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NanoDrop Micro-UV/Vis Spectrophotometer

NanoDrop One

User Guide

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About the Spectrophotometer



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

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

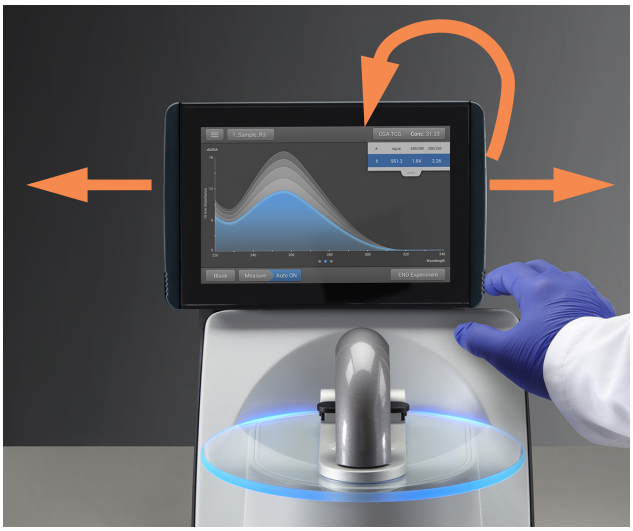
The NanoDrop One system comes with preloaded software and a touchscreen display. NanoDrop One PC Control software can be installed on a local PC and used to control the instrument and view data. The instrument can be connected to an optional printer with a USB cable or to a remote printer through an Ethernet connection or wireless network.

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

Features

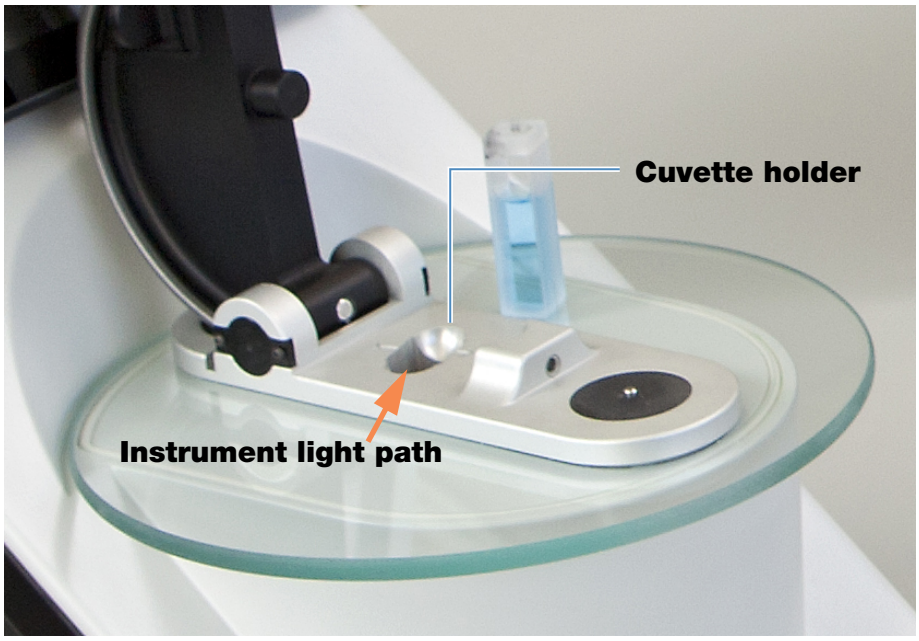
TheNanoDrop One^{C} spectrophotometer features the patented micro-volume sample retention system. The NanoDrop One^{C} also features a cuvette holder for analyzing dilute samples using standard UV-Visible cuvettes.

Touchscreen



TheNanoDrop One^{C} comes with a built-in, 7-inch high-resolution touchscreen preloaded with easy-to-use instrument control software. The touchscreen can slide left or right to accommodate personal preference, and tilt forward or back for optimal viewing

Cuvette Holder



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

USB-A port

One USB-A port is located on the front of the instrument and two more USB-A ports are located on instrument back panel.

Accessories

This section lists the accessories included for use with the NanoDrop One^{C}.

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.

Instrument Detection Limits



Measurement Location	Pathlength (mm)	Upper Detection Limit (10 mm Equivalent Absorbance)
Pedestal	1.0	12.5
	0.2	62.5
	0.1	150
	0.05	300
	0.03	550
Cuvette	10	1.5
	5	3
	2	7.5
	1	15

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Instrument Set up

Register Your Instrument

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

To register your instrument

1. Do one of the following:
   - From any PC that is connected to the Internet, use any web browser to navigate to our website.

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 software on a personal computer (PC). An Internet connection is required to download software.

To install or update NanoDrop One software on a PC

1. Insert the USB flash drive containing the installer software into an available USB port on your PC, or open the installation folder downloaded from the internet.
2. Launch **Start.exe** and click **Install**. The software installer will run.

To install or update NanoDrop One software on the instrument

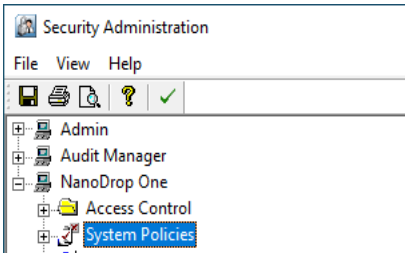
1. Copy the .zip file with the new software from your computer to a USB storage device. **Do not attempt to unzip the folder**.
2. Insert the USB device into any USB port on the NanoDrop One instrument.
3. From the instrument Home screen, tap **Settings** > **System** > **Update Software** and choose the latest version of software.

Setting Up User Account Control (Optional)

User account control is managed using the Security Administration application. The Thermo Scientific Security Administration software for NanoDrop One may be purchased for instruments used in labs requiring 21 CFR Part 11 compliance. When you launch Security Administration, you will need to enter your Windows log-in information.

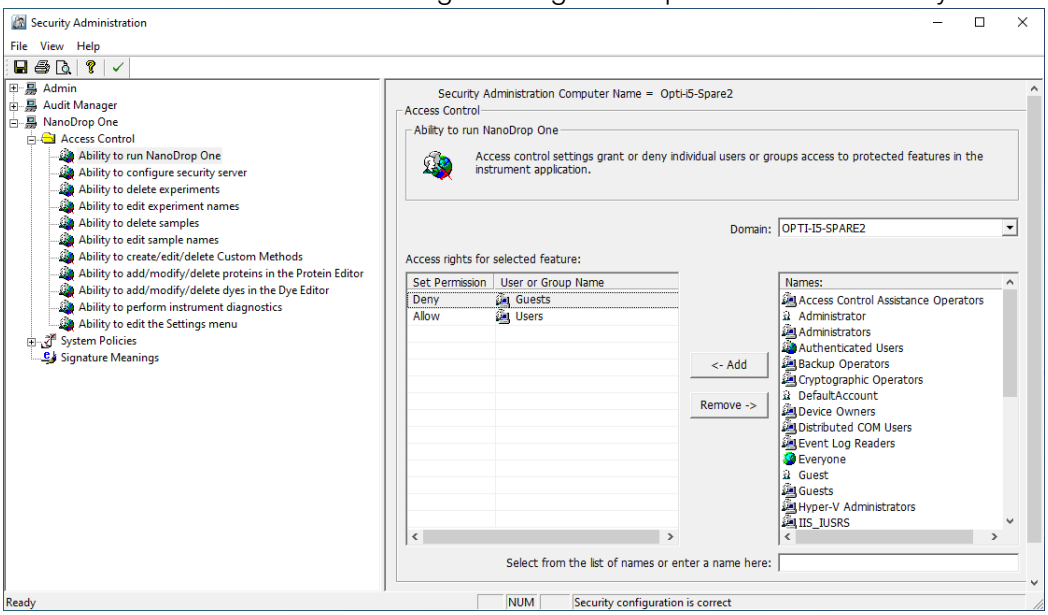
User Account Control

Launch the Security Administration application and select NanoDrop One from the directory on the left to reveal **Access Control** and **System Policies**.



Access control

Access control is used to grant or deny individual users or groups access to protected features in the instrument application. Add and remove users and groups to the access list and set access rights using the drop-down for each entity.



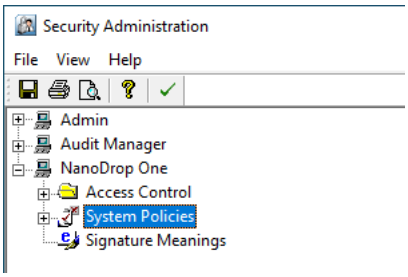
System policies

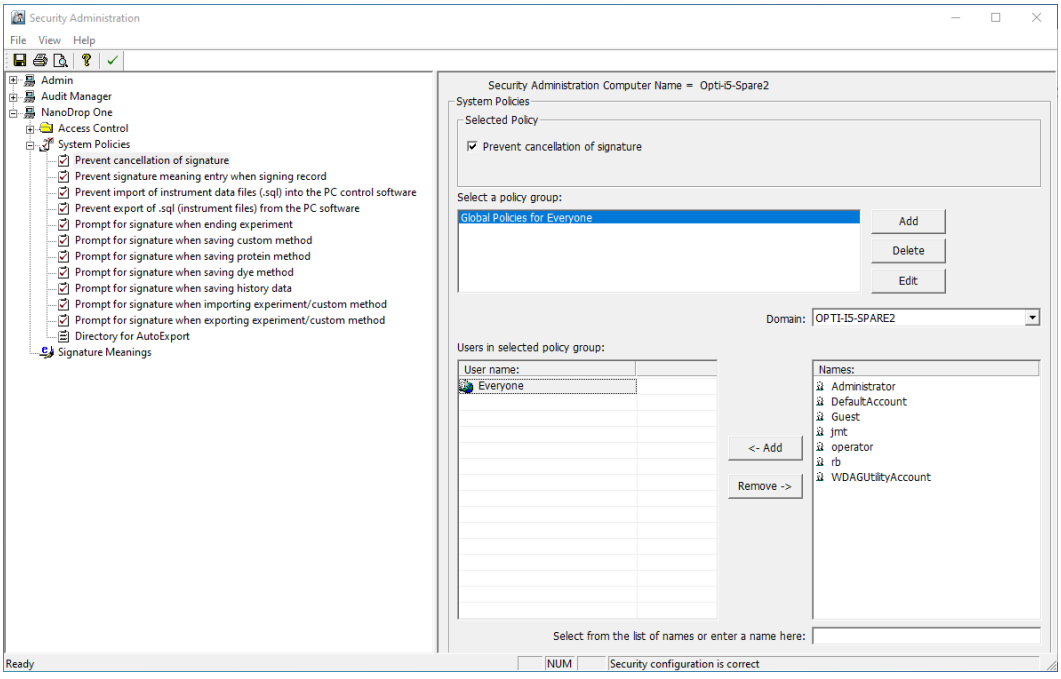
System Policies is used to set options that define the behavior of the client application. See “Security Administration Policies.”

