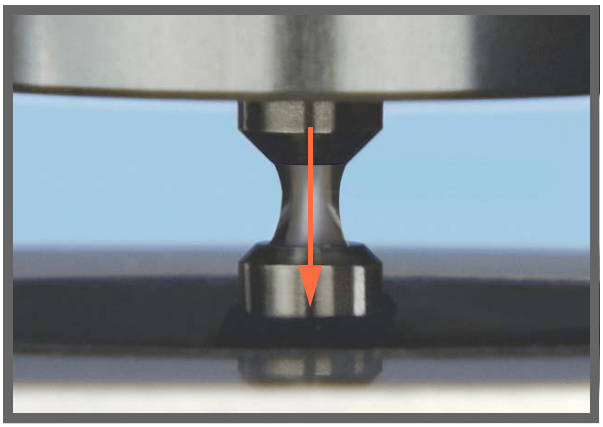
Absorbance Spectrum

Sample Absorbance

Sample Concentration

Baseline Correction

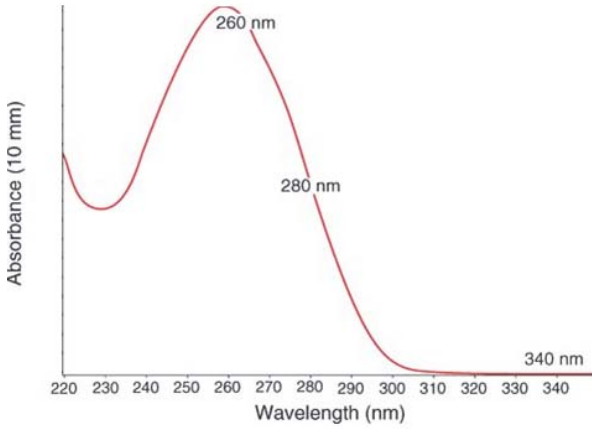




Surface Tension

The NanoDrop One spectrophotometer uses 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.



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

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

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

Beer-Lambert equation

$$A = \varepsilon b c$$

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)

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

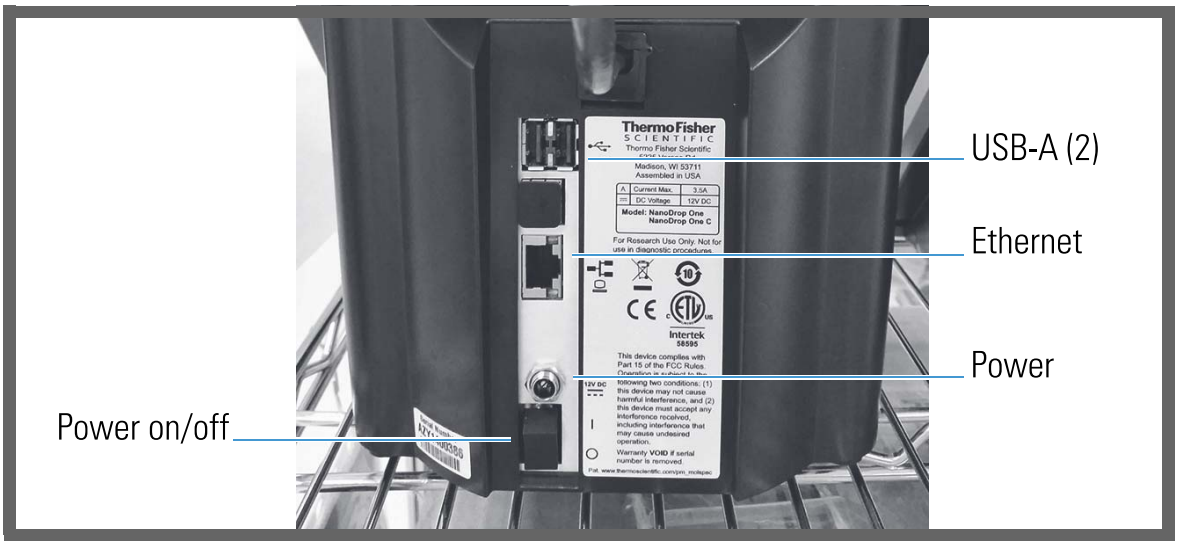
For some applications, the instrument can be set up to apply a baseline correction to each measurement to minimize any offset caused by light scattering particulates in the sample spectra. The correction subtracts the absorbance value at a reference wavelength that is close to zero from the absorbance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

Related Topics

- Instrument Models and Features
- Measure a Micro-Volume Sample

- Calculations for Nucleic Acid Measurements
- Calculations for Protein A280 Measurements

Set Up the Instrument



Connect Power

CAUTION Avoid shock hazard. Each wall outlet used must be equipped with a ground. The ground must be a noncurrent-carrying wire connected to earth ground at the main distribution box.

Connect the provided power cord to a grounded wall outlet. Tap here for more information.

Connect an Accessory

To connect a compatible printer or other compatible accessory such as a USB keyboard and/or mouse to the instrument, use any USB port on the instrument (front, back-left or back-right). See Accessories for information about accessories compatible with the NanoDrop One instruments.

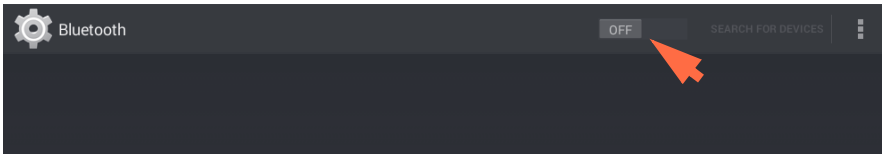
Set Up Bluetooth Connections

Use Bluetooth™ to connect the instrument to one or more Bluetooth (wireless) input devices such as a Bluetooth keyboard, mouse or barcode scanner.

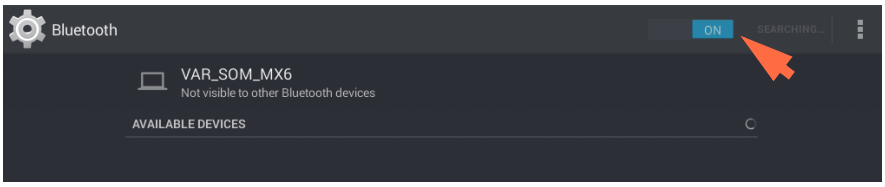
Note Make sure the device is labeled "Bluetooth" and not just "wireless." All Bluetooth devices are wireless but not all wireless devices will run with Bluetooth.

Set up Bluetooth connections on the instrument

- from instrument Home screen, tap (Settings)
- tap System tab
- tap Bluetooth (if Bluetooth is disabled, button in upper right is set to "Off" and no Bluetooth input devices are listed)

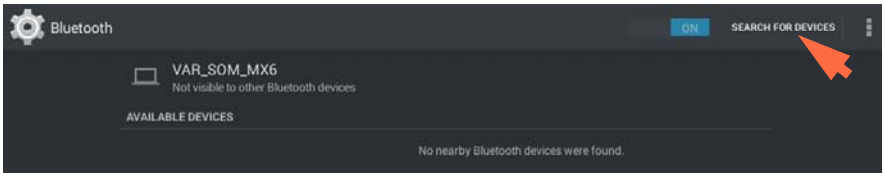


- tap Off button to enable Bluetooth connectivity (button turns blue, changes to "On" and software automatically searches for any available Bluetooth input devices)

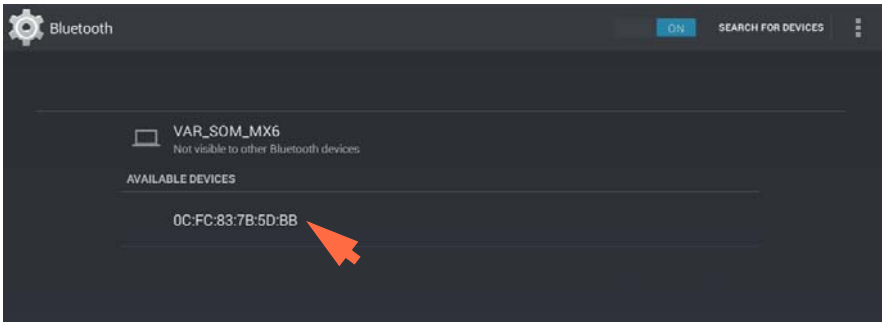


If no Bluetooth devices are found, after a few seconds the message "No nearby Bluetooth devices were found" is displayed

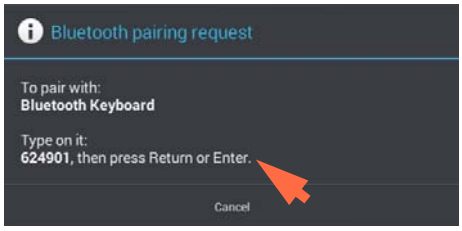
- to add a Bluetooth device, follow manufacturer instructions to pair the device (for example, you may need to hold down a button) and tap Search For Devices on instrument)



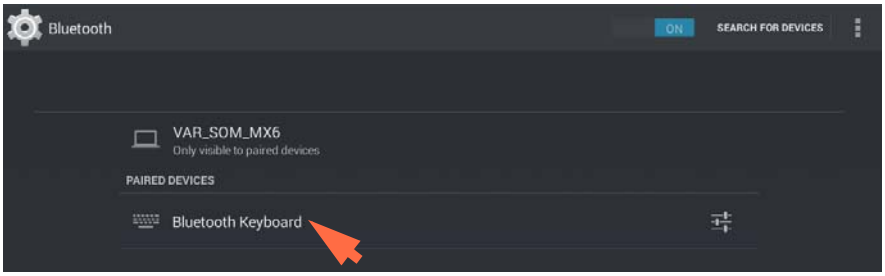
device name should appear in Available Devices list



– to pair device, tap its name in Available Devices list (a pairing request similar to the following may be displayed)

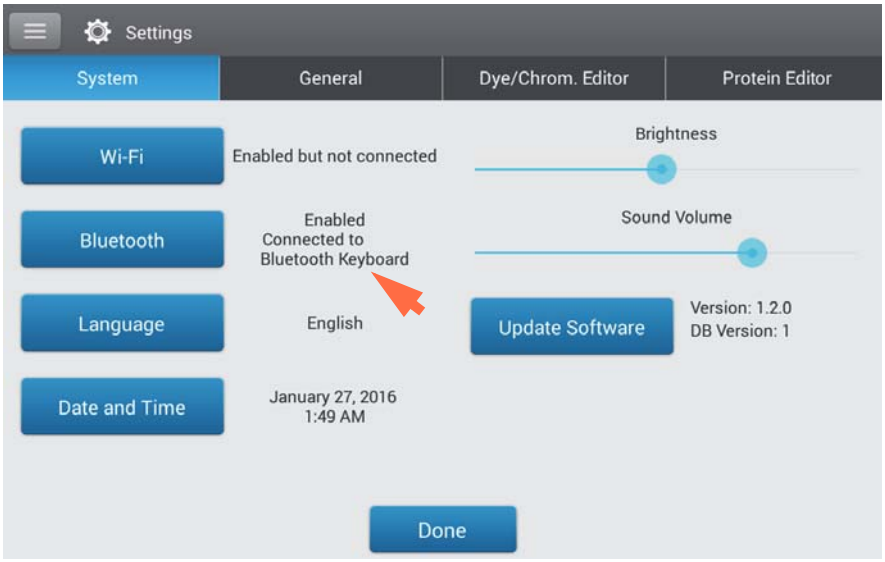


– complete any instructions to pair the device



Note If your Bluetooth device does not pair, restart the device and then repeat the steps above to pair it with the instrument (you may also try turning Bluetooth off and back on). After a device is paired, it remains paired even after the instrument is restarted.

– tap Back (Bluetooth status is displayed at right of Bluetooth button)



– repeat steps above to add another Bluetooth device or tap Done to close Settings

Deselect Bluetooth input device

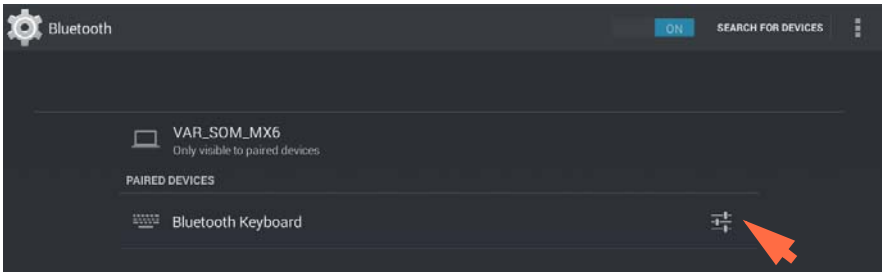
You may want to stop using a Bluetooth device for input without disconnecting or unpairing it. This allows others to easily reselect and use the device for input. For example, if there are multiple connected and paired Bluetooth input devices such as a keyboard and a barcode scanner, follow these steps to select the devices to use or to deselect devices you don't want to use:

– from instrument Home screen, tap 

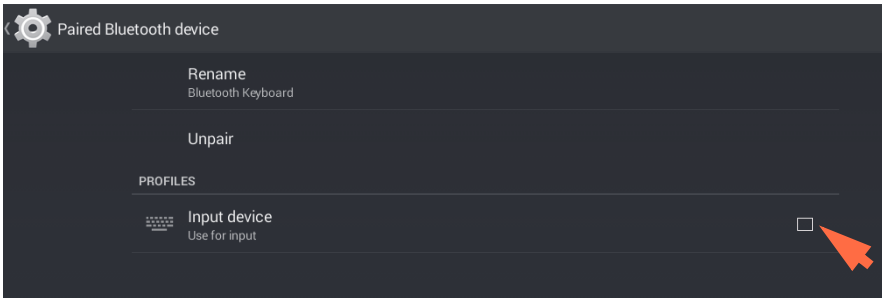
– tap System tab

– tap Bluetooth

– to deselect a paired Bluetooth device such as a keyboard for input, tap its Profiles button 



– deselect Use For Input by clearing it's associated checkbox



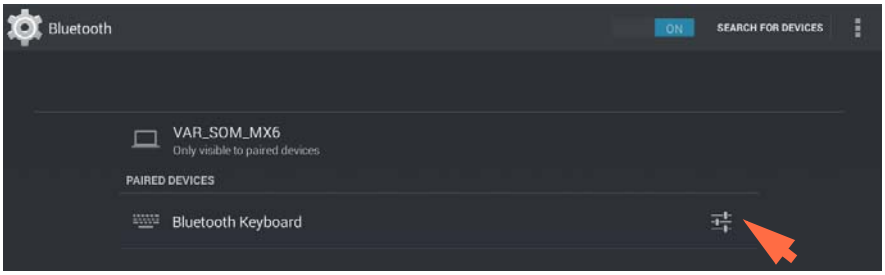
– tap Paired Bluetooth Device in upper left to return to previous screen
– tap Back to return to System settings
– tap Done to close Settings

Note

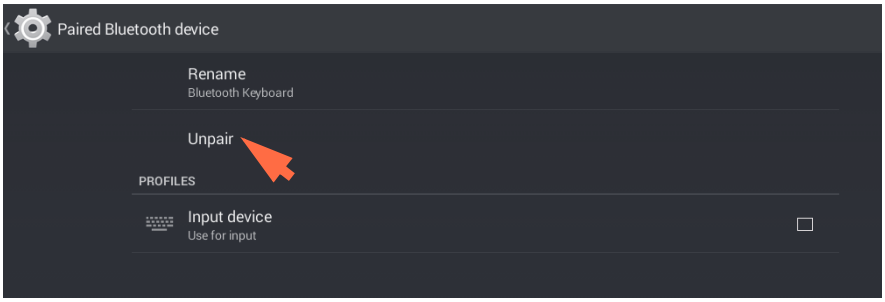
- If no Bluetooth device is selected for input, the instrument relies on the integrated touchscreen keyboard for input.
- To select the device again, follow the steps above and select the device's Use for Input checkbox.

Disconnect Bluetooth device

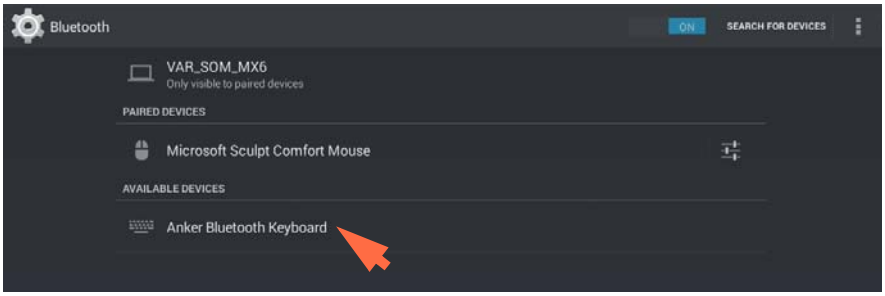
– from instrument Home screen, tap 
– tap System tab
– tap Bluetooth
– to disconnect paired Bluetooth device, tap its Profiles button 



– tap Unpair



device is no longer listed under “Paired Devices” but remains in Available Devices list



– tap Back to return to System settings

– tap Done to close Settings

Set up Ethernet Connection

The instrument Ethernet port can be used to set up a wired connection between the instrument and a personal computer (or PC). The connected computer can then be used to store or view data acquired with the NanoDrop One instrument. (NanoDrop One Viewer software must be installed on the computer.)

Tools needed:

- Standard (straight through) Ethernet cable (CAT5e or newer is recommended)

Note If the computer is an older model, you may need a crossover Ethernet cable instead. Most newer model computers are designed to automatically detect and work with both cable types. However, a straight through cable will provide best performance.

Set up Ethernet connection

– connect Ethernet cable between Ethernet port on instrument back panel (see image above) and Ethernet port on computer

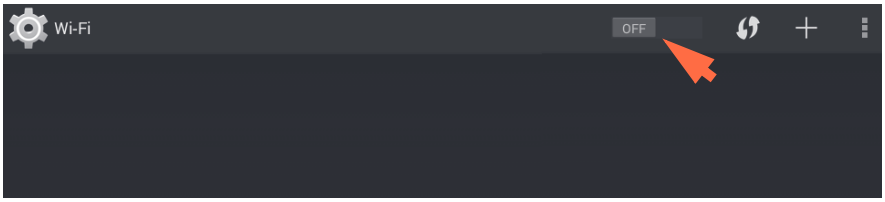
Set up Wireless Connections

Use Wi-Fi™ to connect the instrument to a remote computer through a wireless local area network (WLAN). The remote computer can then be used to store or view data acquired with a NanoDrop One instrument.

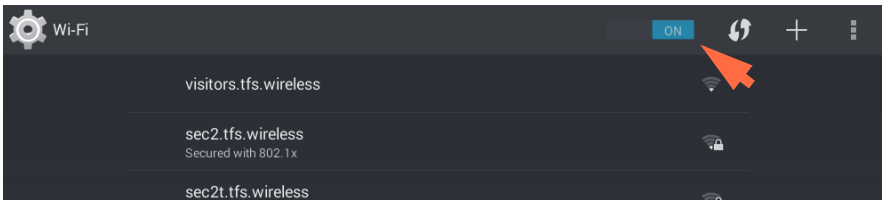
Note To store or view collected data on a connected computer using W-Fi, NanoDrop One Viewer software must be installed on the remote computer and the computer must be configured for Wi-Fi data storage. The instrument must also be connected to the remote computer's network host and have Wi-Fi enabled.

Select Wi-Fi network on the instrument

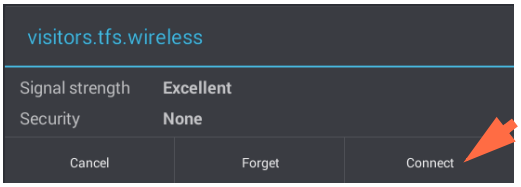
- from instrument Home screen, tap  (Settings)
- tap System tab
- tap Wi-Fi (if Wi-Fi is disabled, button in upper right is set to "OFF" and no wireless networks are listed)



- tap button to enable Wi-Fi and display available Wi-Fi networks

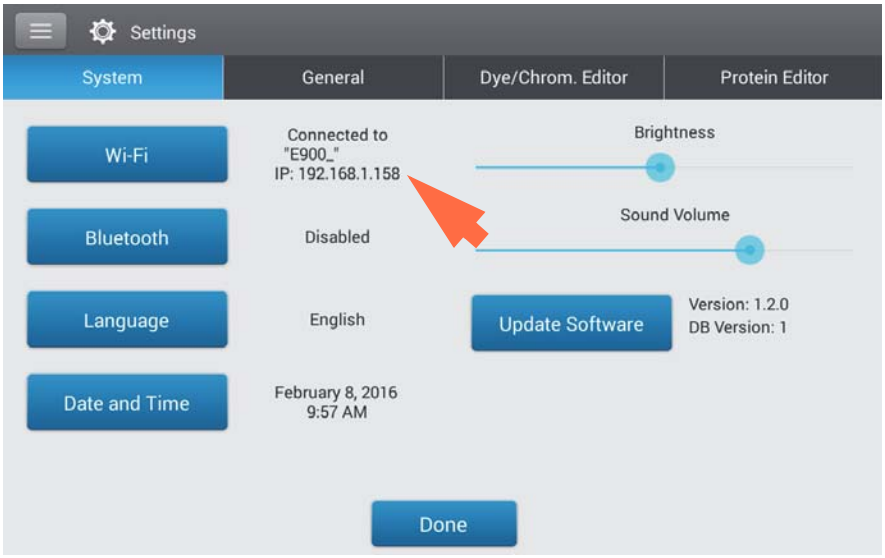


- select remote computer's Wi-Fi network host and tap Connect (here is an example)



– tap Back to exit Wi-Fi setup (if the connection is successful, the instrument is assigned an IP (Internet Protocol) address, which appears at the right of the Wi-Fi button as in the example below)

Note Some Wi-Fi networks may require an identity, password or other information before you can connect to them, or they may be anonymous (that is, you may have to search for them by name). For more information, see the system administrator at your work site.

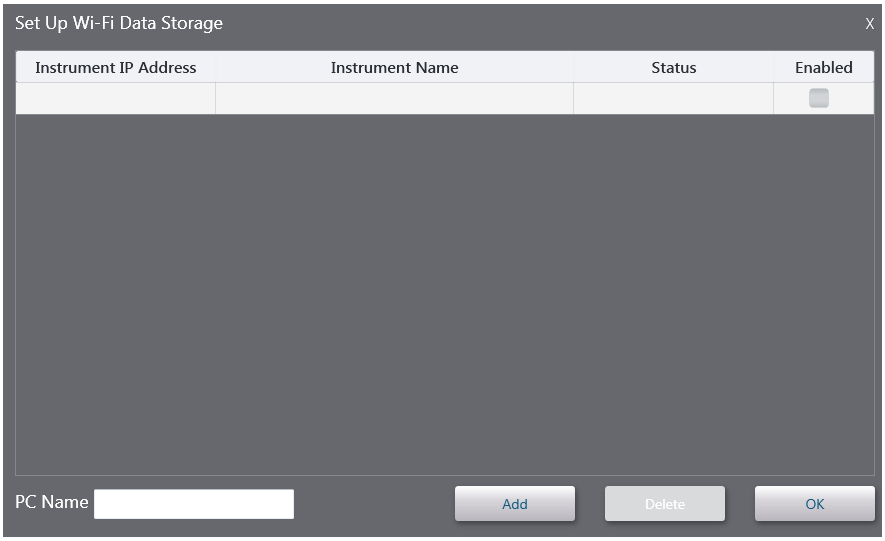


– record IP address (you will need to enter it on the remote computer in the next section)

– tap Done to exit Settings

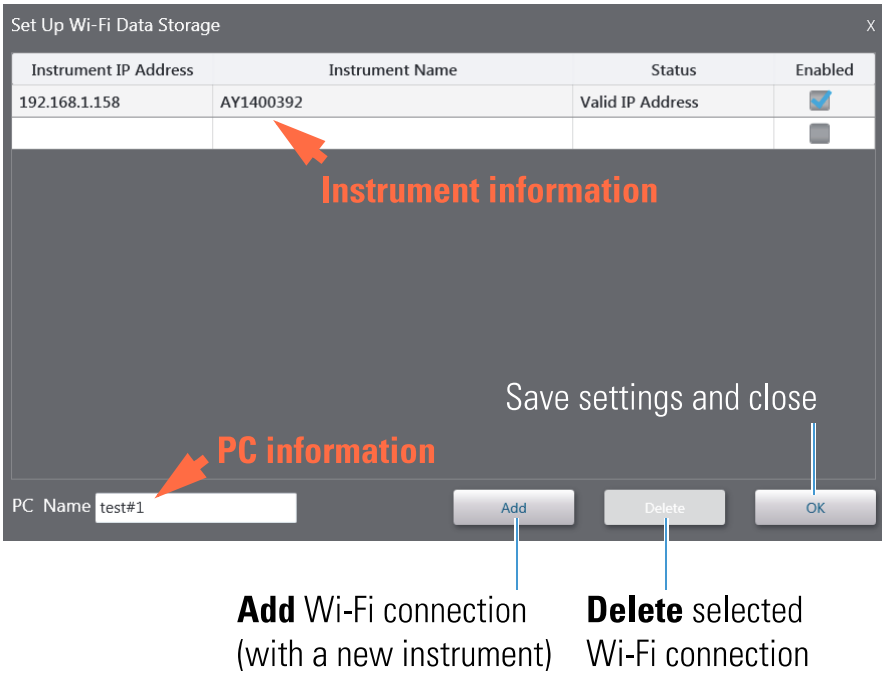
Configure Wi-Fi data storage on the remote computer

- from remote computer, open NanoDrop One Viewer software
- choose File (menu) > Set Up Wi-Fi Data Storage (the following screen is displayed)



- enter the following information:
  - Instrument IP address (displayed on instrument in Settings > System, see previous section; if IP address is valid, Status column shows "Valid IP Address")
  - unique Instrument Name (in case there are multiple instruments in the same lab on the same network)
  - PC Name, such as the computer's assigned name or an invented name (the name you enter will appear in the "select a data storage location" list box on the instrument (see the next section)

– make sure the instrument's Enabled button is selected (see example below)



– to set up another instrument, tap Add and then repeat steps above

NOTICE You can add multiple Wi-Fi addresses to this list to make it easy to switch computers. However, only one Wi-Fi connection should be enabled during data collection to ensure the data integrity.