Security Administration Policies

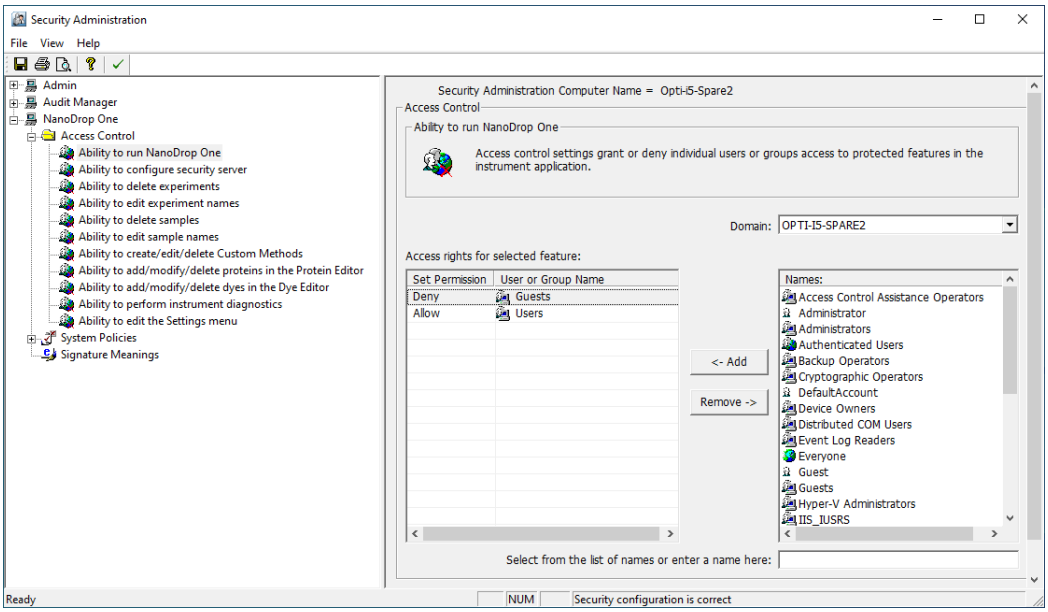
System policies allow you to assign data and method creation and deletion and editing privileges for users and groups.

Launch the Security Administration application and select **NanoDrop One**-> **System Policies**





You can add, delete, or edit policy groups and enable or disable the group's users permission to delete data. Select **NanoDrop One**-> **Access Control**.



When you are finished, select **Save**. Changes will take effect the next time NanoDrop One is launched.

Technical Support

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Telephone: 302 479 7707
Toll Free: 1 877 724 7690 (U.S. & Canada only)
Fax: 302 792 7155
E-mail: nanodrop@thermofisher.com
Website: www.thermofisher.com/nanodrop

For International Support, please contact:

Contact your local distributor. For contact information go to:

http://www.thermofisher.com/NanoDropDistributors

If you are experiencing an issue with your system, refer to the troubleshooting information. If the issue persists, contact us. If you are outside the U.S.A. and Canada, please contact your local distributor.

If your instrument requires maintenance or repair, contact us or your local distributor.

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Application Measurement Ranges

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

Sample Type	Lower Detection Limit	Upper Detection Limit	Typical Reproducibility[a]
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
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 IgG by Protein A280	0.06 mg/mL (pedestal) 0.006 mg/mL (cuvette) 0.03 mg/mL (pedestal) 0.003 mg/mL (cuvette)	825 mg/mL (pedestal) 402 mg/mL (pedestal)	±0.10 mg/mL (for 0.10–10 mg/mL samples); ±2% for samples >10 mg/mL
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=%)

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Nucleic Acid Applications

Measure dsDNA, ssDNA or RNA

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

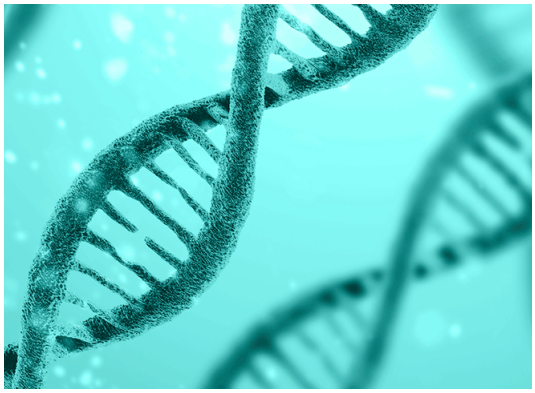
Measure dsDNA, ssDNA or RNA

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.

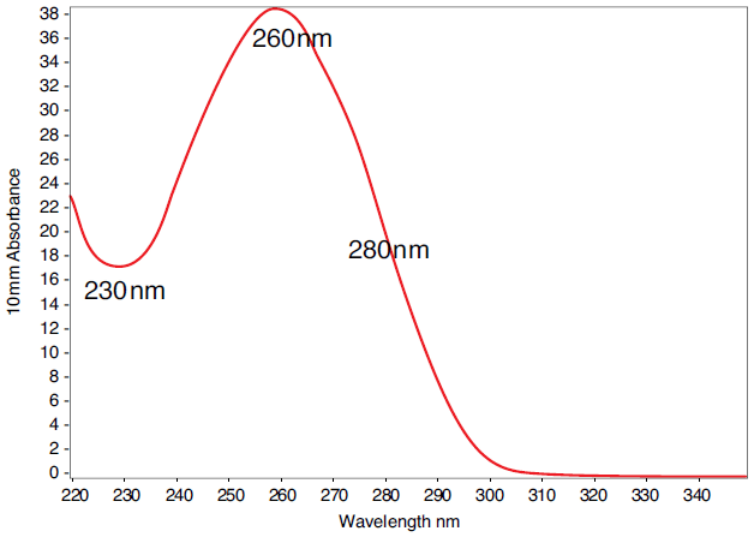
To measure nucleic acid

1. From the Home screen, select the **Nucleic Acids** tab and select **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.

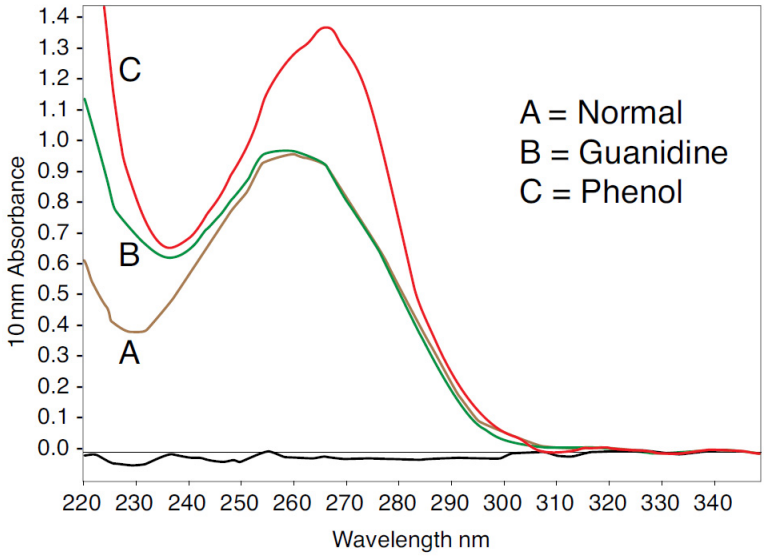
   Tip: If using a cuvette, make sure to align the cuvette light path with the instrument light path.
4. Tap **Blank** and wait for the measurement to complete.

   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.