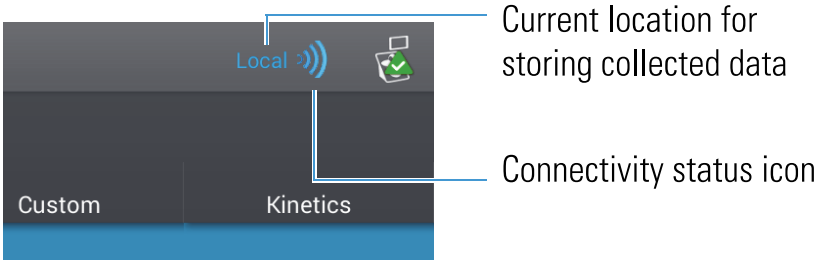
– to remove an item from the list, tap to select the row and then tap Delete

– when finished, choose OK to close Wi-Fi Data Storage setup

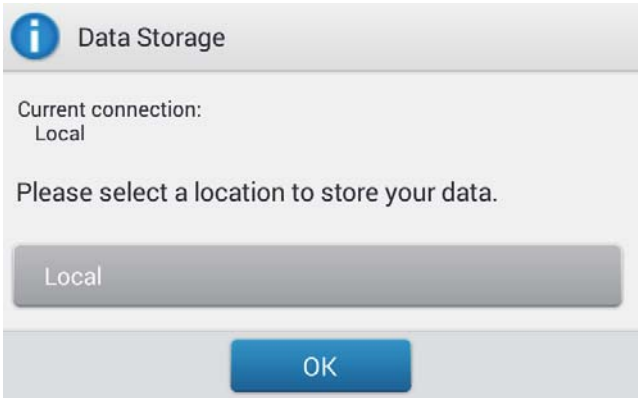
Select location for saving collected data

– from instrument Home screen, tap Connectivity Status icon 

Note The Connectivity Status icon is active, i.e., blue, only when the instrument is connected to a personal computer (PC) with an Ethernet cable or through a properly configured wireless network, as in the example below.



the Data Storage message box is displayed as in the example below

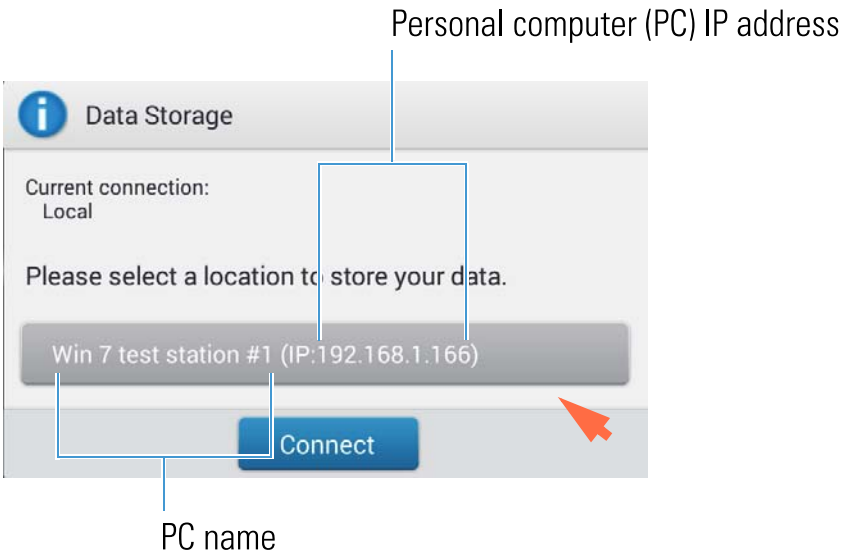


– select an available option below:

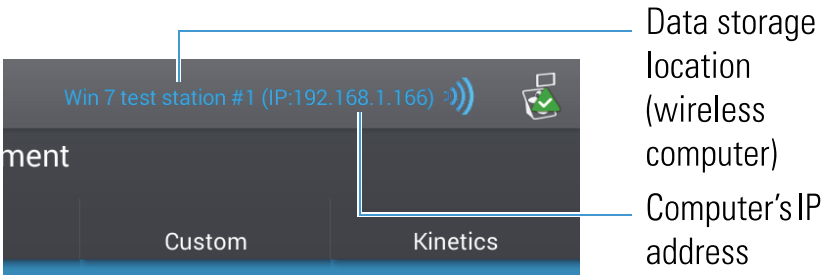
- to store all subsequently acquired measurement results only in the NanoDrop One database on the instrument, set Data Storage to **Local** (see example above).
- to store all subsequently acquired measurement results in the NanoDrop One Viewer database on a computer connected to the instrument with an Ethernet cable, set Data Storage to **Direct-Connect PC*** (see Set Up Ethernet Connection for details).
- to store all subsequently acquired measurement results in the NanoDrop One Viewer database on a computer connected to the instrument through a wireless network, set Data Storage to the **computer's assigned name*** (see Set Up Wi-Fi Connections for details).

* The Ethernet and wireless options listed above will also store data on the instrument as a backup.

Here is an example of a wireless configured destination computer selected for data storage



– tap Connect (or OK if connection had already been established) to close message box (new data storage location appears adjacent to Connectivity Status icon)



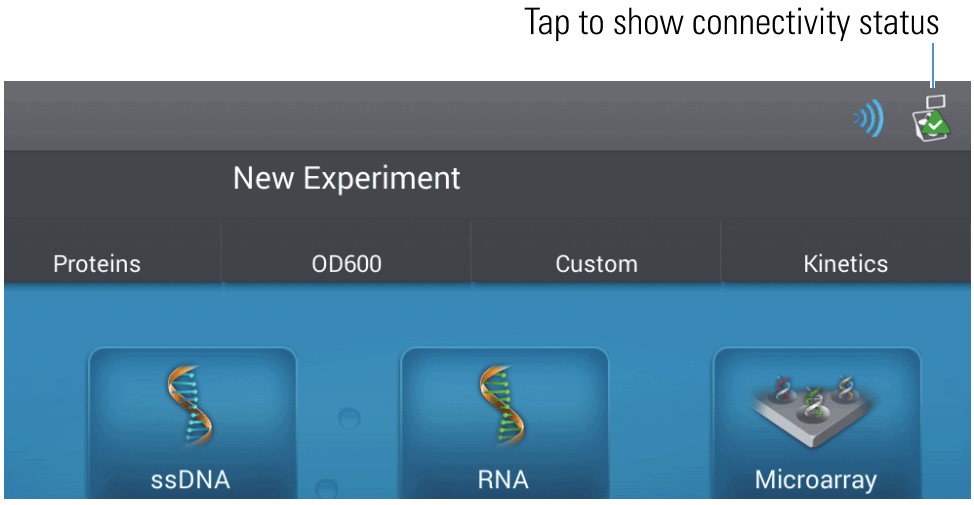
all subsequently acquired measurement results are saved in the NanoDrop Viewer database on the selected computer, and in the NanoDrop One database on the local instrument.

Note

- The NanoDrop One Viewer software does not need to be running for data from the instrument to be saved there.
- If the wireless or Ethernet connection is interrupted during a measurement, data storage switches back to the local instrument with no loss of data.
- Custom methods and Kinetics methods must reside on the connected computer when the data are configured for remote storage.
- When the instrument is connected to a computer with an Ethernet cable or through a wireless network, the Data Viewer icon on the instrument Home screen is unavailable. (You cannot use the instrument to view the NanoDrop One database on a connected computer,)

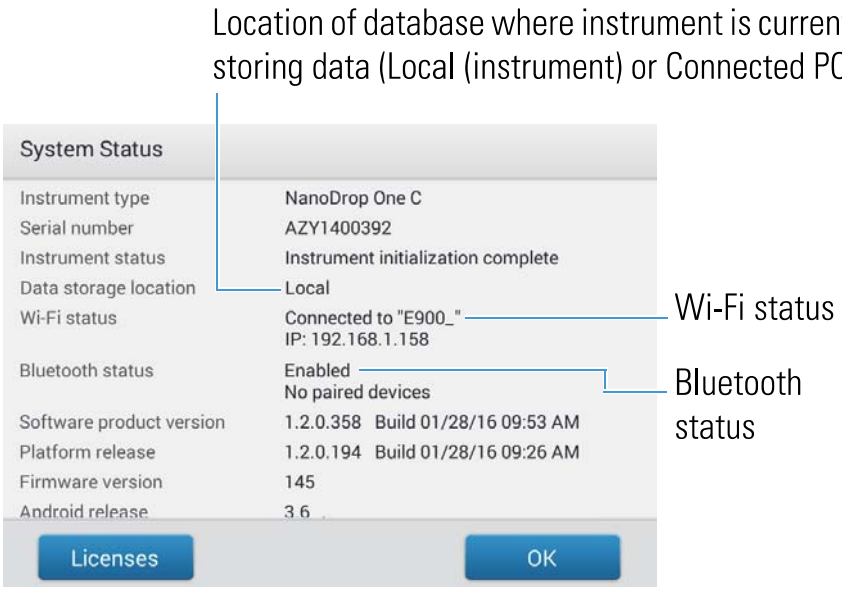
Assess Instrument Connectivity

Use the System Status icon at the top right of the instrument Home screen to quickly assess the instrument's connectivity status including Bluetooth, Ethernet and Wi-Fi:



Show connectivity status

– tap  on instrument Home screen to open System Status box



– tap OK to exit System Status

Operating Specifications

The instrument operates reliably when the room environment meets these specifications:

- operating temperatures: 5 °C - 35 °C (41 °F - 95 °F)
- relative humidity (non-condensing): 20-80%

Locate the instrument away from air vents and exhaust fans to minimize evaporation.

Note If operating the instrument at the low end of the recommended humidity range, use adequate sample volume to avoid evaporation.

After the instrument is installed, you can leave it turned on.

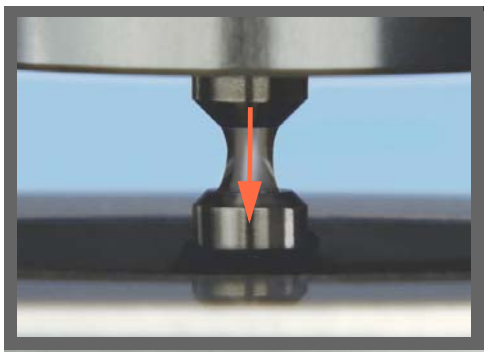
Related Topics

- Safety and Operating Precautions
- Instrument Models and Features

- Optional Accessories
- Instrument Settings

Measure a Micro-Volume Sample

The NanoDrop One spectrophotometer uses 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. Tap here for details.



Supplies needed

- NanoDrop One or NanoDrop One^{C} spectrophotometer
- lint-free laboratory wipes
- calibrated precision pipettor (0–2 µL)
- sample material resuspended in appropriate buffer solution (see Preparing Samples)
- pure buffer solution for blanking instrument (see Choosing and Measuring a Blank or watch multimedia training What is a blank?)

Best practices for micro-volume measurements

Cleaning pedestals for daily operation

- Before first measurement, clean both pedestals with a new laboratory wipe.
- Run a blanking cycle to verify pedestals are clean.
- After each measurement, clean both pedestals with new wipe to prevent carryover.
- After each set of measurements, clean pedestals with DI H2O (see Clean pedestals between users)
- Recondition pedestals periodically to maintain their hydrophobic property.

Pipetting Samples

- Use recommended sample volumes to ensure proper liquid column formation.
- Use calibrated precision pipettor (0–2 µL volume range) with well-fitting, low-retention precision tips to apply sample material to instrument for measurement.

  If using low accuracy (0-10 µL) pipettor, use 2 µL sample volumes.
- Use new tip for each blank and sample aliquot.
- Use new aliquot of sample for each measurement.
- If solvents are used, make sure they are compatible with the pedestals. (see "Compatible Solvents" in Hazardous Materials).

Recommended sample volumes

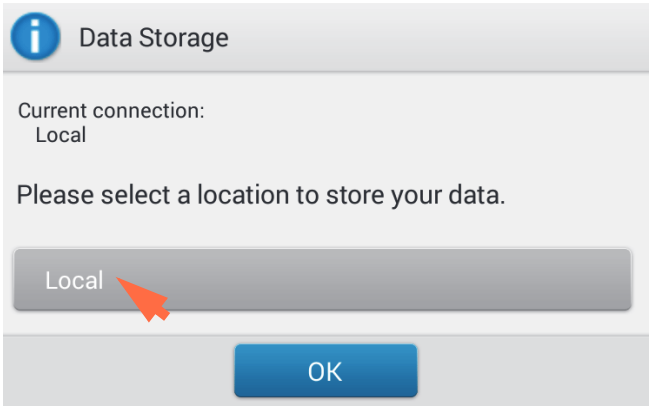
Application	Sample Volume
Nucleic acid (aqueous solution)	1 µL[a]
Purified protein	2 µL
Other protein applications such as Bradford or BCA	2 µL
Microbial cell suspensions	2 µL

[a] Use 2 µL for samples that contain materials that may reduce surface tension such as a surfactant.

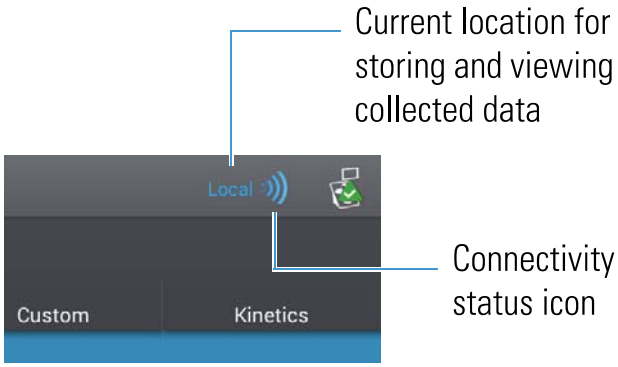
To measure a micro-volume sample

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.

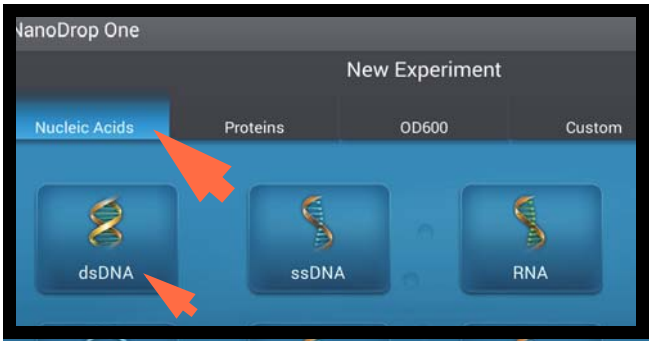


1. If the instrument has a working Ethernet or wireless connection to a personal computer (PC), the Connectivity Status icon is blue and shows the currently selected location for storing and viewing data collected with the instrument.



   If the Connectivity Status icon is blue, tap the icon and set Data Storage to Local as shown at the left.

2. From the instrument Home screen, select an application tab such as Nucleic Acids and tap an application name such as dsDNA or RNA.



3. Lift the instrument arm and clean the upper and lower pedestals with new laboratory wipe.

4. Measure a blank:
   - Pipette 1–2 µL blanking solution onto the lower pedestal and quickly lower the arm.
   - Tap **Blank** and wait for the measurement to complete.

     Tip: If Auto-Blank is On, blank measurement starts automatically after you lower the arm.
   - Lift the arm and clean both pedestals with a new laboratory wipe.

5. Measure the first sample:
   - Pipette 1-2 µL sample solution onto the pedestal and quickly lower the arm (see Recommended Sample Volumes for more information).
   - Start the sample measurement:
     - if Auto-Measure is On, lower arm
     - if Auto-Measure is off, lower arm and tap **Measure**
   - When the sample measurement is completed, the spectra and reported values are displayed.

Tap to end experiment

If one of these symbols appears next to a sample ID, tap the symbol for any alerts or additional information about the measurement:

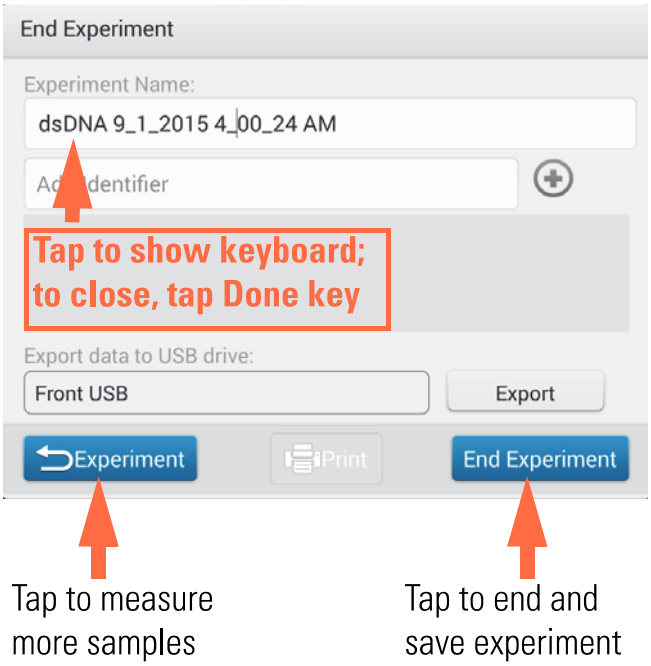
 contaminant information available

 on-demand technical support available

 invalid result

6. To measure another sample:
   - Lift the arm.
   - Clean both pedestals with new wipe.
   - Load the next sample and quickly lower the arm.
   - Start the sample measurement.
   - Wait for the measurement to complete.

   The new spectrum replaces the previous one on the spectral display and the new reported values appear under the previous ones in the table. (Drag tab down to show both sets of data.)



7. When you are finished measuring samples:
   - Tap **End Experiment.**
   - Enter an experiment name (tap Experiment Name box, use displayed keyboard to type name, tap Done key), or leave the default experiment name
   - Tap End Experiment
   - Lift the arm and clean both pedestals with a new wipe.

     If finished with the instrument for the day, clean the pedestals with DI H2O (see Clean pedestals between users).

Acquired data are automatically saved in an experiment with the entered name. In the default configuration, experiments are stored in a database on the local instrument according to acquisition date, experiment name, application used and any assigned labels (see Manage identifiers on the instrument).

Related Topics

- Micro-Volume Sampling—How it Works
- Absorbance Detection Limits
- Prepare Samples and Blanks
- Auto-Measure and Auto-Blank
- Acclaro Sample Intelligence
- Cleaning the Pedestals
- Search Experiment Database
- Export Data
- Measure a Sample Using a Cuvette

Measure a Sample Using a Cuvette

The NanoDrop One^{C} spectrophotometer includes a cuvette holder for measuring dilute samples, colorimetric assays, cell cultures and kinetic studies. The cuvette system offers an extended lower detection limit and an optional 37 °C heater and micro-stirrer.

Supplies needed

- NanoDrop One^{C} spectrophotometer
- lint-free laboratory wipes
- two compatible cuvettes
- sample material resuspended in appropriate buffer solution (see Preparing Samples)
- pure buffer solution for blanking instrument (see Choosing and Measuring a Blank or watch multimedia training What is a blank?)

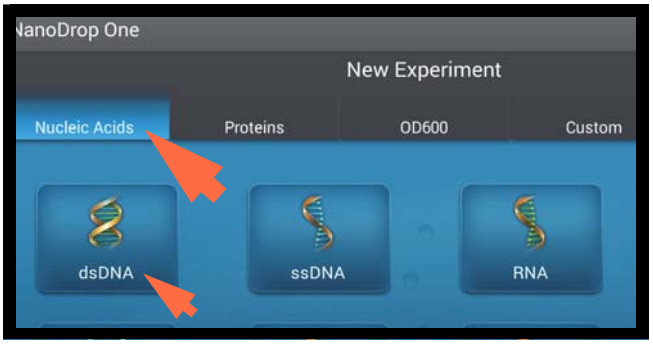
Best practices for cuvette measurements

- The instrument arm can be up or down for cuvette measurements.
- Use 10 mm, 5 mm, 2 mm or 1 mm cuvettes up to 48 mm tall.
- Clean and dry cuvette after each measurement.
- Use cuvettes that are free of scratches and avoid fingerprints which may affect results.
- Use quartz cuvettes or UV-grade plastic cuvettes to measure samples with analysis wavelengths in the UV range (<340 nm).
- Micro, semi-micro, and ultra-micro cuvettes should be masked.
- Fill cuvettes with enough blanking or sample solution to cover instrument optical path (2 mm sample beam is 8.5 mm above cuvette bottom).
- Lift instrument arm and make sure cuvette holder is free of debris.
- When inserting quartz or masked plastic cuvettes, align cuvette light path with instrument light path.

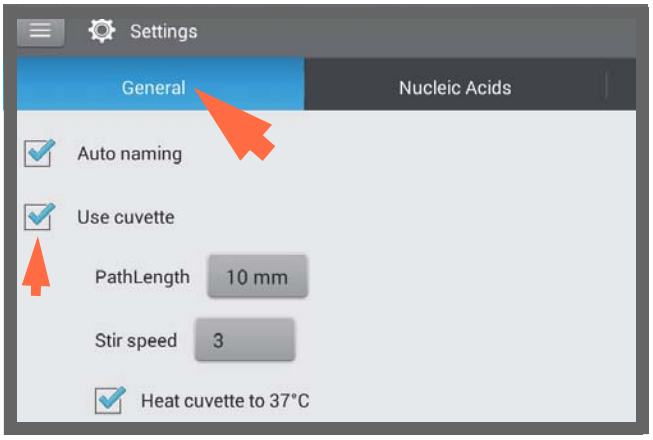
To measure a sample using a cuvette

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.

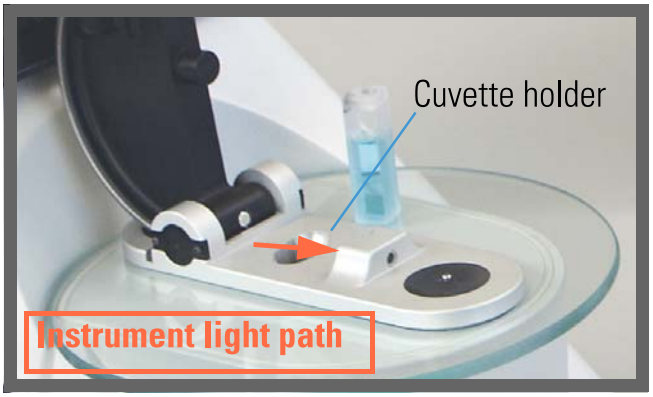


1. From the Home screen, select an application tab such as Nucleic Acids and tap an application name such as dsDNA or RNA.

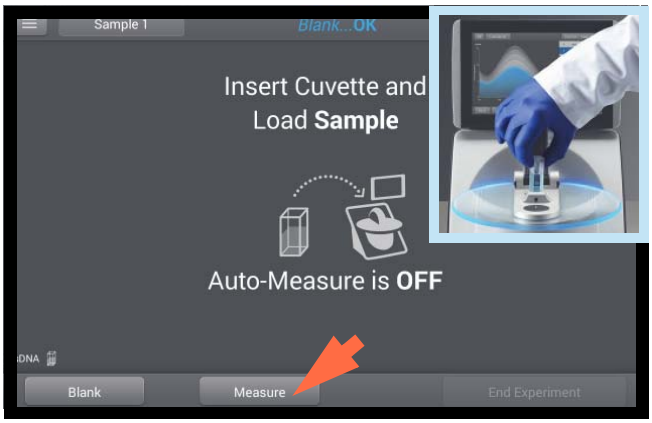


2. Specify the cuvette options:
   - From Home screen, tap  (Settings).
   - tap **General**.
   - select **Use Cuvette**.
   - set **Pathlength** to pathlength (width) of cuvette (see cuvette manufacturer for specifications).
   - set stirrer and heater if desired.
   - tap **Done**.

   See General settings for details.



3. 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.
   - Tap **Blank** and wait for the measurement to complete.



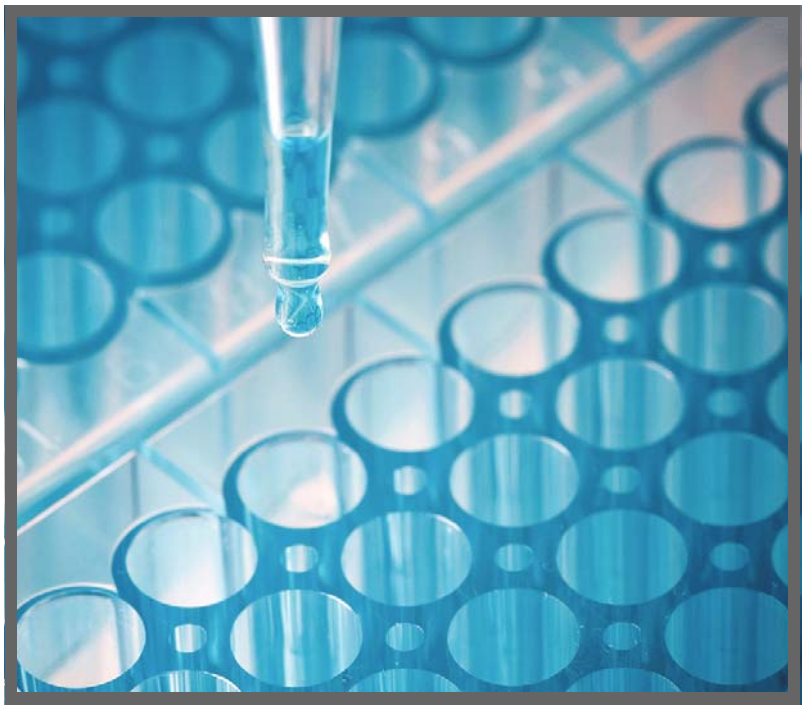
4. Measure a sample:
   - Fill clean cuvette to same height with sample solution.
   - Replace blanking cuvette with sample cuvette, making sure to align light paths.
   - Tap Measure.
   - Wait for measurement to complete.
   - Remove cuvette.
   - Clean cuvette according to manufacturer specifications.

Related Topics

- Instrument Models and Features
- Absorbance Detection Limits
- Prepare Samples and Blanks
- Acclaro Sample Intelligence
- Instrument Settings
- Search Experiment Database
- Export Data
- Measure a Micro-Volume Sample

Prepare Samples and Blanks

Preparing Samples



- Isolate and purify samples before measuring them with the instrument. Commercial sample isolation kits are available for these purposes, or use an in-house protocol. After purification, analyte of interest is typically dissolved in aqueous buffer solution before it is measured.

  Tip: Any molecule that absorbs light at analysis wavelength will contribute to total absorbance value used to calculate sample concentration.

- Ensure final analyte concentration is within instrument's absorbance detection limits.

- For micro-volume measurements, gently (but thoroughly) vortex each sample before taking a measurement.

  Tip: Heat highly concentrated or large molecule nucleic acid samples, such as genomic or lambda DNA, to 63 °C (145 °F) before vortexing them.

- Avoid introducing bubbles when mixing and pipetting. For more information, watch multimedia training Effects of Bubbles in Samples.

Note Samples dissolved in extremely volatile solvent such as hexane may work best with cuvette sampling option (NanoDrop One^{C} instruments only).

Choosing and Measuring a Blank

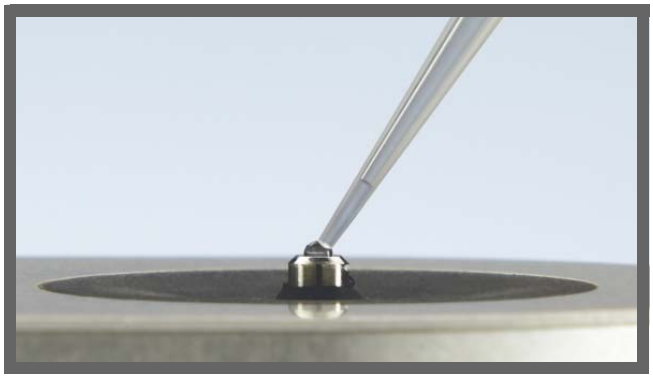
The buffer used to resuspend a sample analyte can contribute absorbance. Blanking minimizes any absorbance contribution due to the buffer components from the sample measurement. The resulting sample spectrum represents the absorbance of only the analyte of interest. For more information, watch the multimedia training What is a blank?

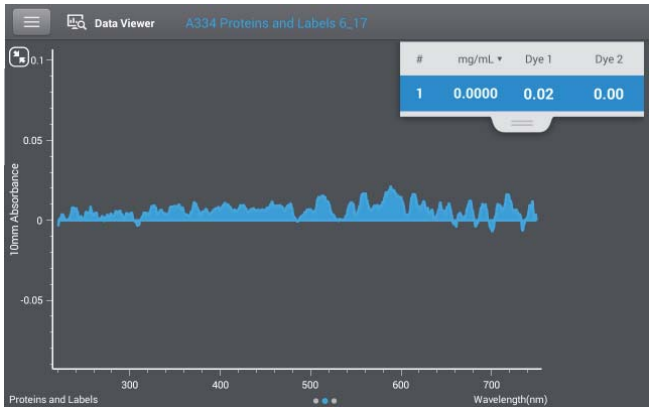
For best results:

- For most applications, blank with the same buffer solution used to resuspend the analyte of interest. The blanking solution should be a similar pH and ionic strength as the analyte solution. For details, see “To measure samples” in the application used.
- Measure new blank before each set of samples. It is not necessary to blank the instrument before each sample measurement unless the samples are dissolved in different buffer solutions.
- Measure a new blank every 30 minutes.
- Run a blanking cycle to assess the suitability of your blanking solution before using it to perform sample measurements. For a quick demonstration, watch the multimedia training Evaluating a Blanking Solution for Suitability.