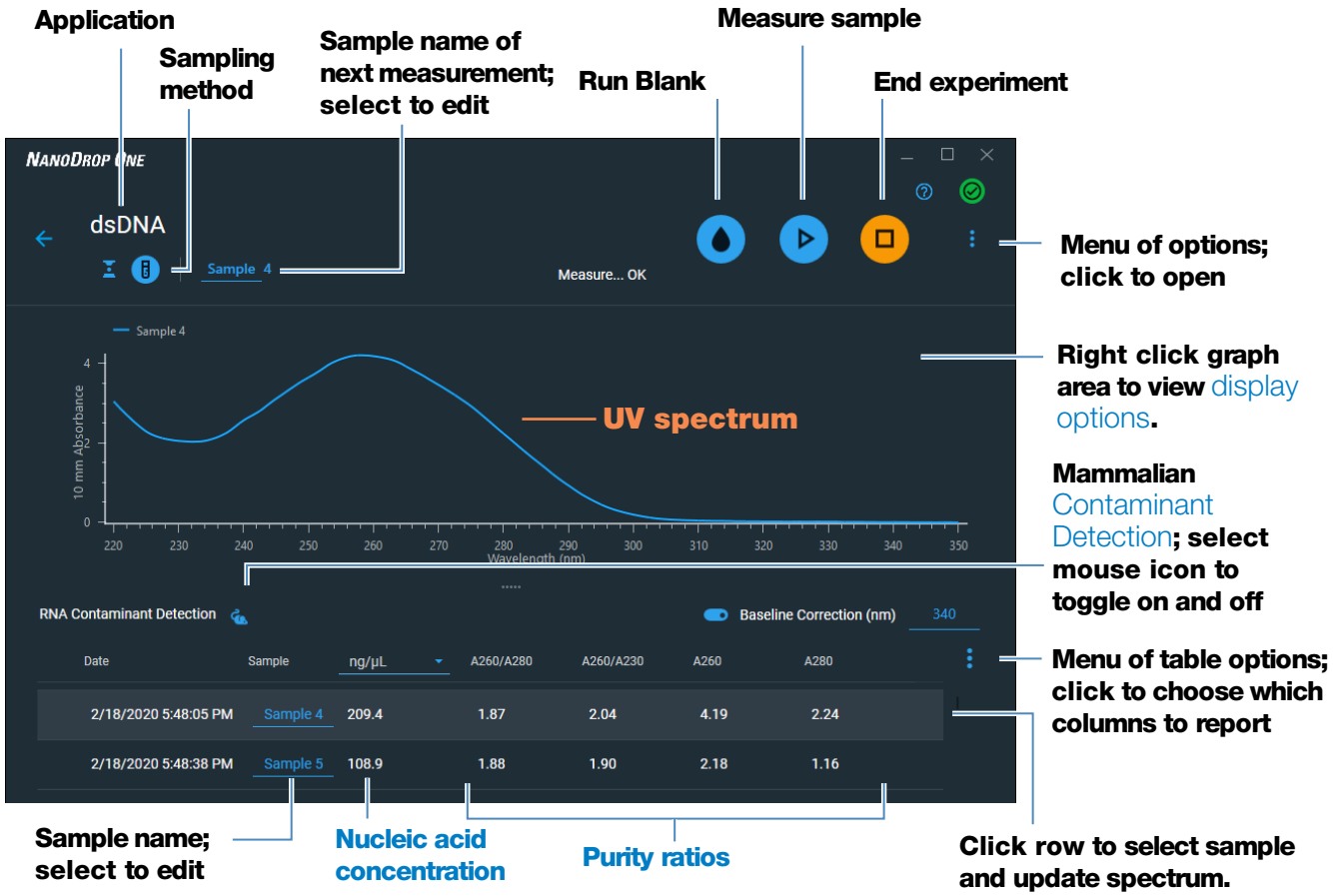
Related Topics

- 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

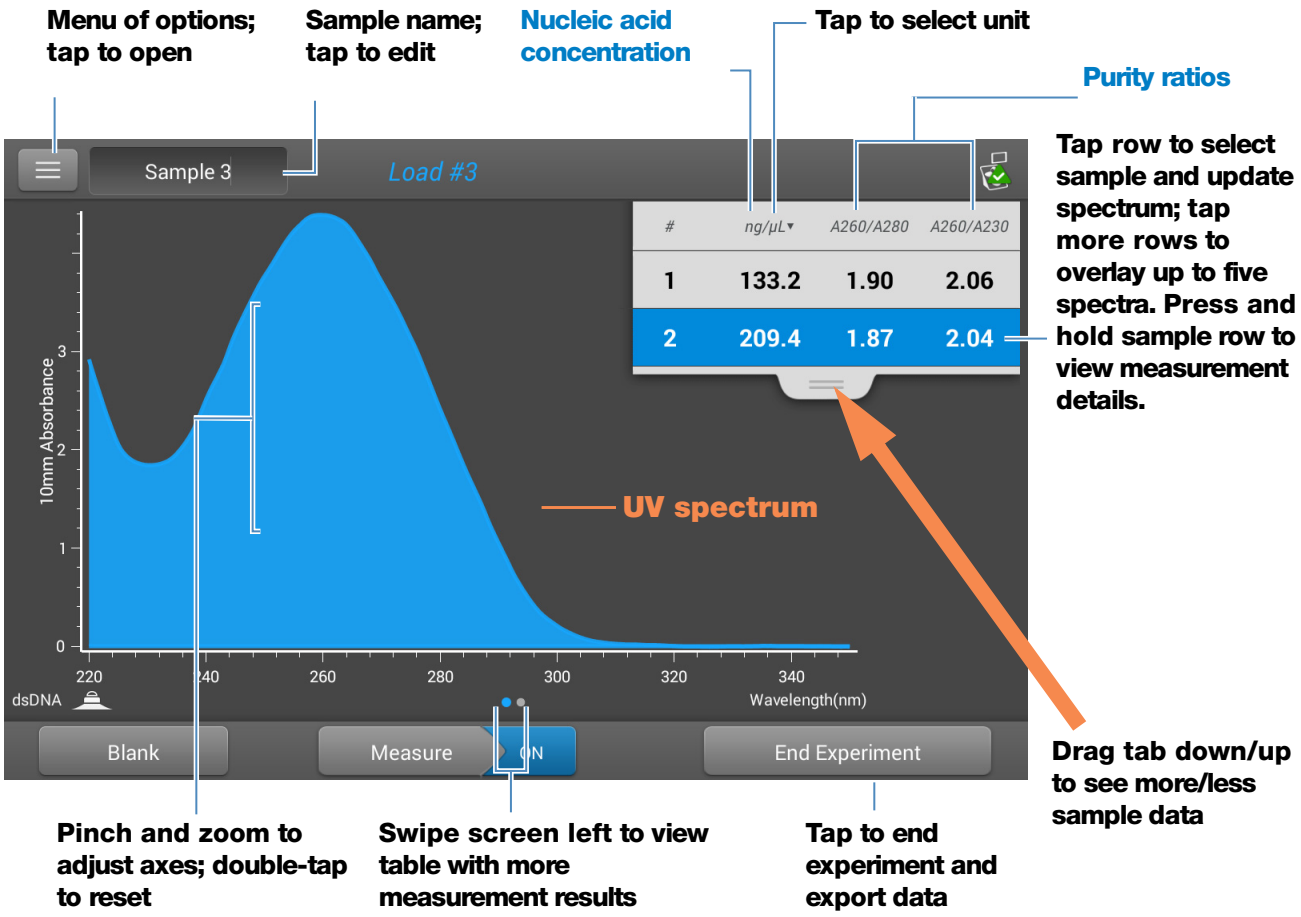
For each measured sample, the dsDNA, ssDNA and RNA applications show the UV absorbance spectrum and a summary of the results. Below is an example of the measurement screen of the PC control software:



Measurement screen of PC Control software

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

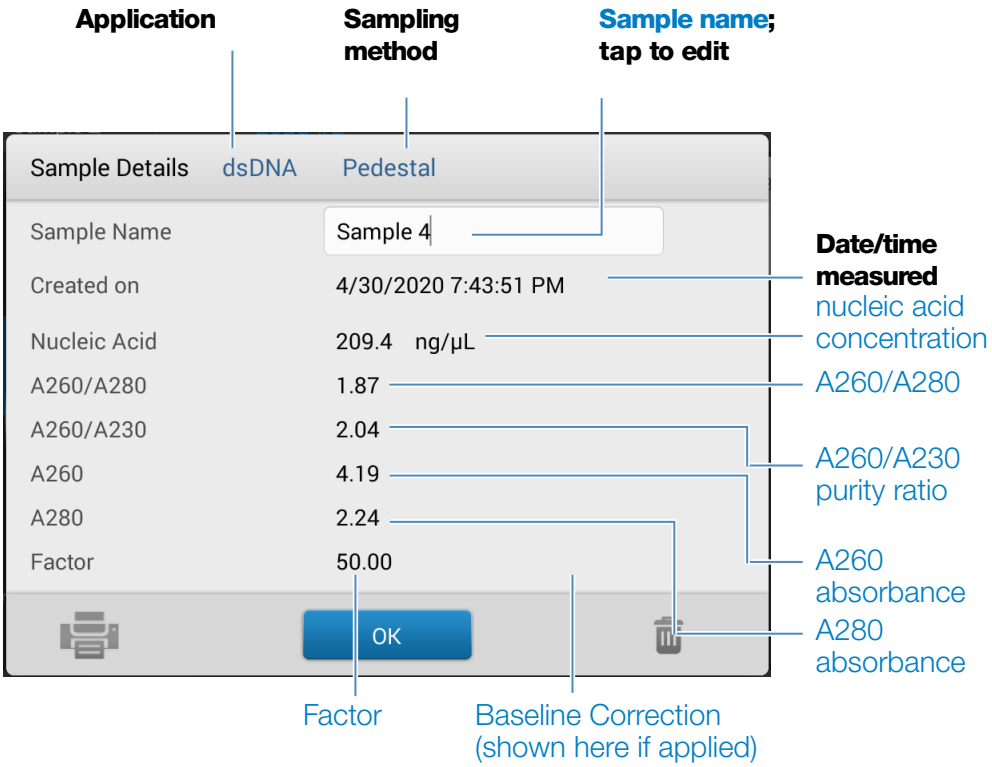
The following is an example of the measurement screen of the local control:



Measurement screen of NanoDrop One local control software

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, that is, pedestal or cuvette)
- sample name
- created on (date sample measurement was taken)
- nucleic acid concentration
- A260/A280
- A260/A230
- A260
- A280
- factor
- baseline correction

Settings 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.

Calculations for Nucleic Acid Measurements

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."

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)

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.

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.

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

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).

- **Factor**. Used in conjunction with Beer's Law to calculate sample concentration

- **Contaminant** - If a contaminant was identified by the Acclaro software, the contaminant will be displayed in this column.

- **A260 absorbance**.

- **A280 absorbance**.

- **Baseline correction**.

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.

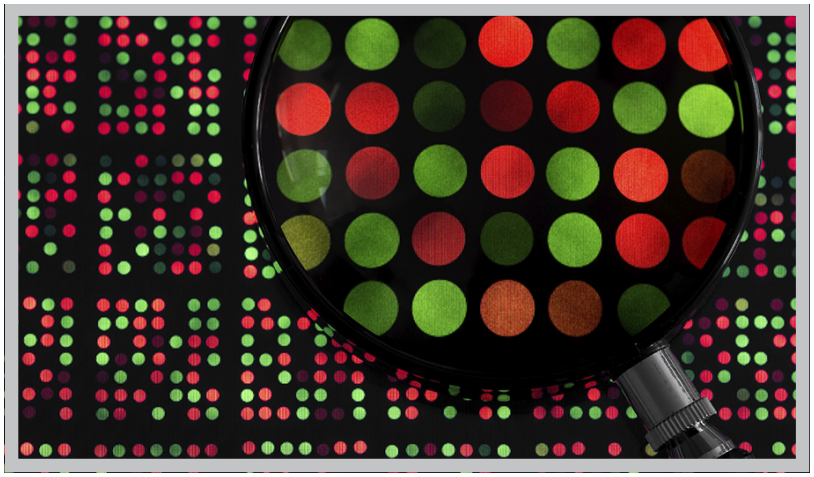
Measure Microarray Samples

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

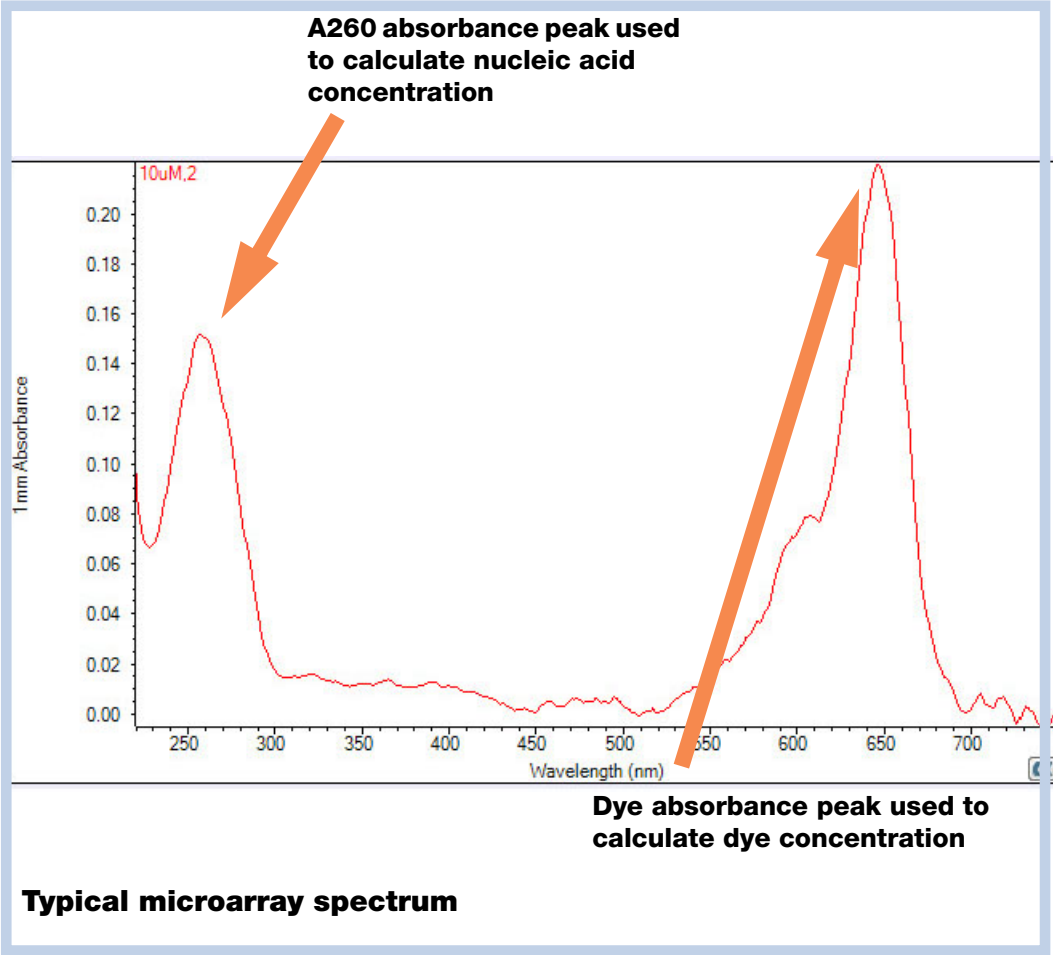
1. From the Home screen, select the **Nucleic Acids** tab and select **Microarray**.
2. Specify the sample type and factor and the type of dye(s) used.

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

   Tip: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements.)
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 select **Measure**.
   - Cuvette: Select **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, select **End Experiment**.
9. Lift the arm and clean both pedestals with a new wipe, or remove the sample cuvette.