  The resulting spectrum should vary no more than 0.04 A (10 mm equivalent) across the spectrum, especially at the analysis wavelength as in the example at the right.

  If the resulting spectrum is greater than 0.04 A around the analysis wavelength, that buffer solution may interfere with the sample analyses, especially for low concentration samples. See below for details.





Good blanking buffer (measured abs < 0.04)

Problems associated with blanking

- Residual sample was left on pedestal or in cuvette before blank measurement was performed. (Resulting sample spectra may exhibit negative absorbance values, indicating blank had more absorbance than sample in that region of spectrum.)
- Blank measurement exhibits higher absorbance than unknown sample at analysis wavelength. (If buffer used as blank differs in composition from that used to resuspend sample, measurement results will be incorrect.)
- Sample was inadvertently used to blank instrument. (Resulting sample spectra may exhibit negative absorbance values or, in some cases, resemble a mirror image of a typical pure nucleic acid or protein spectrum as in example at right.)

Protein sample solution used to blank instrument results in “mirror image” spectrum

Solutions for blanking problems

- Thoroughly clean and/or recondition both pedestals and then:
  - rerun blanking cycle, or
  - measure new blank using new aliquot of appropriate buffer solution, then measure new aliquot of unknown sample
- For most applications, blank with the same buffer solution used to resuspend the analyte of interest. The blanking solution should be a similar pH and ionic strength as the analyte solution. For details, see “To measure samples” in the application used.
- If blanking problems persists, use an application that may be more suitable such as a fluorescence assay using the NanoDrop 3300 or a colorimetric assay if measuring proteins.

Run a Blanking Cycle

Run a blanking cycle to verify the following:

- instrument is operating normally (with flat baseline)
- pedestals are clean (i.e., no dried-down sample material on pedestals)
- absorbance contribution of buffer solution you plan to use for sample analyses

Supplies needed

- lint-free laboratory wipes
- calibrated precision pipettor (0–2 µL)
- buffer solution for evaluation

To run a blanking cycle

For quick demonstration, watch multimedia training Evaluating a Blanking Solution for Suitability.

NOTICE

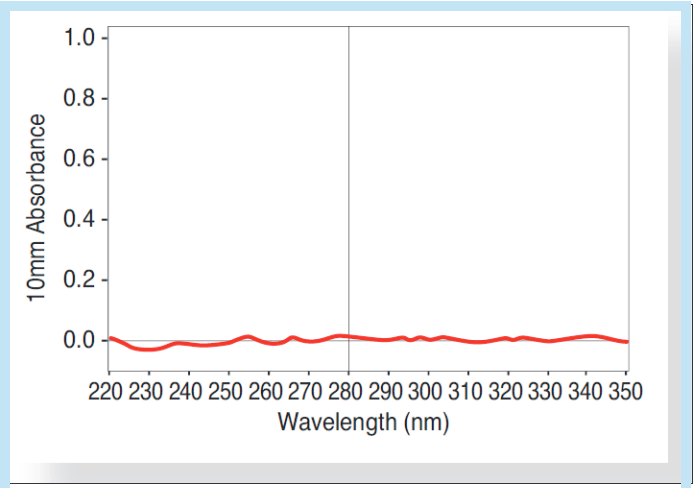
- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

1. From the Home screen, select an application tab such as Nucleic Acids and tap an application name such as dsDNA or RNA.
2. Lift the instrument arm and clean the upper and lower pedestals with new laboratory wipe.
3. Measure a water blank:
   - Pipette exactly 1 µL deionized water (DI H_2O) onto the lower pedestal and lower the arm.
   - Tap **Blank** and wait for the measurement to complete.
   - Lift the arm and clean both pedestals with new laboratory wipe.
4. Measure the buffer solution:
   - Pipette 1-2 µL buffer solution onto the pedestal and lower the arm.
   - Start the sample measurement:
     - if Auto-Measure is On, lower arm
     - if Auto-Measure is off, lower arm and tap **Measure**
   - Wait for measurement to complete.

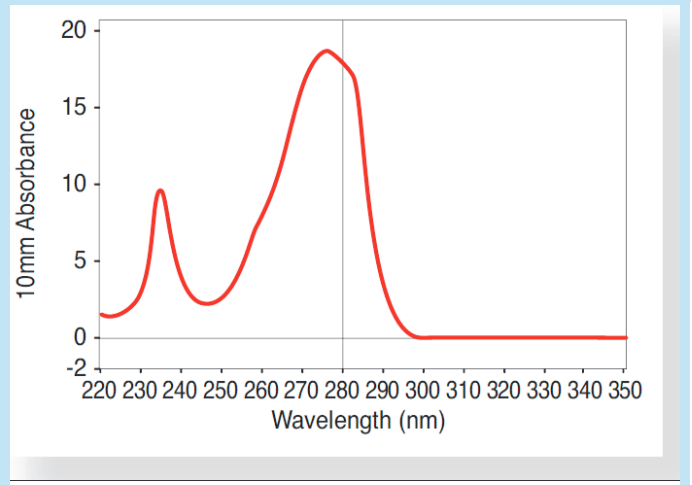
   The resulting spectrum should vary no more than 0.04 A from the baseline at the analysis wavelength (260 nm for nucleic acids; 280 nm for proteins).

   If your spectrum does not meet these criteria, repeat steps 2–4.

   If spectrum is still outside specifications, see Solutions for Blanking Problems.
5. When you are finished with the blanking cycle, tap **End Experiment**.
6. Lift the arm and clean both pedestals with a new wipe.



Example spectrum of buffer suitable for Protein A280 protein quantification



Example spectrum of buffer unsuitable for Protein A280 protein quantification

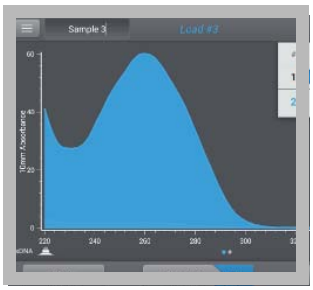
Related Topics

- Absorbance Detection Limits

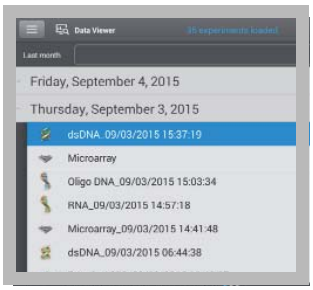
- Effects of Bubbles in Samples
- What is a Blank?
- Evaluating a Blanking Solution for Suitability
- Maintaining the Pedestals
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette

Basic Instrument Operations

Home Screen



Measurement screens

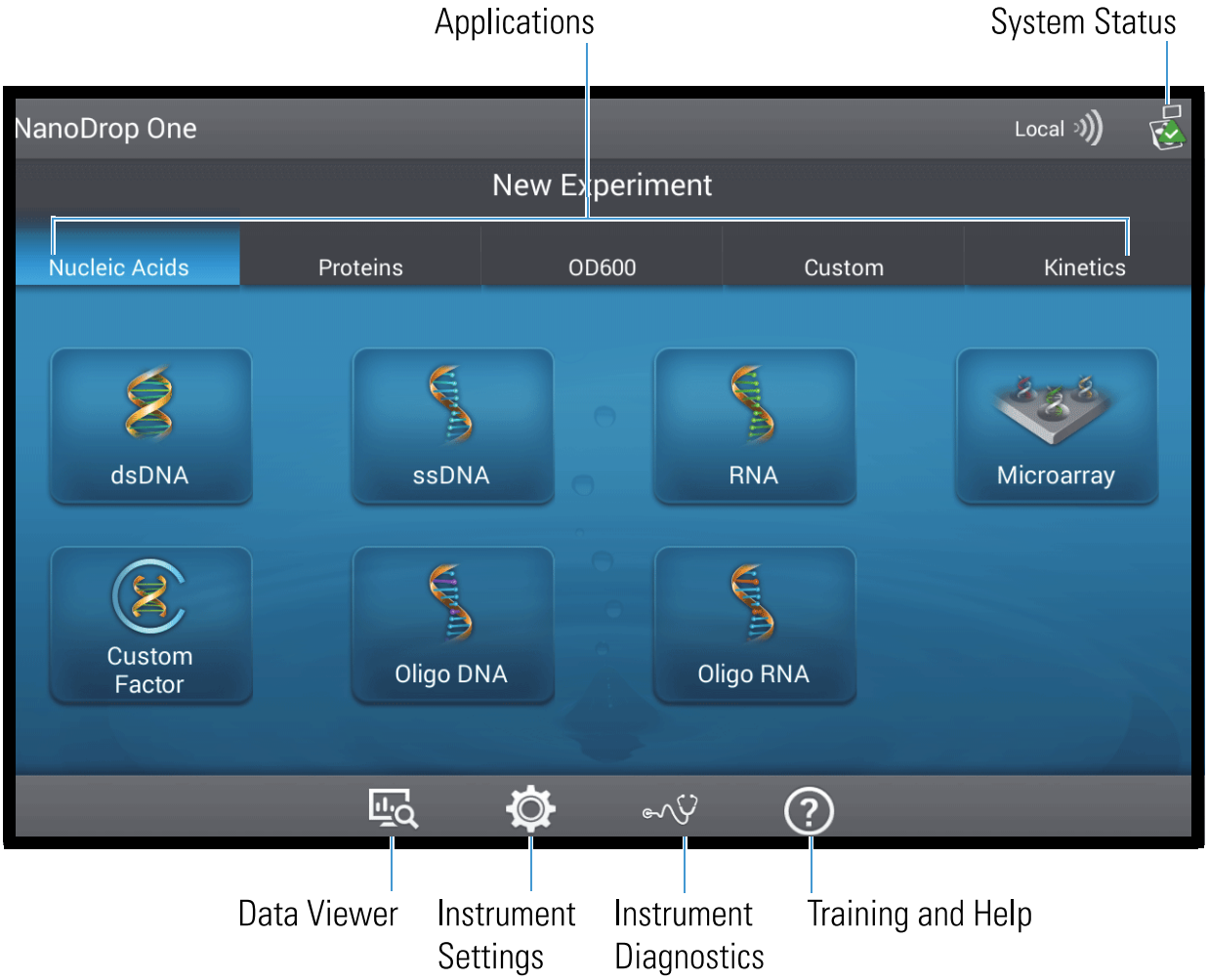


Data Viewer

General Operations

NanoDrop One Home Screen

These operations are available from the NanoDrop One Home screen.



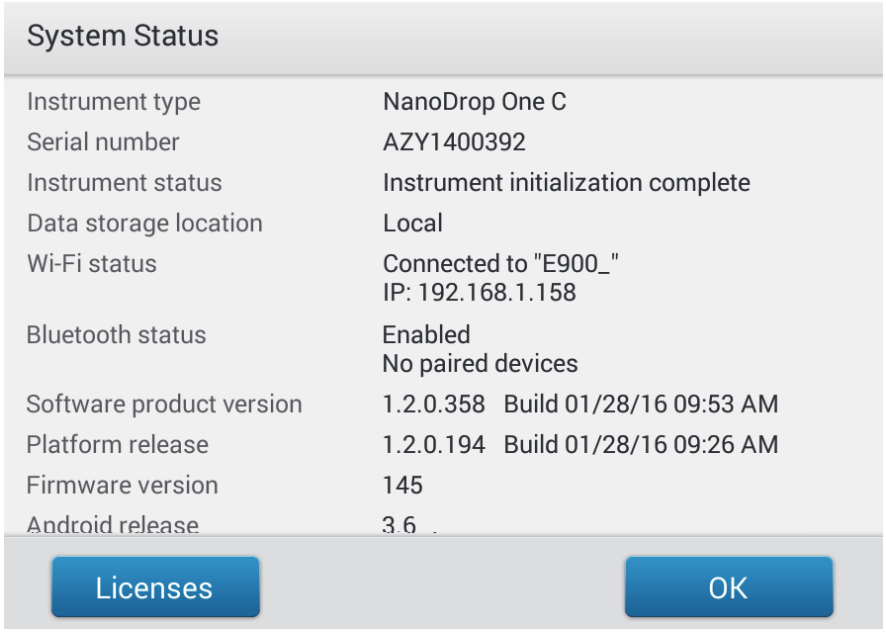
Applications

The NanoDrop One offers a broad range of applications for measuring samples with the instrument. To select an application, tap an Application tab such as Nucleic Acids and then tap an application name such as dsDNA.

Tap here for detailed information about each available application.

System Status

Tap  on the instrument Home screen to open the system status box. Here is an example:



The available information is described below.

Instrument type	Instrument model (NanoDrop One or NanoDrop One^{C})
Serial number	Instrument serial number
Instrument status	Current status of the instrument
Data storage location	Indicates location of database where instrument is currently storing data. These options are available: • Local (instrument) • Connected PC* (personal computer connected through Ethernet cable or wireless network) * the Ethernet and wireless options listed above also store data on the instrument as a backup.
Wi-Fi status	Status of WiFi connections for the instrument ("Connected to...", "Enabled and not connected" or "Disabled")
Bluetooth status	Status of Bluetooth connections for the instrument ("Connected to...", "Enabled-[list of any paired devices]" or "Disabled")
Software product version	Version of NanoDrop One software installed
Platform release	Platform release to support NanoDrop One instruments

Firmware version	Version of instrument firmware installed
Android release	Custom version of Android release
Android version	Version of Android operating system software installed

Data Viewer

Tap  on the Home screen to view any data acquired earlier today, last week, last month, last six months, last year or in a specific date range. Tap here for more information about the Data Viewer on the instrument.