Typical microarray spectrum

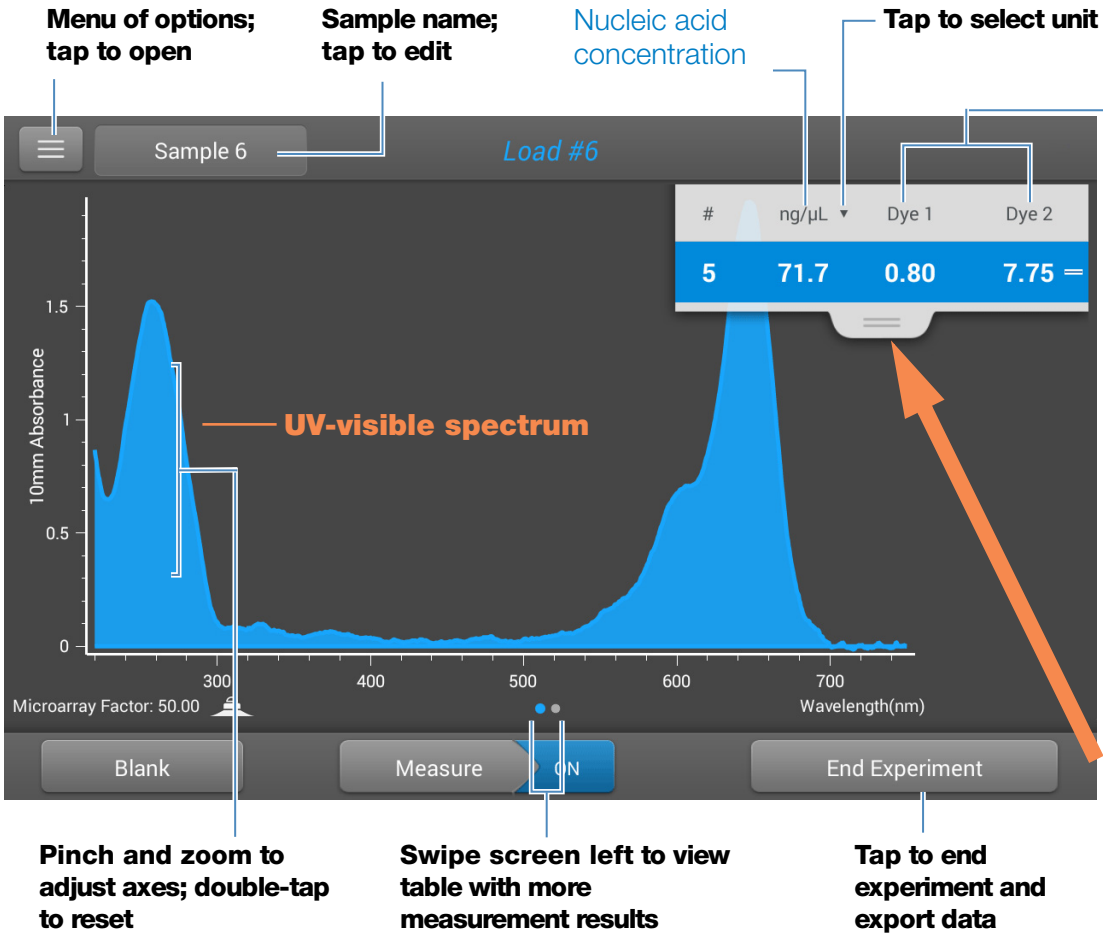
Related Topics

- 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 (Local Control)

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 850 nm (absorbance value at 850 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.

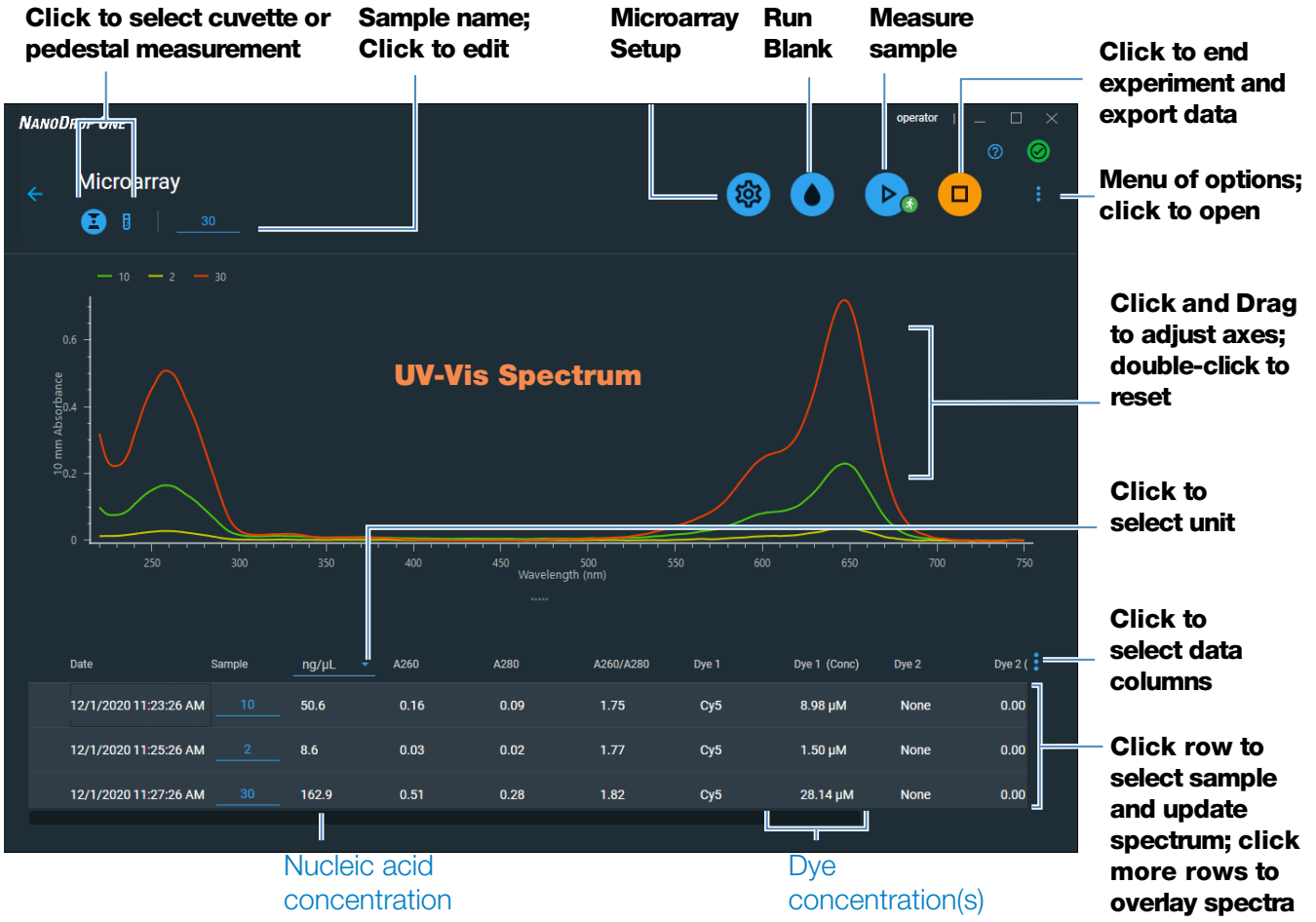
Microarray reported values

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

Microarray measurement screen (PC Control)

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 850 nm (absorbance value at 850 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.

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 Editor.

Setting	Available Options	Description
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.

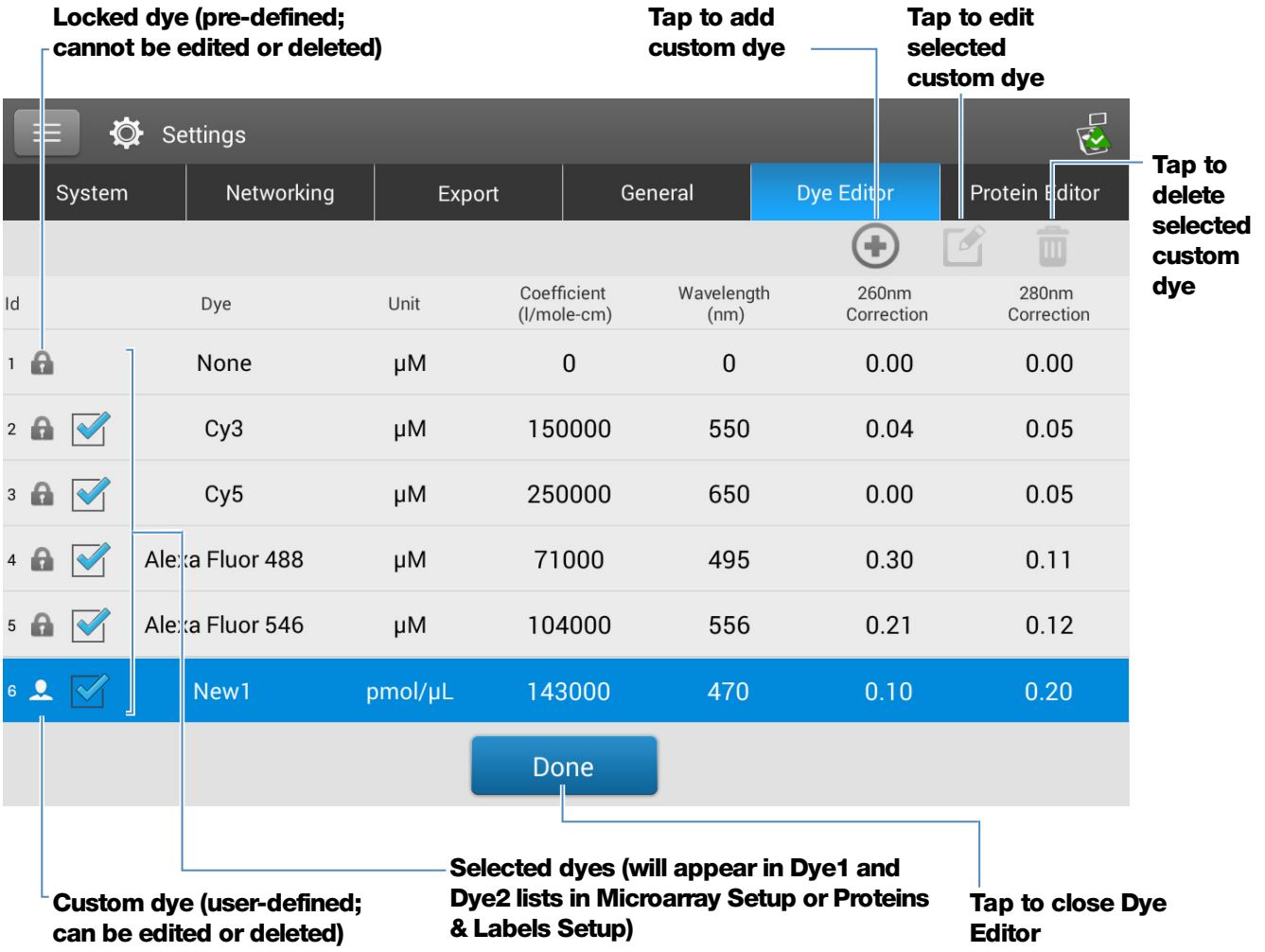
Dye/chromophore editor

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, select Settings **> Dye Editor**
- from the Microarray or Proteins & Labels measurement screen, tap > **Settings > Dye Editor**

Dye Editor

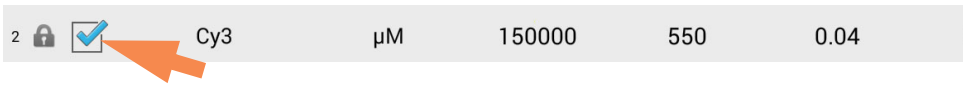


These operations are available from the Dye/Chromophore Editor:

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 350 nm and 850 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.

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.