Note The instrument will only allow you to view data in its local NanoDrop One database. When the instrument is connected to a computer with an Ethernet cable or through a wireless network, the Data Viewer icon on the instrument Home screen is unavailable.

Instrument Settings

Tap  on the Home screen to access general instrument settings such as WiFi and using cuvettes. Tap here for detailed information about all available instrument settings.

Instrument Diagnostics

Tap  on the Home screen to verify instrument operation. Instrument diagnostics should be run periodically according to the recommended maintenance schedule. Tap here for information about how to run the available instrument diagnostics.

Training and Help

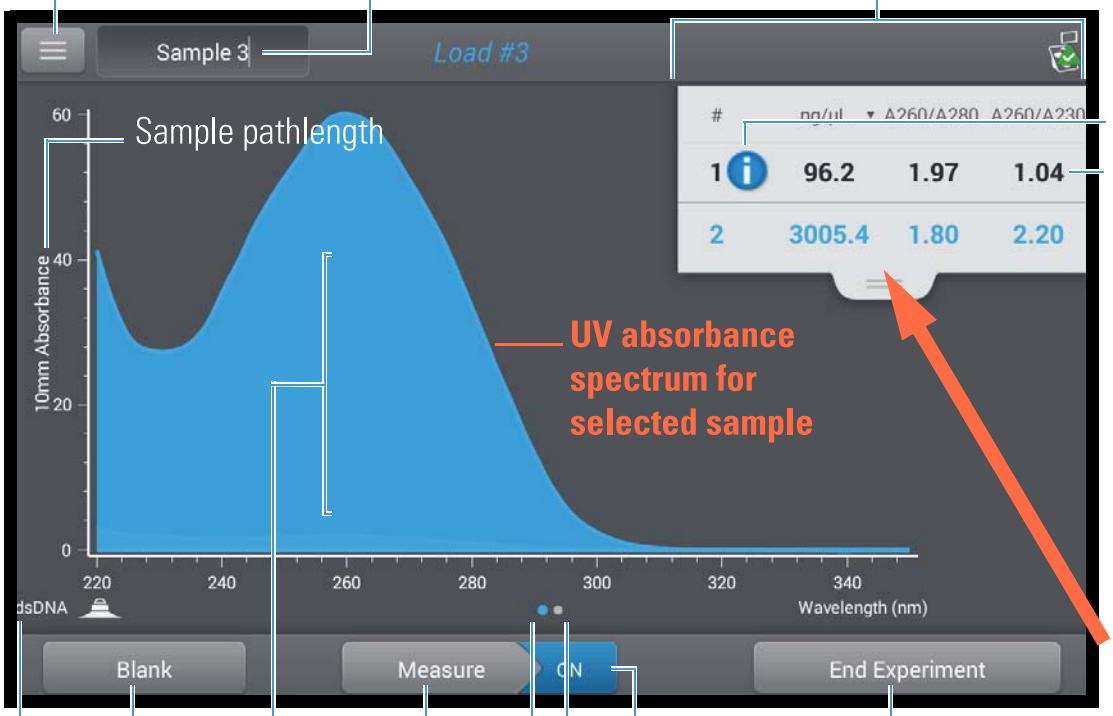
Tap  on the Home screen to access this Help system. The NanoDrop One software comes with comprehensive embedded training and support. Tap here for information on how to navigate the available information.

Related Topics

- Applications
- Set Up the Instrument
- NanoDrop One Data Viewer
- Instrument Settings
- Instrument Diagnostics
- About this Help System
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette

NanoDrop One Measurement Screens

These operations are available from any measurement screen within an Application.



Menu

Tap ☰ in any measurement screen to see the available menu options.

Home	Return to NanoDrop One Home screen
[application] Setup	View or change settings for selected application
Settings	View or change instrument settings
Print	Print selected measurement results

Sample Name

Tap the Sample Name field in any measurement screen to edit the sample name.

When Auto-Naming is On (see General Settings), each sample is automatically assigned a sample name using the default base name followed by a unique number starting with "1." The first time this appears is after the first blank measurement and before the first sample measurement in each experiment as shown below.

In this 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.

Note If you edit the sample base name during an experiment when Auto-Naming is selected, the assigned sample ID numbers restart.

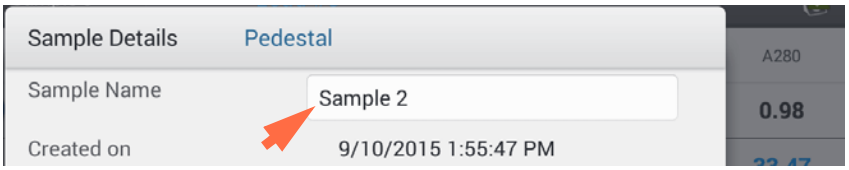
Edit default sample base name

After you measure a blank and before the first sample is measured:

- tap Sample Name field to display keyboard
- enter new base name
- tap Done key

Edit sample name

- from Home screen, tap  to open Data Viewer
- select experiment
- swipe left to show data table
- press and hold sample name to show Sample Details box
- tap Sample Name field to display keyboard



– enter new sample name
– tap Done key

Measurement Results

The types of results that appear in the measurement screens depend on the selected application. For details, see:

Applications > [application group] > Measure [application name] > Reported Results

Here is an example for dsDNA.

Absorbance Spectrum

For each measured sample, each application shows the UV or UV-visible absorbance spectrum and a summary of the results. The vertical axis shows absorbance in absorbance units (A). The horizontal axis shows wavelength in nm.

Sample Pathlength

All applications display the sample pathlength along the spectrum's vertical axis. Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10.0 mm pathlength equivalent.

Measurement Alerts

The Acclaro Sample Intelligence technology built into the NanoDrop One instruments provides important features to help you assess sample integrity. Tap a Sample Intelligence icon in the software to view its associated information. For more information, tap a link below.

contaminant analysis is available to help qualify a sample before use in downstream applications

on-demand technical support is available for measurements that are atypical or very low concentrations

invalid-results alert

Blank

Tap Blank to measure a blank for the selected experiment.

A blank must be measured before each group of similar samples. The blank solution is typically the pure buffer that was used to resuspend the sample. For more information, see Choosing and Measuring a Blank.

Measure

Tap Measure to measure a sample for the selected experiment.

Samples must be properly isolated and prepared before they can be measured with the instrument and the concentration must be within the instrument's absorbance detection limits. For more information, see Preparing Samples. and Measure a Micro-Volume Sample or Measure a Cuvette Sample and Absorbance Detection Limits.

Note The Measure button is enabled after a valid blank measurement is completed.

Auto-Measure and Auto-Blank

Speed up sample analysis with the NanoDrop One Auto-Measure and Auto-Blank features, which cause the instrument to start the measurement immediately after you lower the instrument arm. These options eliminate the need for repetitive Measure or Blank operations for large batches of samples.

Note Auto-Measure and Auto-Blank are available for micro-volume measurements only.

Auto-Measure

To select or deselect Auto-Measure, from any sample measurement screen, tap the On or Off button at the right of the Measure button.

Auto-Blank

To select or deselect Auto-Blank, from any blank measurement screen, tap the On or Off button at the right of the Blank button.

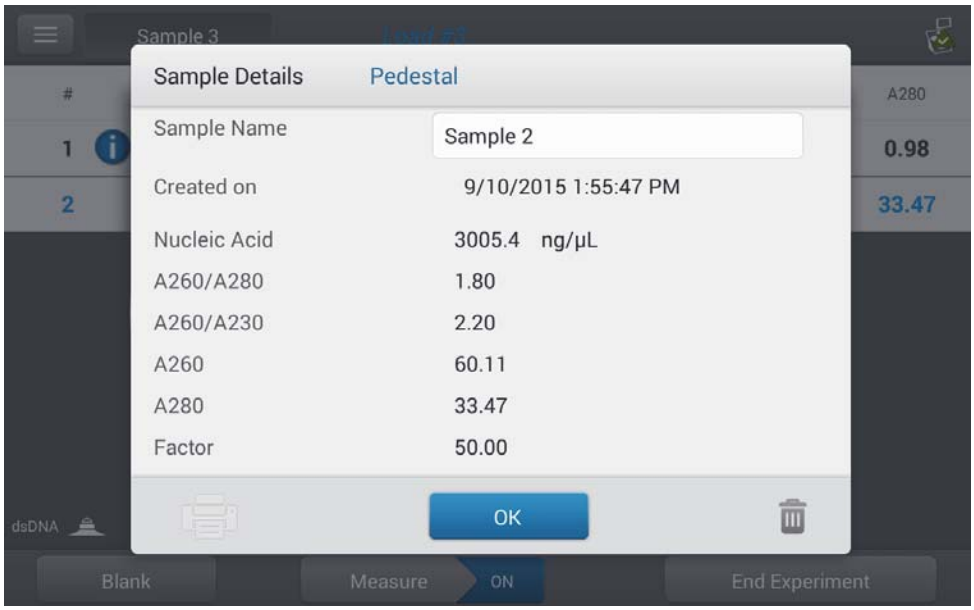
End Experiment

Tap End Experiment when you are ready to name and save your experiment, add a label to help you locate the experiment later (see Manage identifiers on the instrument), or export the data.

Note The End Experiment button is enabled after the first sample measurement is completed.

Sample Details

Press and hold a sample row in any measurement screen or data table to show the sample details, which include all available measurement results and associated details for the selected sample. Here is an example:



Note You can also edit the sample name from the Sample Details box.

Data Table

Swipe left in any measurement screen to see the data table for the current experiment. The data table contains the measurement results for all samples in the experiment. The image below highlights the available features.

Related Topics

- Measure Nucleic Acids
- Measure Proteins
- Instrument Settings
- Print Data
- Acclaro Sample Intelligence

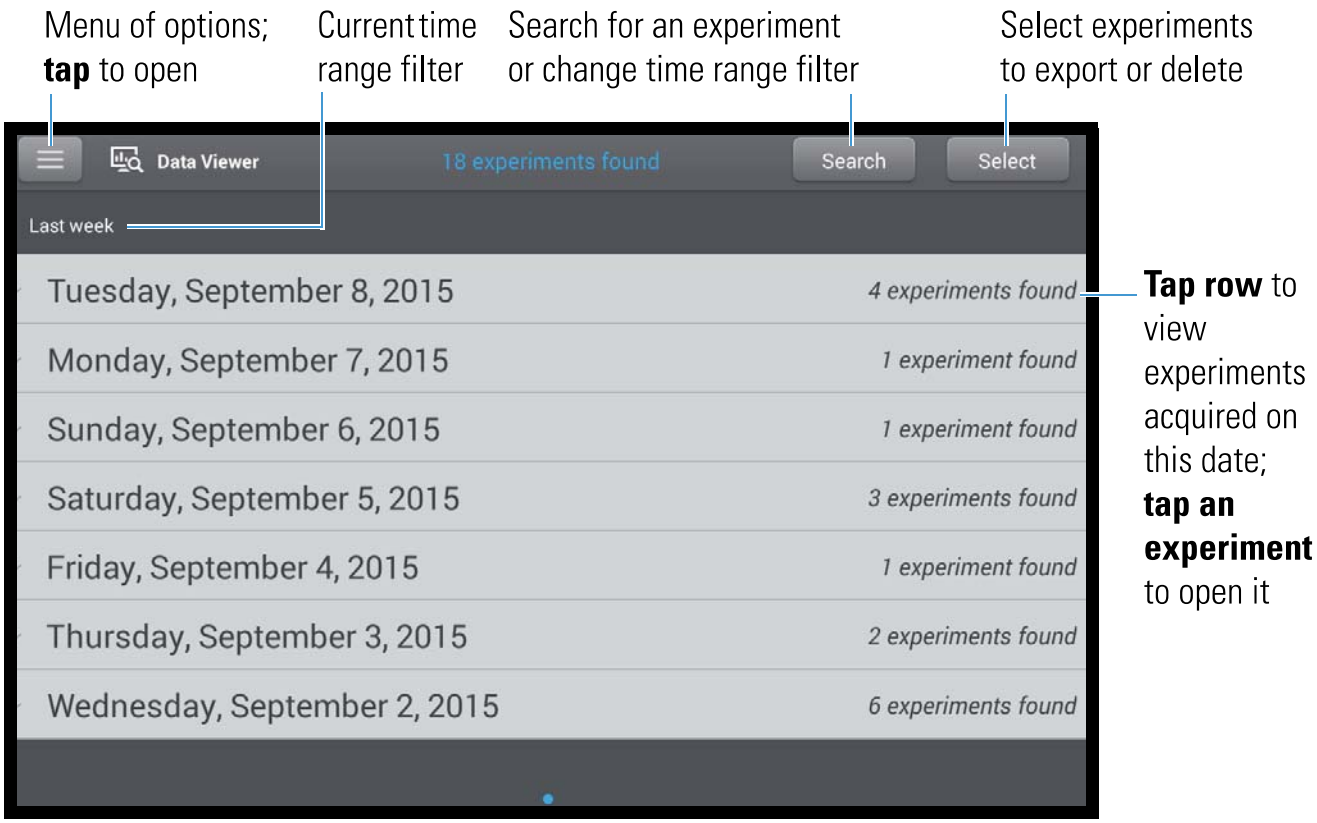
- Prepare Samples and Blanks
- Search Experiment Database
- Export Data
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette

NanoDrop One Data Viewer

The Data Viewer opens the database that stores sample data measured with the instrument. The data are saved according to acquisition date, experiment name, application used and any assigned labels (see Manage Identifiers).

From the Data Viewer, you can locate and select any experiment to see the measurement data it contains, or export selected experiments to a variety of locations and formats.

These operations are available from the Data Viewer.