Measured Values

A260 absorbance

Note: The absorbance value at 850 nm is subtracted from all wavelengths in the spectrum. As a result, the absorbance at 850 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 850-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

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

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 850 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

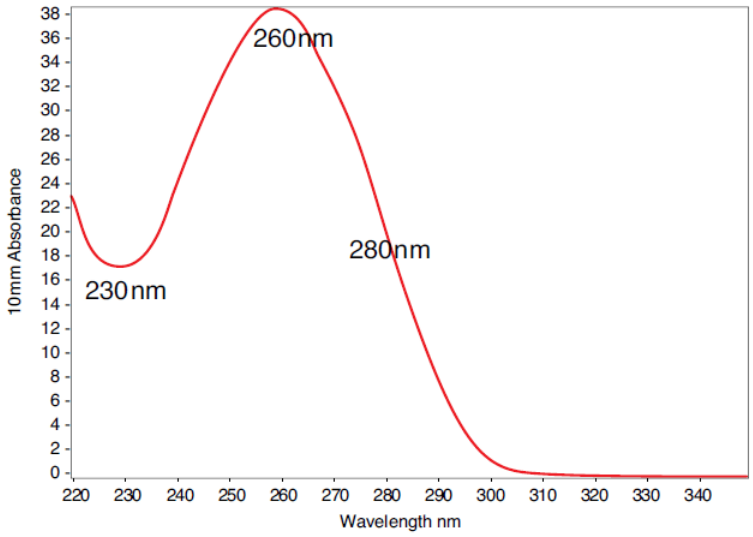
1. From the Home screen, select the **Nucleic Acids** tab and select **Custom Factor**.

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

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

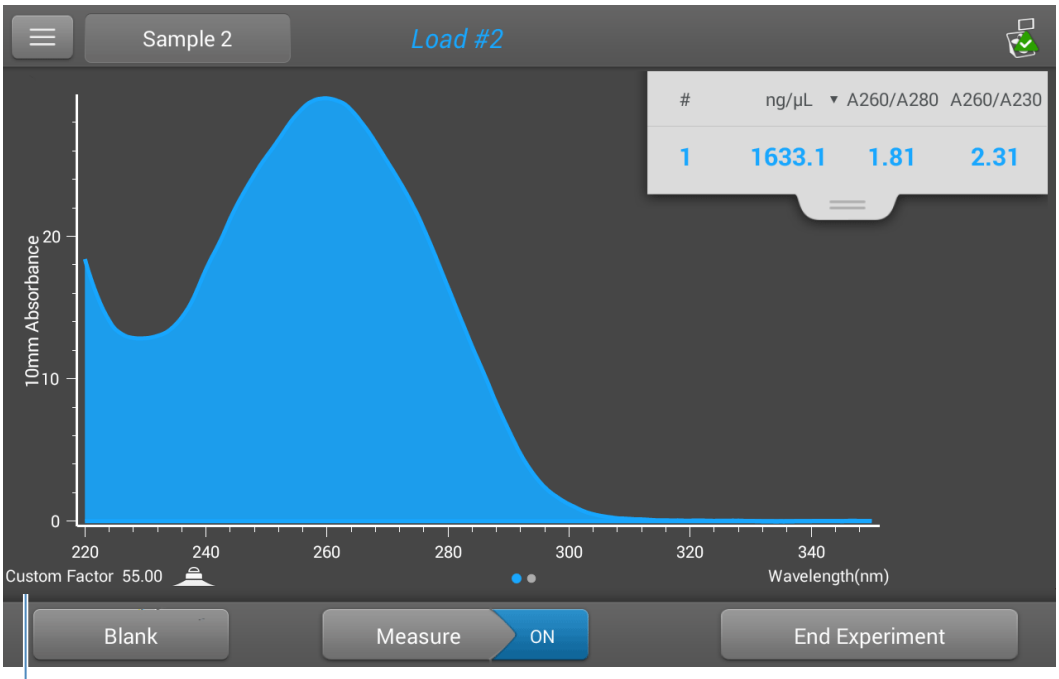
Related Topics

- 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, from the local control, tap ☰ > **Custom Factor Setup.**

When using the PC Control software, from the Custom Factor measurement screen, select the settings icon  to view the **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 = 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. NOTE: Baseline correction is selected from the measurement screen of the PC control software and is not shown in the Custom Factor Setup.

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

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Measure Oligo DNA or Oligo RNA

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

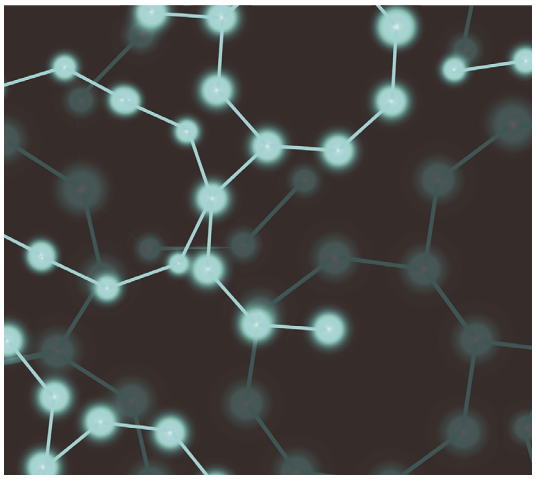
Measure Oligo DNA or RNA

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.

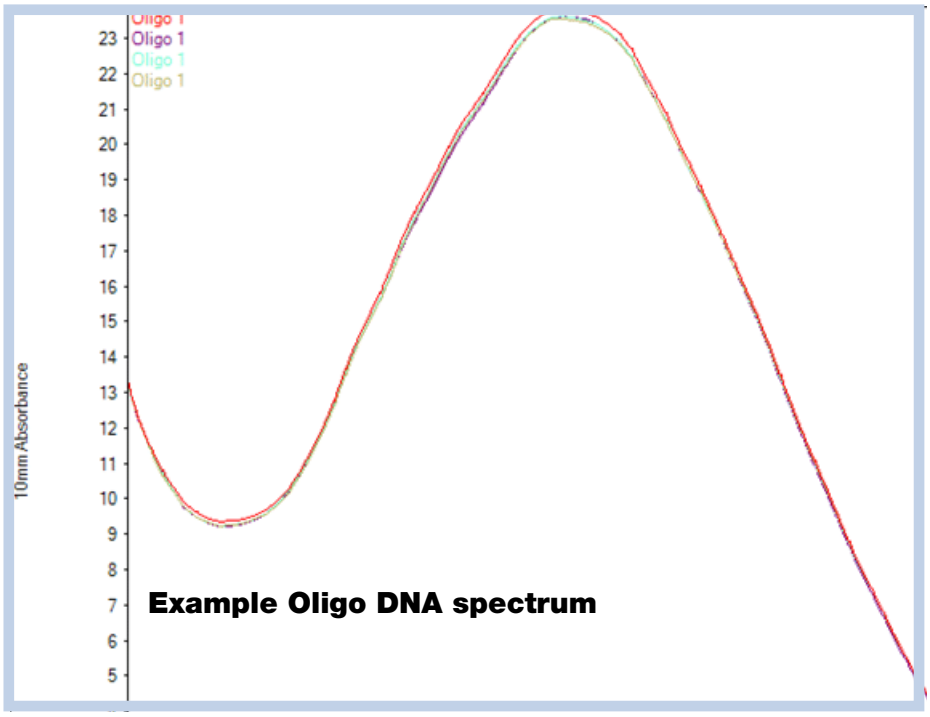
To measure an oligonucleotide sample

1. From the Home screen, select the **Nucleic Acids** tab and select 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.



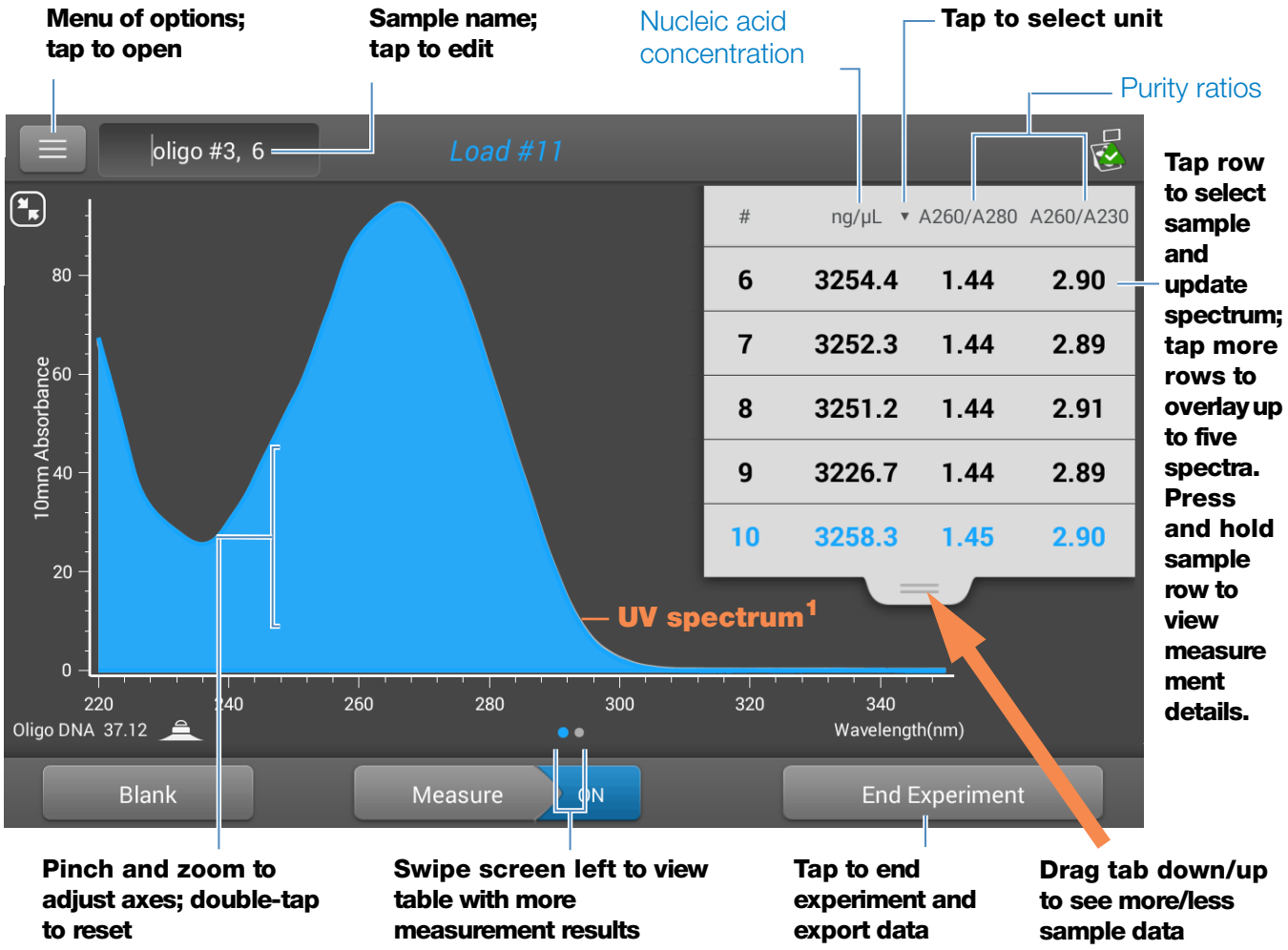
Related Topics

- 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 (Local Control)

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

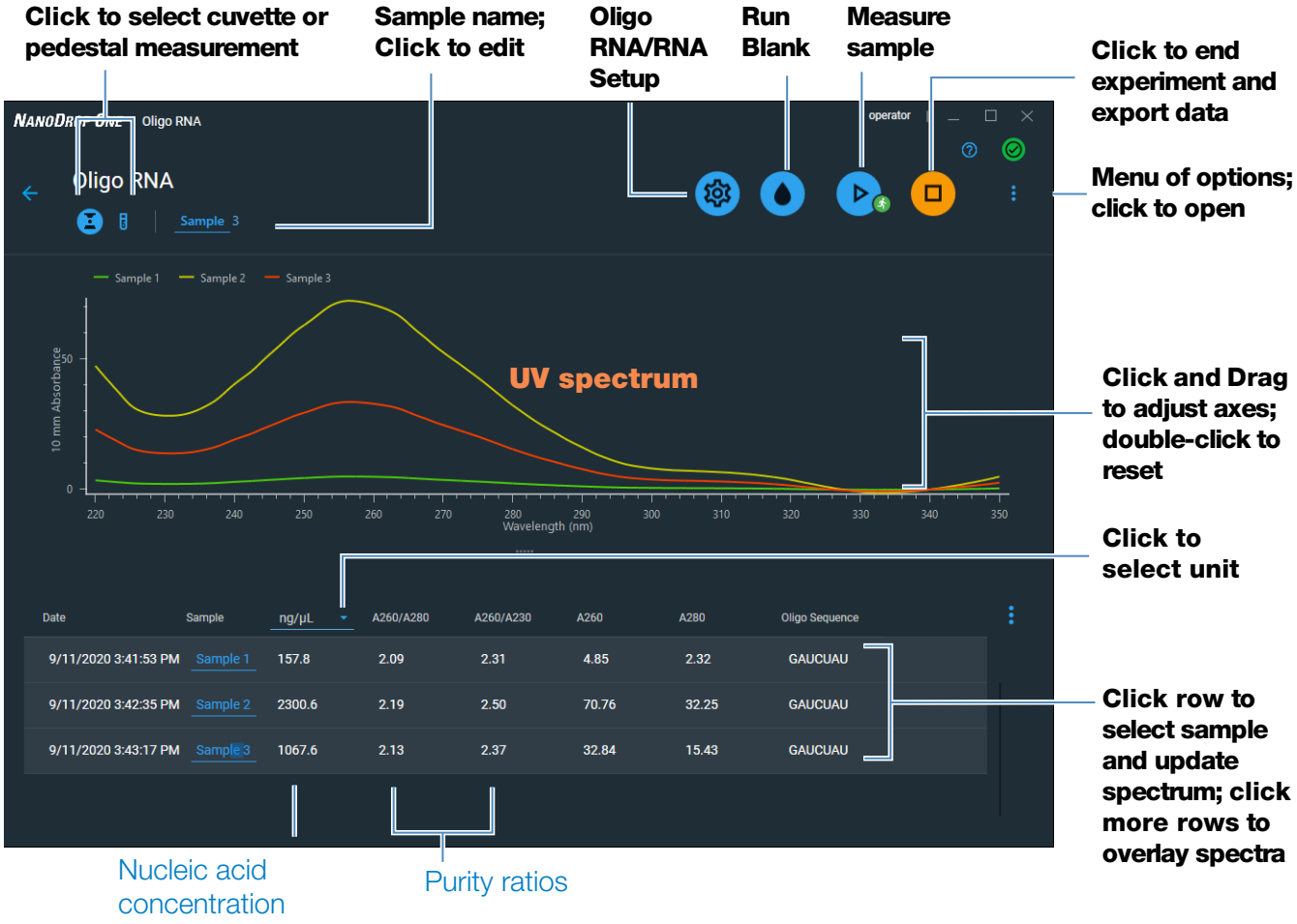
The initial screen that appears after each measurement (see previous image) shows a summary of the reported values. To view all reported values, press and hold the sample row. Here is an example:

- 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.

Oligo DNA and RNA measurement screen (PC Control)

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



Related Topics

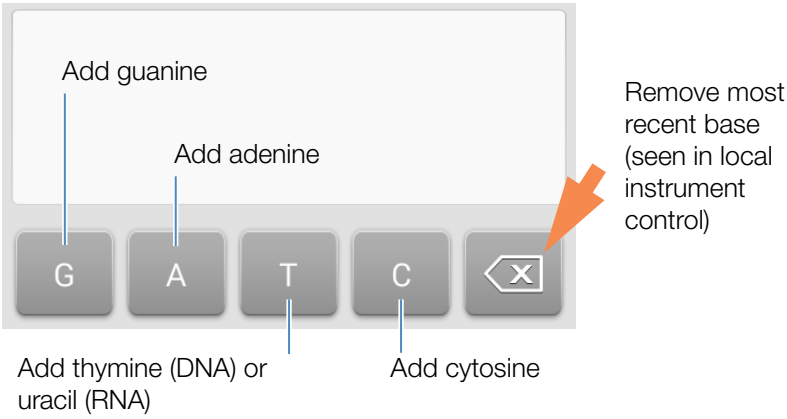
- Basic Instrument Operations
- Oligo Calculations

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.

In local instrument control, from the Oligo measurement screen, tap ☰ > Oligo DNA Setup **Oligo DNA Setup** (or Oligo RNA Setup **Oligo RNA Setup**).

From the PC control software, from the Oligo DNA or RNA measurement screen, select the settings icon to view **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. Tap or click the corresponding keys:  From the PC control software, you can also enter base sequence using the keyboard, or by copy and pasting a sequence from another application.
		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: $f = 1/(\varepsilon_{260} * b)$ 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.

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
$\varepsilon_1 = \varepsilon_{\text{nearest neighbor}}$
$\varepsilon_2 = \varepsilon_{\text{individual bases}}$
$\varepsilon_3 = \varepsilon_{\text{modifications, such as fluorescent dyes}}$

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

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Protein Applications

Measure Protein A280

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

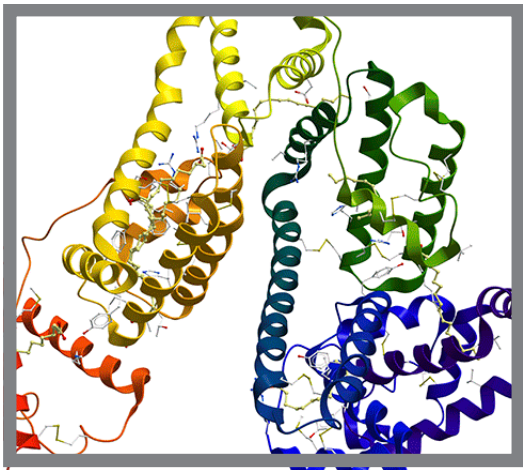
Measure A280 Proteins

Reported Results

Settings

Detection Limits

Calculations



Measure Protein Concentration at A280

Use the Protein A280 application to quantify purified protein samples 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.

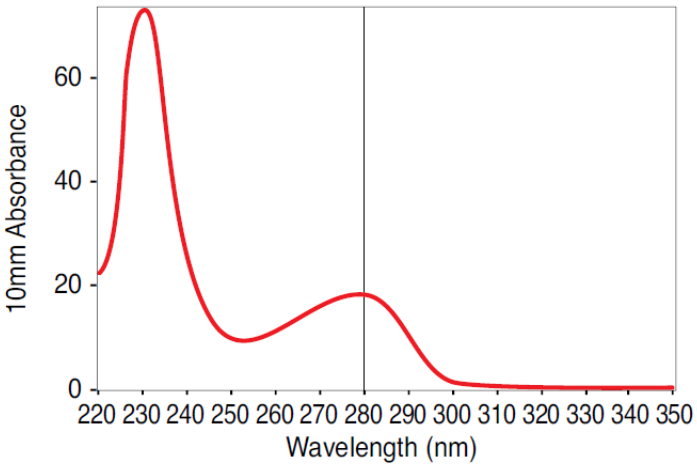
To measure a Protein A280 sample

1. From the Home screen, select the **Proteins** tab and select **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.

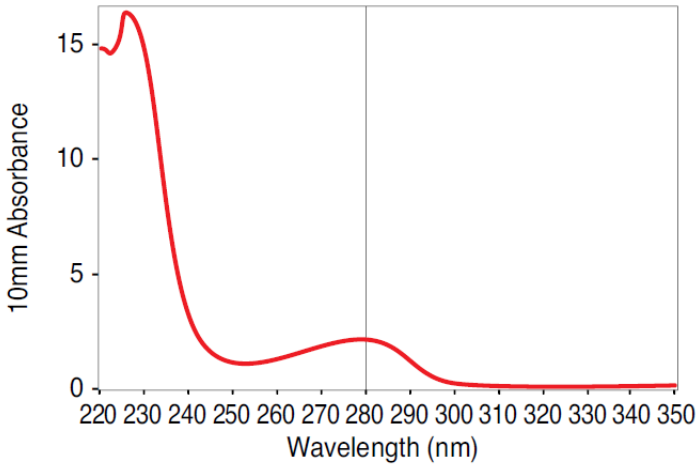
   Tip: If using a cuvette, make sure to align the cuvette light path with the instrument light path.
4. Tap **Blank** and wait for the measurement to complete.

   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.

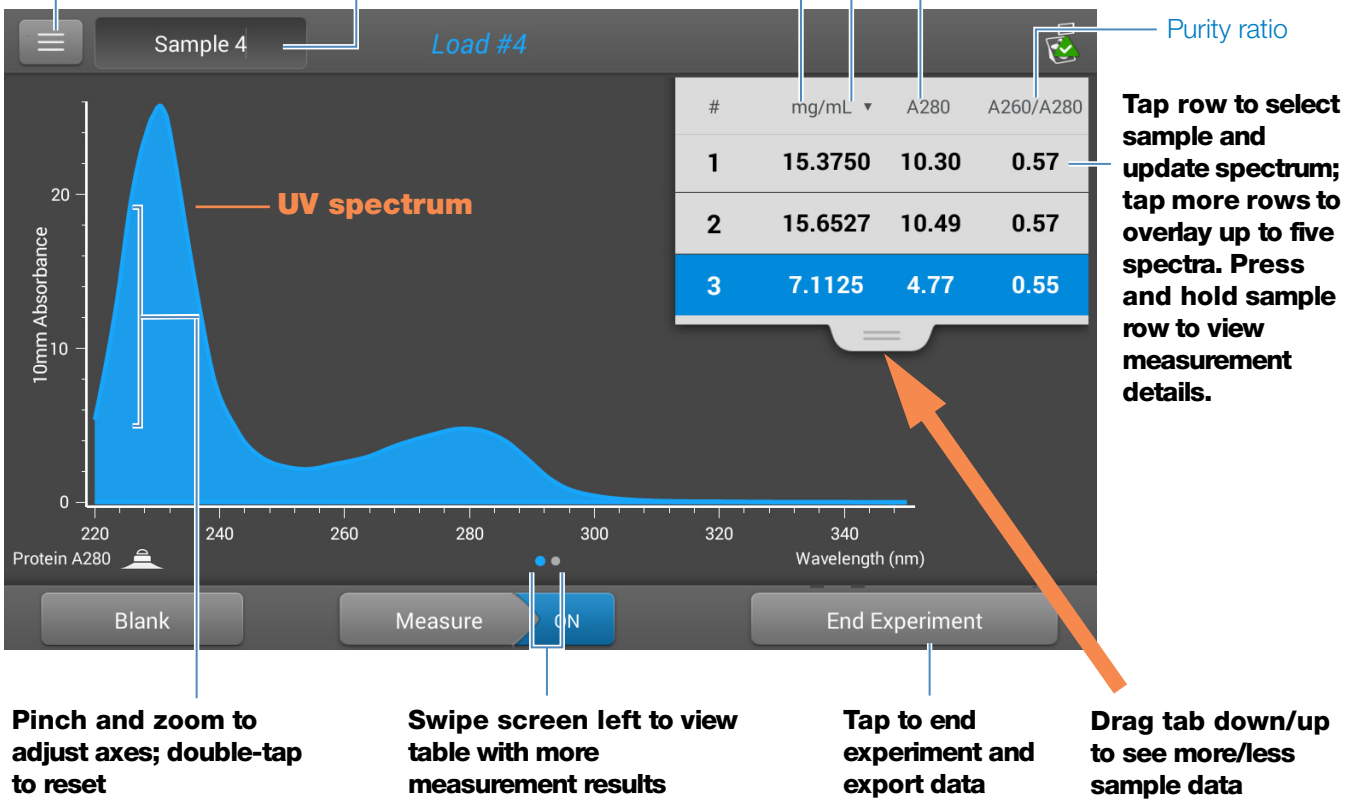
Related Topics

- 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 (Local Control)