Open Data Viewer

Whether you collect one sample or many in a row, after you choose End Experiment, the acquired data are automatically saved in an experiment with an experiment name. In the default configuration, experiments are stored in the NanoDrop One database on the local instrument according to acquisition date, experiment name, application used and any assigned labels.

Use the Data Viewer to open the database on the local instrument in order to view acquired spectra and associated data from any experiment at any time.

Open instrument database of measurement results

– to open NanoDrop One database on instrument, tap  (Data Viewer) on instrument Home screen

> **Note** The Data Viewer icon is not available on the instrument Home screen when the instrument is connected to a computer with an Ethernet cable or through a wireless network (see Setting Up the Instrument for details).

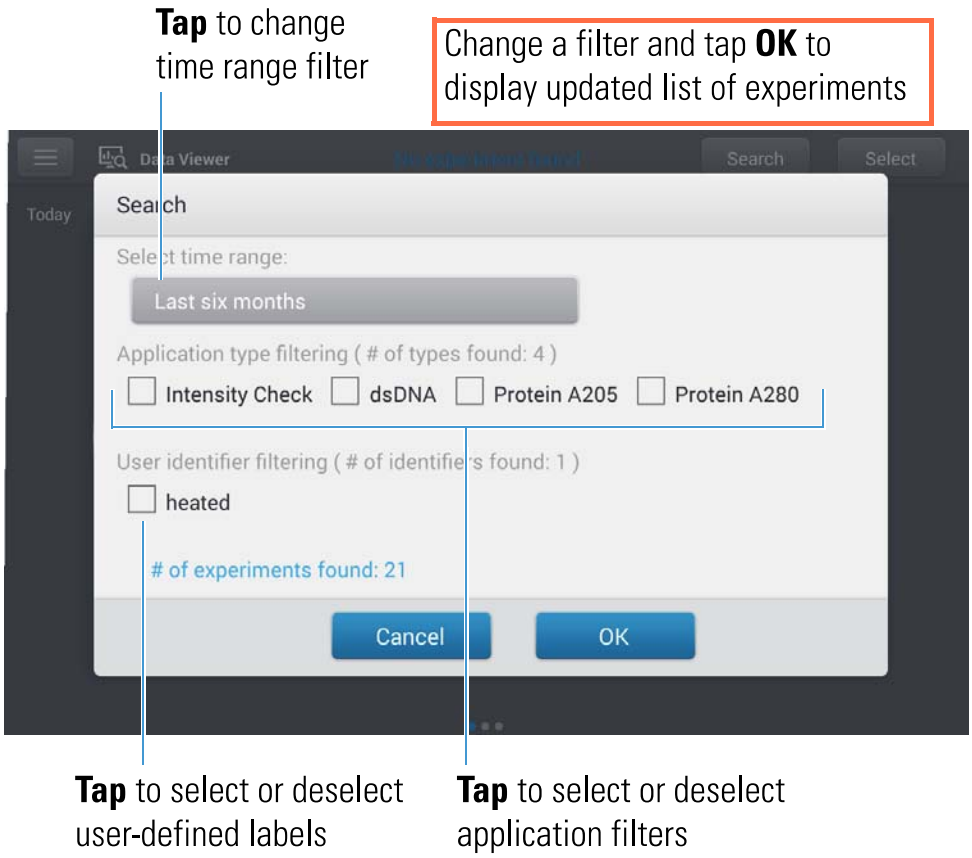
Menu

Tap  in the Data Viewer to see the available menu options.

Home	Return to NanoDrop One Home screen
Settings	View or change instrument settings
Import	Import data from a USB flash drive
Disk Status	View remaining space available for storing measurement data on the instrument

Search Experiment Database

Tap Search in the Data Viewer to search the selected database for an experiment or to change the time range or other search filters. The database is filtered using the current settings in the Search box. Filters include time range, application type and any user-defined labels (see Manage Identifiers for information about adding and deleting labels). Here is an example:



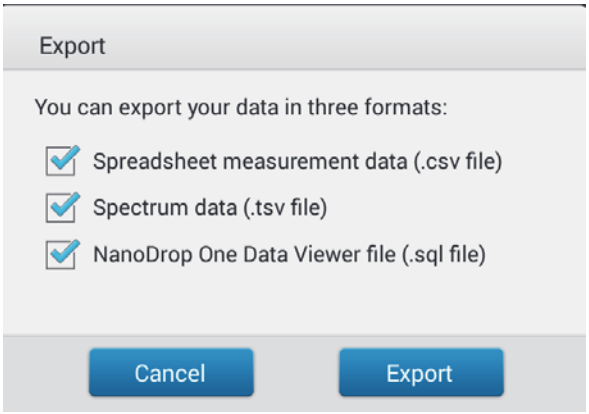
Export Selected Experiments

Use Select in the Data Viewer to select experiments to be exported.

Export selected experiments

- tap row in Data Viewer to list experiments acquired on that date, or use Search feature to find experiment
- insert USB memory device into available USB port on instrument (front, back-left or back-right)
- tap Select

- tap to select one or more experiments to export (tap again to deselect an experiment)
- tap Export
- select one or more formats to export to (see “Export Data” in General Operations for details)



- tap Export
- after “Export Success” message, tap OK

Delete Selected Experiments

Use Select in the Data Viewer to select experiments to be deleted.

Delete selected experiments

- tap row in Data Viewer to list experiments acquired on that date, or use Search feature to find desired experiment
- tap Select
- tap to select one or more experiments to delete (tap again to deselect an experiment)
- tap Delete and OK

NOTICE Deleted data cannot be recovered.

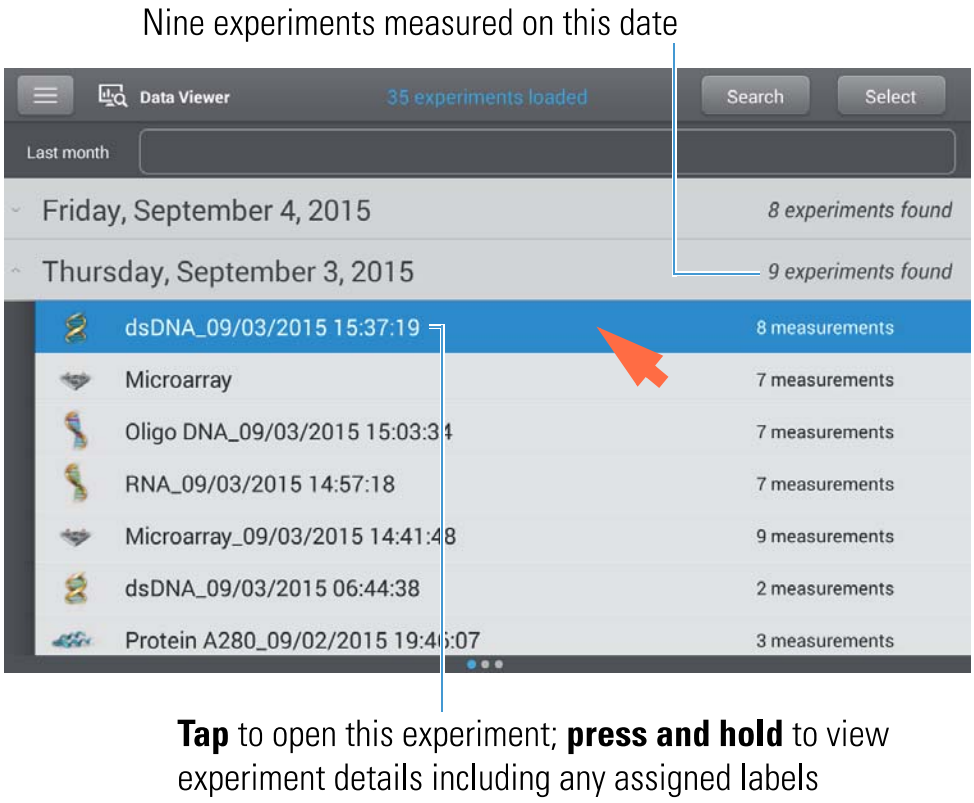
Open Experiment and View Associated Data

Use the Data Viewer to locate and open any experiment to see the measurement data it contains.

Open an experiment

- tap row in Data Viewer to list experiments acquired on that date, or use Search feature to find desired experiment
- tap experiment name to open the experiment

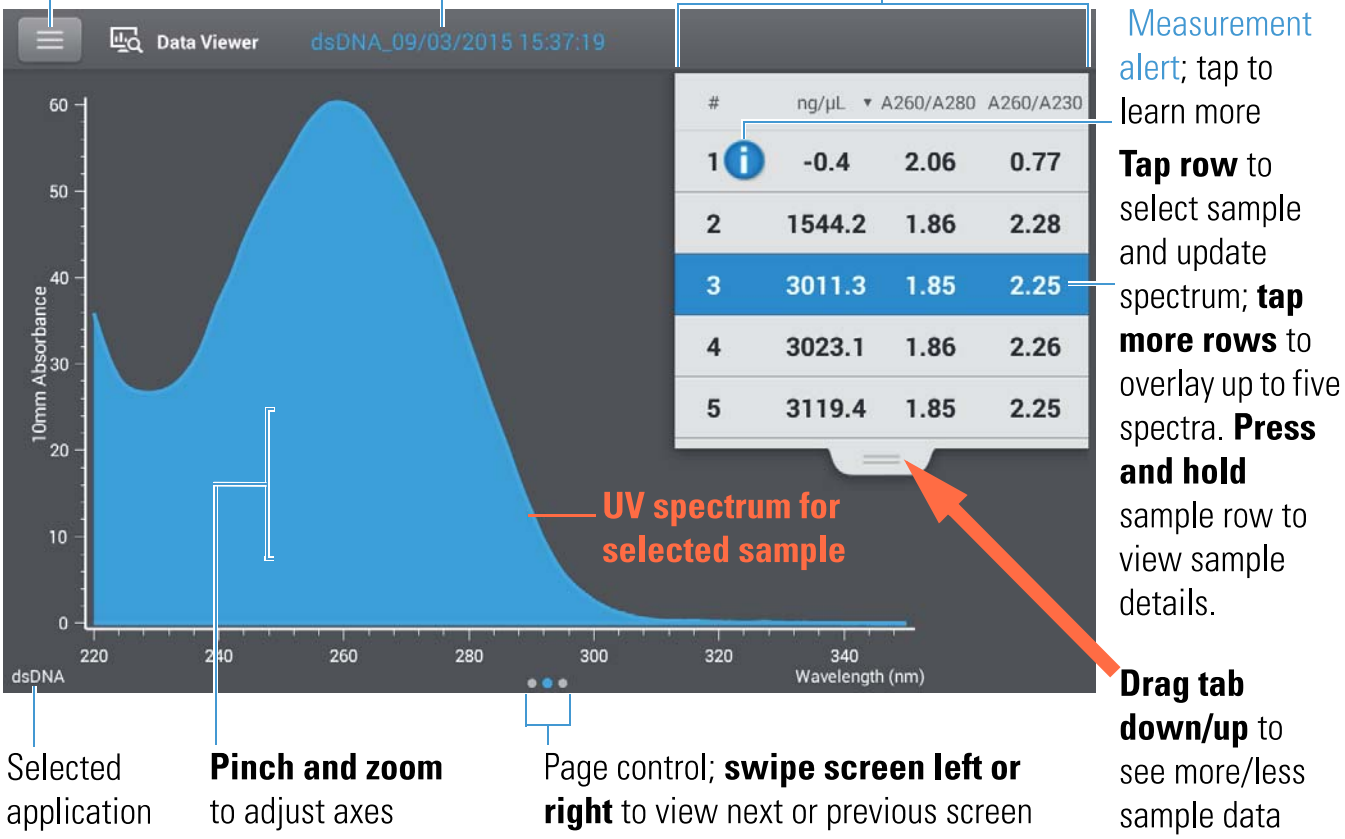
Here is an example:



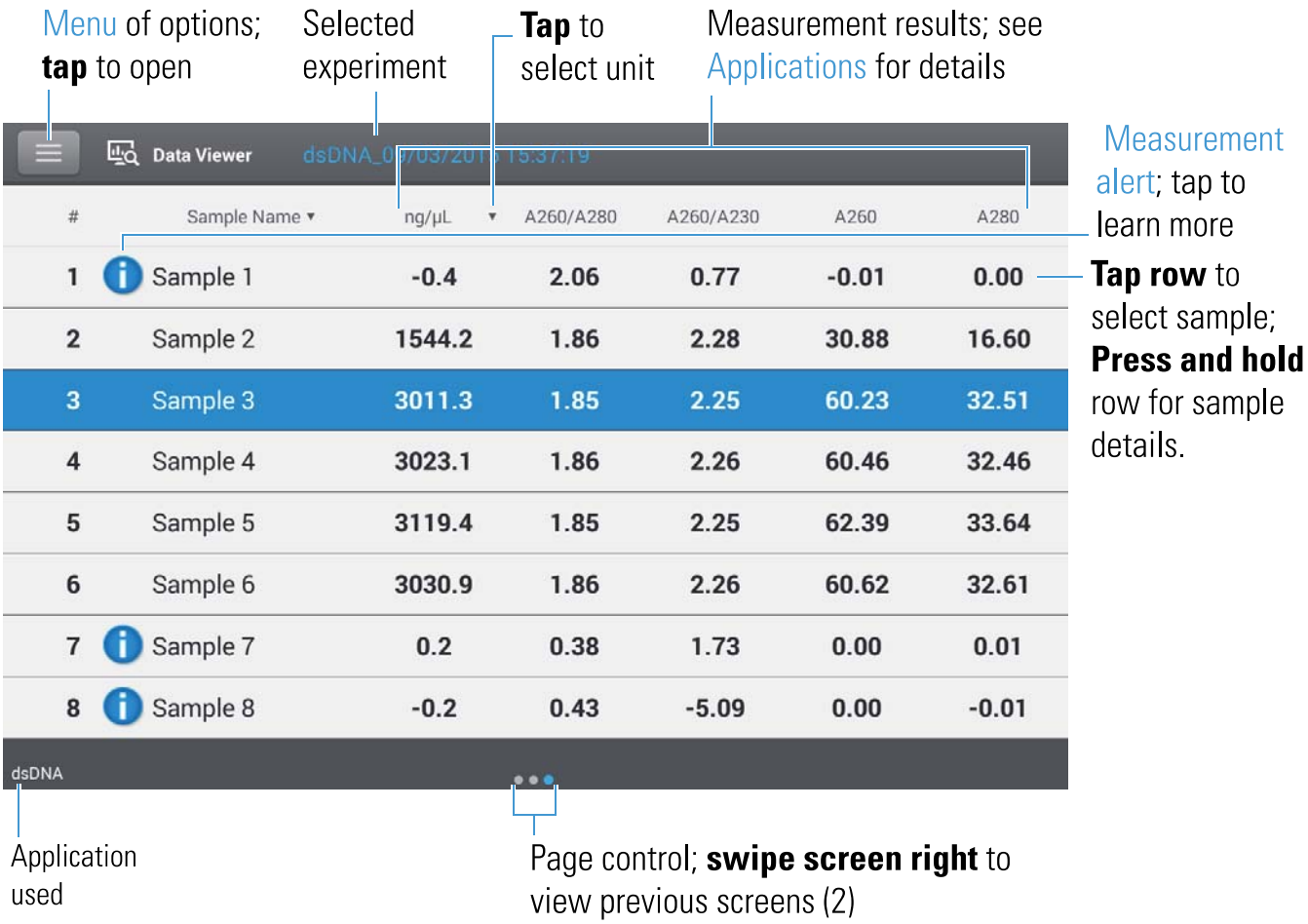
The Data Viewer provides measurement data as spectral data and data tables, similar to what you see after you complete a measurement.

Note The data shown are dependent upon the application used to measure the samples (nucleic acids in these examples). For more information, see the application details.

Spectral data—
After you open an experiment, the software shows the UV or UV-visible absorbance spectrum and a summary of the associated data for the first sample measurement, much like it appears during a measurement. The image below describes the available features.



Data Table—
Swipe left in any Spectral Data screen to see the data table for the current experiment. The data table contains the measurement results for all samples in the experiment. The image below describes the available features.



Menu

Tap ☰ from any Spectral Data or Data Table screen to see the available menu options.

Home	Return to NanoDrop One Home screen
Manage Identifiers	Add or delete labels for selected experiment to make it easier to find (see Manage identifiers on the instrument)
Export	Export selected experiment to USB device
Print	Print selected measurement results (Print option appears only when one or more measurements results are selected in Data Table)

Settings	View or change instrument settings
Disk Status	View remaining space available for storing measurement data on the instrument

Related Topics

- Instrument Settings
- Search Experiment Database
- Export Data
- Print Data

NanoDrop One General Operations

These operations are available from any measurement screen or from the Data Viewer.

Manage Identifiers (on the instrument)

You can add one or more "identifiers" (i.e., labels or metadata tags) to an experiment to make the experiment easier to find. Labels can be added from the NanoDrop One software running on the instrument, or from the NanoDrop One Viewer software installed on a personal computer (see Manage Identifiers on a PC).

Use the Data Viewer to add labels to experiments, assign existing labels, view assigned labels and remove or delete labels on the instrument. You can filter the list of experiments in the Data Viewer based on one or more user-defined labels.

Label new experiment when you save it

- after the last sample has been measured, tap 

- in End Experiment box, tap Add Identifier field
- use displayed keyboard to enter label and tap 
- tap Done key
- tap End Experiment

Label experiment in Data Viewer

- from Home screen, tap  to open Data Viewer
- tap to open an experiment
- tap  and choose Manage Identifiers
- in Manage Identifiers box, tap Add Identifier field
- use displayed keyboard to enter label and tap 
- tap Done key
- tap OK

View assigned labels for an experiment

- from Home screen, tap  to open Data Viewer
- press and hold selected experiment to see Experiment Details

Find labeled experiments

- from Home screen, tap  to open Data Viewer
- tap Search
- in Search box, select date range, select application (only applications that have associated data are shown), select one or more identifiers from scrollable list and tap OK

Remove a label

- from Home screen, tap  to open Data Viewer
- tap to open an experiment
- tap  and choose Manage Identifiers
- in Manage Identifiers box, select label and tap  .
- tap OK

Export Selected Measurements

You can export measurement data from one or more experiments when you save the experiment, or afterwards from the Data Viewer.

Note Data exported during a save are still saved to a database (local or remote, depending on the Data Storage setting; see Select location for saving or viewing collected data for more information).

Measurement data can be exported in three formats:

- as a comma-separated values (.csv) file, containing the measurement results and details
- as a tab-separated values (.tsv) file, containing x,y coordinates for every spectral data point
- as a NanoDrop One Viewer (.sql) file, containing spectra and measurement results that can be imported to the NanoDrop One Viewer software running on a personal computer

The filenames are the same as the experiment names. The files are stored in a folder named "NanodropOne" followed by the instrument serial number. (Use System Status to view instrument serial number.)

If you select multiple experiments for export in the CSV and TSV formats, each exported experiment has a corresponding CSV and TSV file. If the SQL option is also selected, the exported SQL file contains all the selected experiments.

Use any spreadsheet or word processing application to open a CSV or TSV file. Here is an example of several sample measurement results in CSV format:

	A	B	C	D	E	F	G	H	I	J
1	Date	Sample Name	Nucleic Acid	A260/A280	A260/A230	A260	A280	Nucleic Acid Factor	Baseline (nm)	
2	4/21/2015 15:37	Sample 1	0.3	0.7	0.56	0.01	0.01	33	340	
3	4/21/2015 15:42	Sample 2	0.37	0.94	0.86	0.01	0.01	33	340	
4	4/21/2015 15:44	Sample 3	0.43	0.98	0.74	0.01	0.01	33	340	
5	4/21/2015 15:44	Sample 4	0.18	2.1	0.83	0.01	0	33	340	
6	4/21/2015 15:45	Sample 5	0	0.07	0.02	0	0	33	340	
7	4/22/2015 8:57	Sample 6	-0.52	2.11	0.66	-0.02	-0	33	340	
8										

Note The types of data exported are dependent upon the application used to measure the samples (nucleic acids in this example). For more information, see the application details.

The SQL file can be opened only using our NanoDrop One Viewer software, and only after the file has been imported.

Data can be exported to a USB device connected to any USB port on the local instrument (front, back-left or back-right), and then transferred to any computer that has an installed spreadsheet or word processing application (for CSV and TSV files) or the NanoDrop One Viewer application (for SQL files).

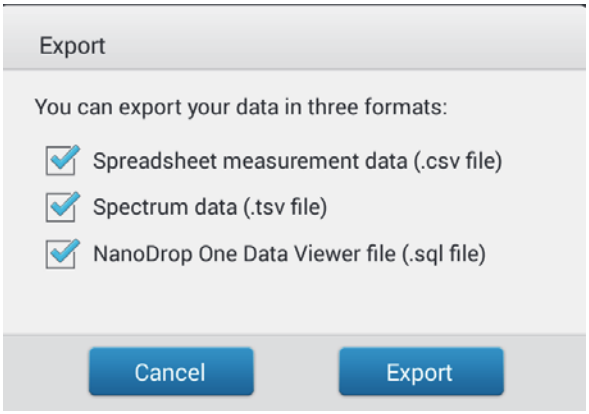
Export data at end of experiment

– insert USB memory device into available USB port on instrument (front, back-left or back-right)

- when finished measuring samples, tap 

- from End Experiment box, tap 

- select one or more formats to export to (see above for details)

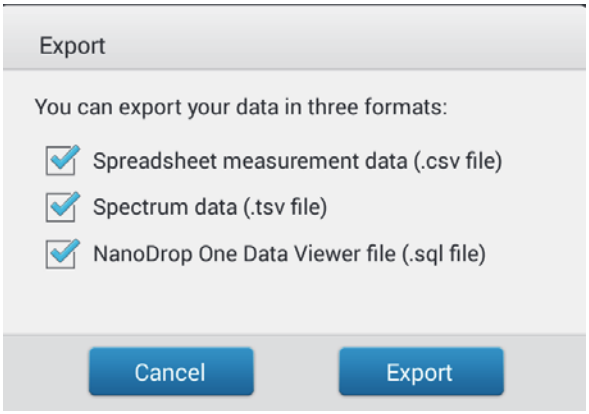


- tap Export
- after "Export Success" message, tap OK
- remove USB device
- tap End Experiment

Export data from Data Viewer

- from Home screen, tap  to open Data Viewer
- tap row in Data Viewer to list experiments acquired on that date, or use Search feature to find experiment
- insert USB memory device into available USB port on instrument (front, back-left or back-right)
- tap Select
- tap to select one or more experiments to export (tap again to deselect an experiment)
- tap Export

– select one or more formats to export to (see above for details)



– tap Export

– after "Export Success" message, tap OK

Delete Selected Measurements

You can delete a sample measurement from any experiment.

NOTICE Deleted data cannot be recovered.

Delete data from any measurement screen

– press and hold sample row to open Sample Details box

– tap 

Delete data from Data Viewer

– from Home screen, tap  to open Data Viewer

– tap row in Data Viewer to list experiments acquired on that date, or use Search feature to find desired experiment

– press and hold sample row to open Sample Details box

– tap 

Print Selected Measurements

Connect a compatible printer to the instrument to quickly print measurement results, including spectral data and sample details, to include in a laboratory notebook or post on a bulletin board.

Print data from any measurement screen

- after you have measured a sample, display the measurement results to be printed such as the spectral data or data table (see NanoDrop One Measurement Screens)
- tap to select one or more sample rows to print (tap again to deselect a sample row)
- tap  and choose  Print
- in the Print Information box, choose OK

One label is printed for each selected measurement.

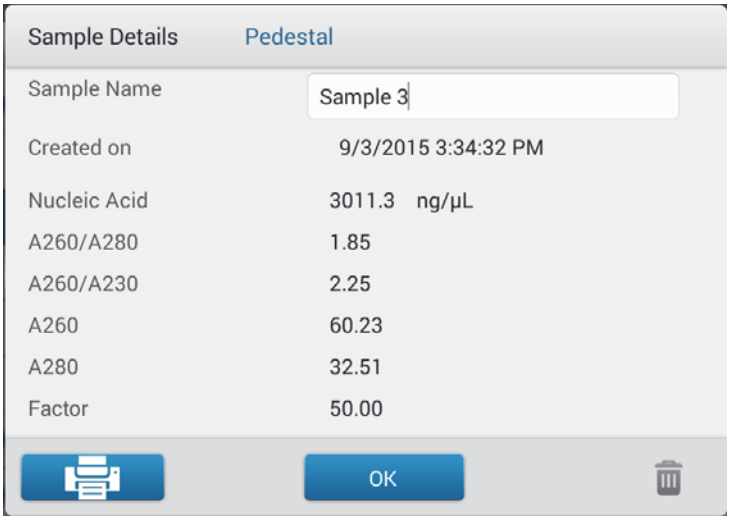
Print data from Data Viewer

- from Home screen, tap  to open Data Viewer
- tap row in Data Viewer to list experiments acquired on that date, or use Search feature to find desired experiment
- tap experiment name to open the experiment
- from the spectral data or data table in any measurement screen or from the Data Viewer, tap to select one or more sample rows to print (tap again to deselect a sample row)
- tap  and choose  Print
- in the Print Information box, choose OK

One label is printed for each selected measurement.

Print sample details

– from the spectral data or data table in any measurement screen or from the Data Viewer, press and hold sample row to open Sample Details box



– tap 

– in the Print Information box, choose OK

One label is printed for this measurement.

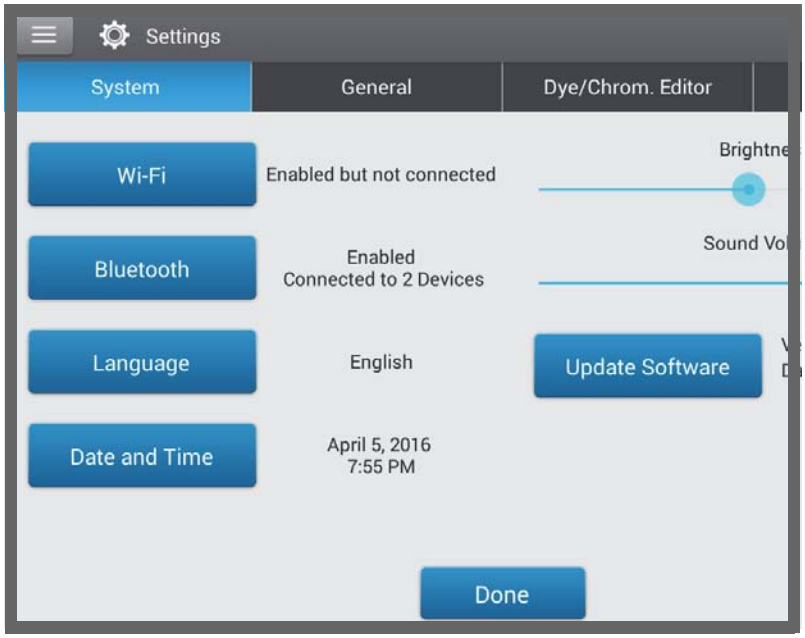
Related Topics

- Instrument Settings
- NanoDrop One Data Viewer
- Search Experiment Database
- Select Experiments to Export or Delete
- Open Experiment

Instrument Settings

View or change instrument settings

- from Home screen, tap
- -or-
- from any measurement screen or the Data Viewer, tap and choose Settings



These instrument settings are available:

System Settings

These options are available:

Wi-Fi	Set up wireless local area network (WLAN) connection on the instrument
Bluetooth	Set up Bluetooth connections to wireless input devices for the instrument such as a wireless keyboard, mouse or barcode scanner
Language	Select language for displaying NanoDrop One software and for any connected input device such as a keyboard, mouse or barcode scanner Notice: Changing the language requires a software restart.