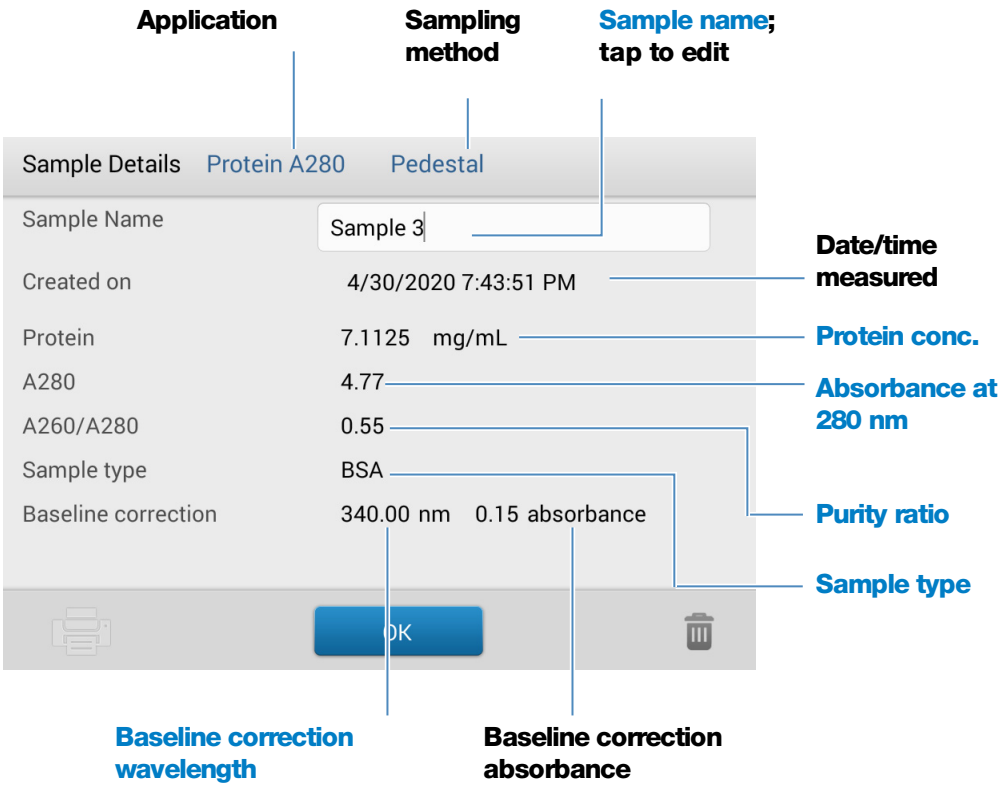
For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example of the Local control screen:



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

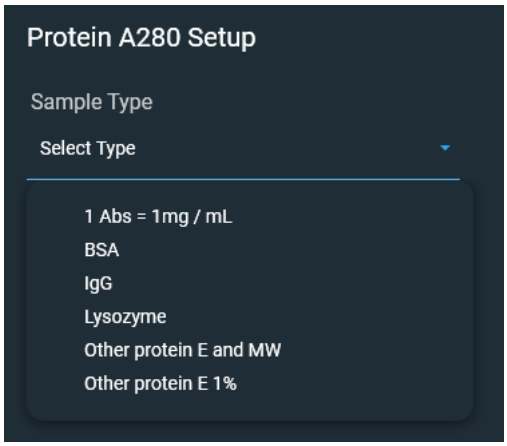
Settings for Protein A280 Measurements

To show the Protein A280 settings, in local instrument control, from the Protein A280 measurement screen, tap ☰ > **Protein A280 Setup.**

From the PC control software, from the Protein A280 measurement screen, select the settings icon ⚙ to view **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 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.

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., ε1% = 10.
	BSA	6.7	Calculates BSA (Bovine Serum Albumin) protein concentration using mass extinction coefficient (ε) of 6.7 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 $M^{-1}cm^{-1}$.

Setting	Available Options	Mass Ext. Coefficient (L/gm-cm)	Description
	IgG	13.7	Suitable for most mammalian antibodies (i.e., immunoglobulin G or IgG). Calculates protein concentration using mass extinction coefficient (ε) 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 (ε) 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 $M^{-1}cm^{-1}$.
	Other protein (ε + MW)	User entered molar extinction coefficient and molecular weight	Assumes protein has known molar extinction coefficient (ε) and molecular weight (MW), where: $(\varepsilon_{molar})*10=(\varepsilon_{percent})*(MW_{protein})$ Enter MW in kiloDaltons (kDa) and molar extinction coefficient (ε) in $M^{-1}cm^{-1}$ divided by 1000 (i.e., ε/1000). For example, for protein with molar extinction coefficient of 210,000 $M^{-1}cm^{-1}$, enter 210.
	Other protein (ε1%)	User entered mass extinction coefficient	Assumes protein has known mass extinction coefficient (ε). Enter mass extinction coefficient in L/gm-cm for 10 mg/mL (ε1%) protein solution.

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

Setting	Available Options	Mass Ext. Coefficient (L/gm-cm)	Description
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.

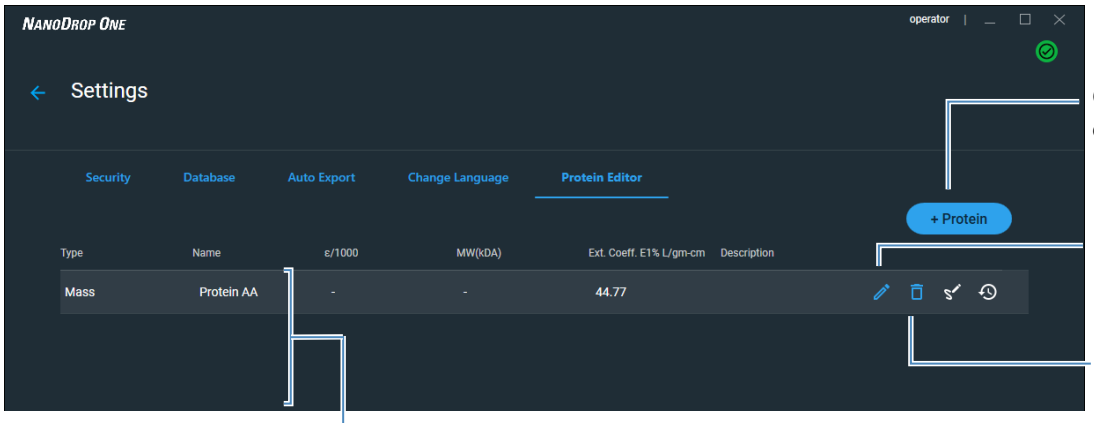
Protein editor

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:

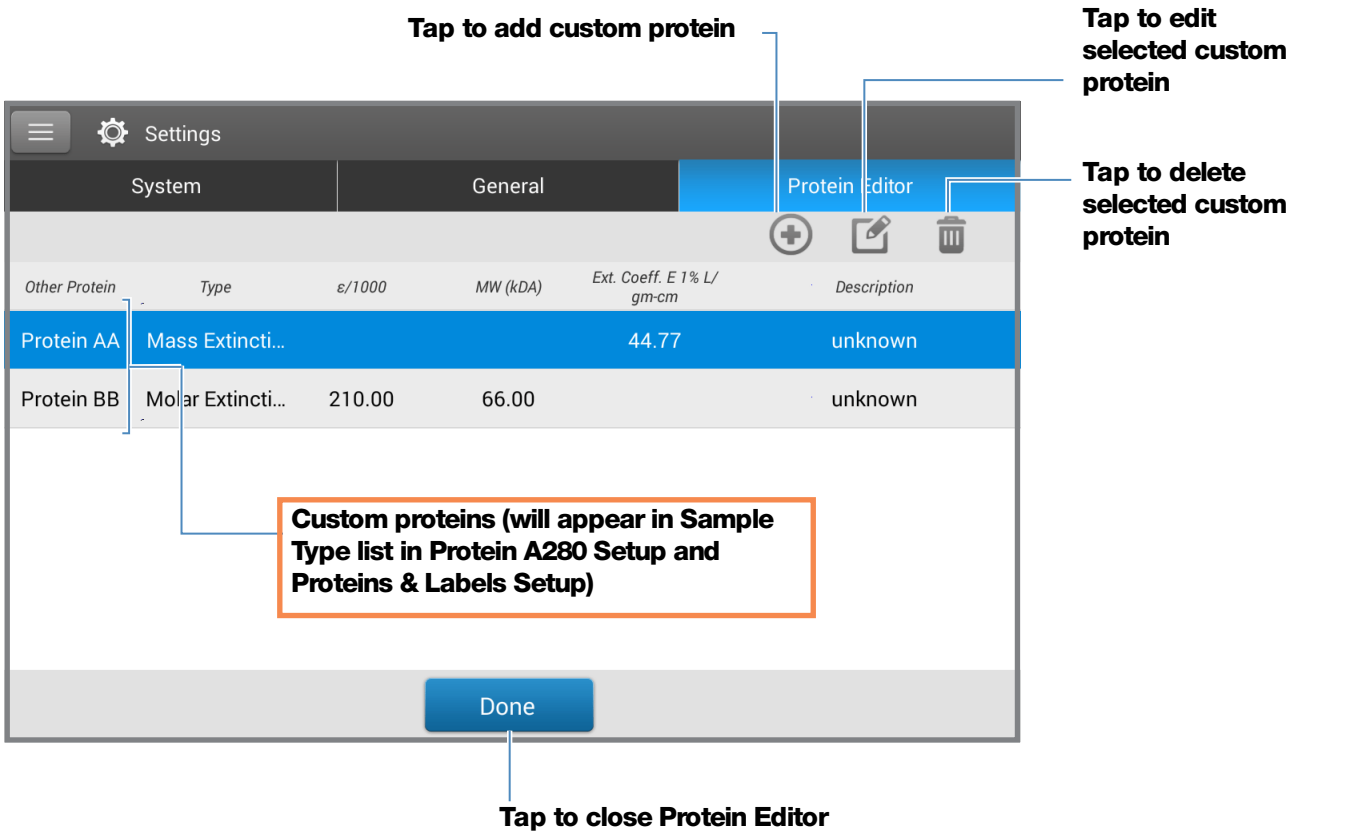
- From the PC control software Home screen, select Settings **Settings > Protein Editor**.

PC Control software Protein Editor



- From the local control Home screen, tap > **Protein Editor**. Alternatively, from the Protein A280 or Proteins & Labels measurement screen, tap > **Settings > Protein Editor**.

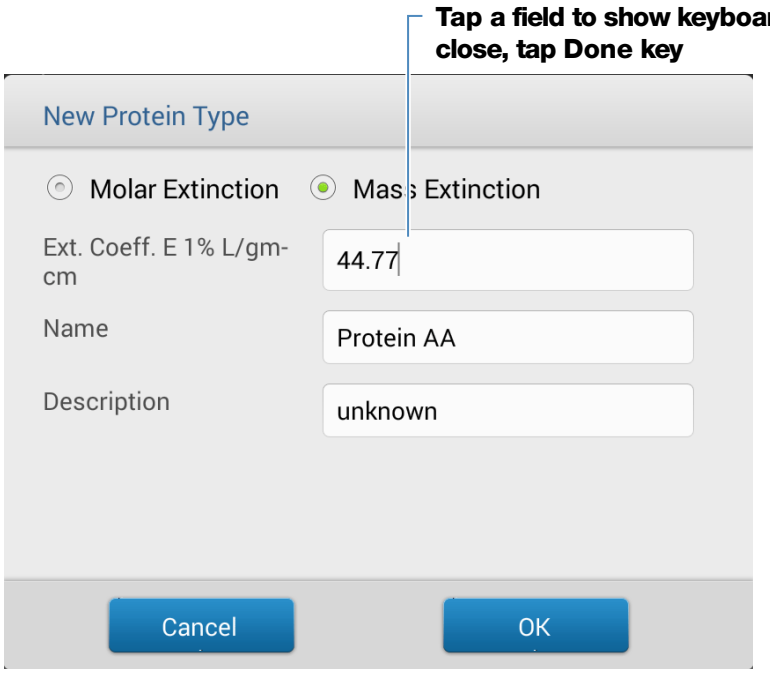
Local Control Protein Editor



These operations are available from the Protein Editor:

Add custom protein

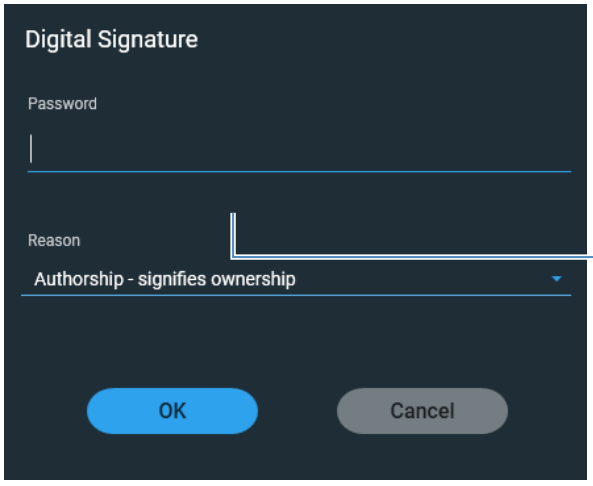
1. In Protein Editor, select or + Protein to show the **New Protein Type** box.
2. Enter a unique **Name** for the new protein (from local control, tap field to display keyboard, tap **Done** key to close keyboard).
3. Enter a **Description** for the new protein.
4. Specify whether to enter **Molar Extinction** 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 (Ɛ1%) protein solution.



- if **Molar Extinction** is selected,
  - enter molar extinction coefficient (ε) in $M^{-1}cm^{-1}$ divided by 1000 (that is, ε/1000). For example, for protein with molar extinction coefficient of 210,000 $M^{-1}cm^{-1}$, enter 210.
  - Enter molecular weight (MW) in kiloDaltons (kDa)

5. From local control, tap **OK** to close the **New Protein Type** box. From PC control software, select **Save**.

6. Enter your password to sign the changes if prompted.



The new custom protein appears in the **Type** list in Protein A280 Setup and Proteins & Labels Setup.

Edit custom protein

1. In Protein Editor, tap to select custom protein
2. Tap  to show the **Edit Protein Type** box
3. Edit any entries or settings
4. Tap **OK**

Delete custom protein

1. In Protein Editor, tap to select a custom protein to delete
2. Tap 

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

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 6.7 for a 1% (10 mg/mL) solution, the equation looks like this:

(550 / 6.7) * 10 = 824.6 (or ~825)

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 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.

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, 6.7 L/gm-cm)
- **IgG** (any mammalian antibody, 13.7 L/gm-cm)
- **Lysozyme** (egg white lysozyme, 26.4 L/gm-cm)
- **Other protein** (ε + MW), user-specified molar ext. coefficient
- **Other protein** (ε1%), 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.

Published Extinction Coefficients

Published extinction coefficients for proteins may be reported as:

- wavelength-dependent molar absorptivity (or extinction) coefficient (ε) with units of $M^{-1}cm^{-1}$
- percent solution extinction coefficient (ε1%) 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.

The equation at the right shows the relationship between molar extinction coefficient (ε_{molar}) and percent extinction coefficient (ε1%).

Conversions Between ε_{molar} and ε1%

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

Example: To determine percent solution extinction coefficient (ε1%) 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:

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

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

$$\varepsilon 1\% = 6.6\ g/100\ mL$$

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

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

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:

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

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

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

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.

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 Protein A205

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

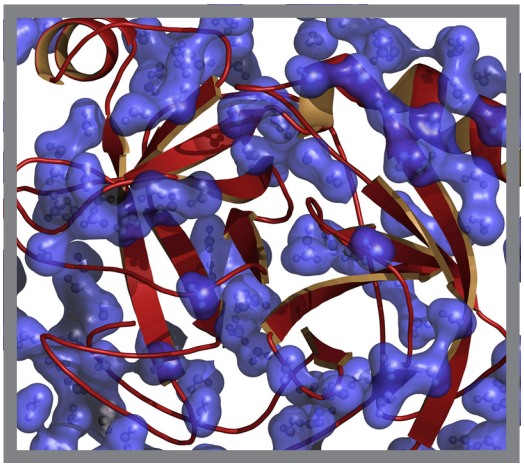
Measure A205 Proteins

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, from the **Proteins** tab, select **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.

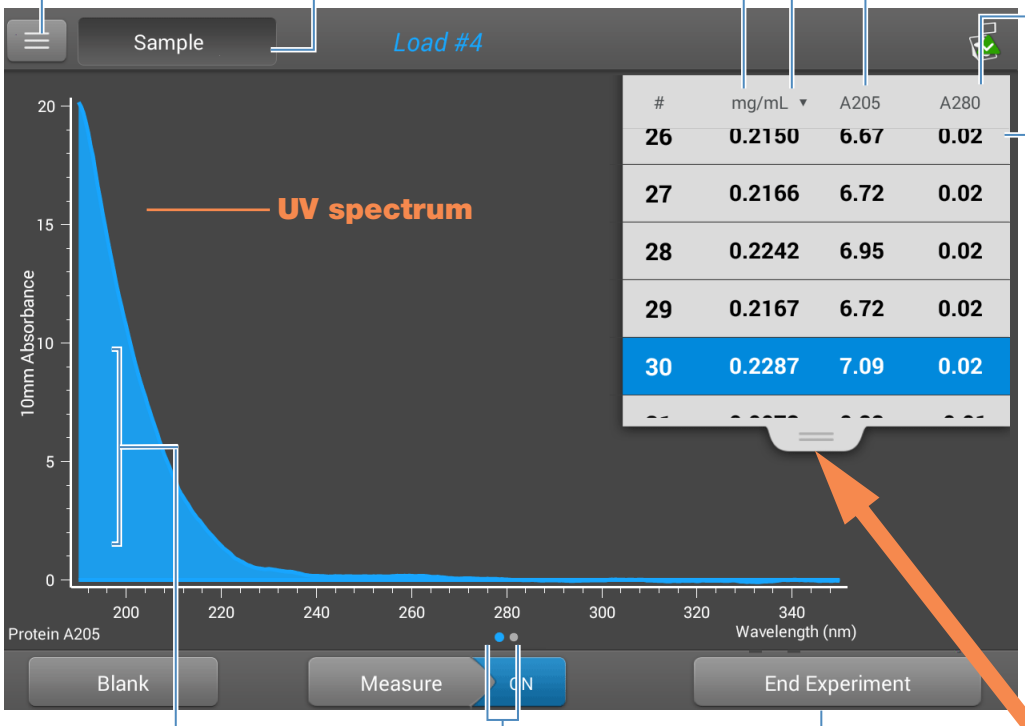
   Tip: If Auto-Blank is On, the blank measurement starts automatically after you lower the arm. (This option is not available for cuvette measurements.)
5. Lift the arm and clean both pedestals with a new laboratory wipe, or remove the blanking cuvette.
6. Pipette 2 µL sample solution onto the pedestal 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.

Protein A205 Reported Results

Protein A205 measurement screen (Local Control)

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



Menu of options; tap to open

Sample name; tap to edit

Protein concentration

Tap to select unit

Absorbance at 205 nm

Absorbance at 280 nm

Tap row to select sample and update spectrum; tap more rows to overlay up to five spectra. Press and hold sample row to view measurement details.

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

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

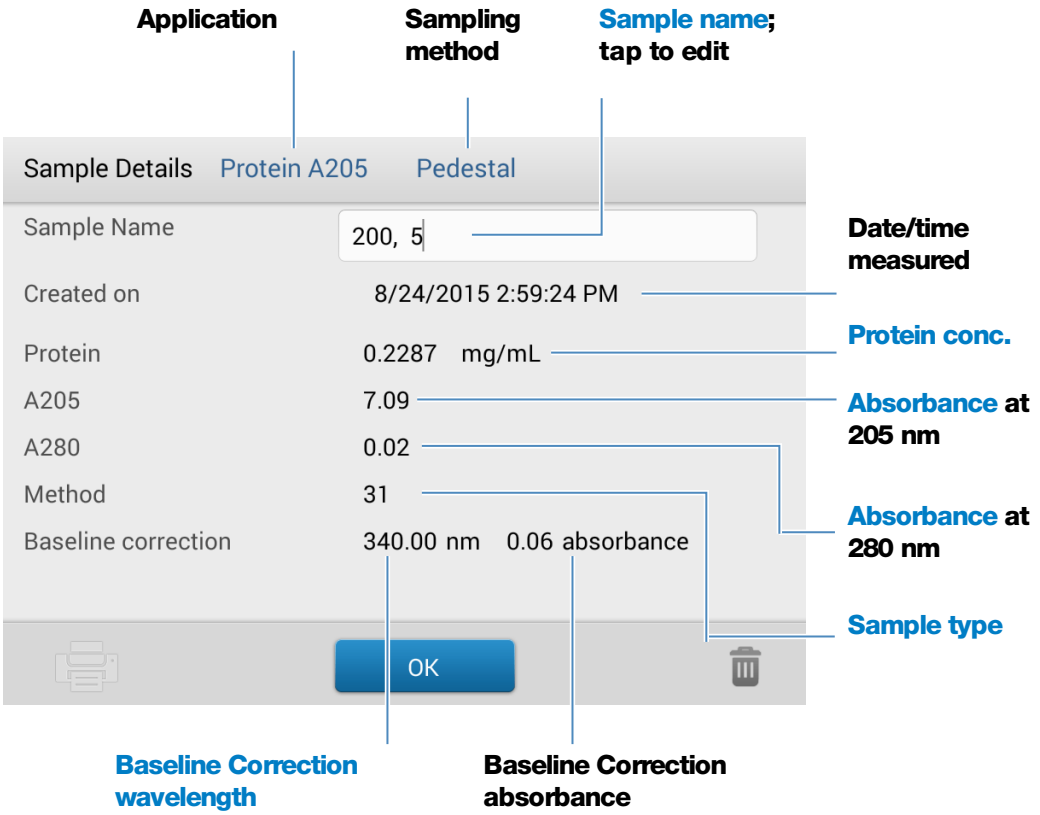
Swipe screen left to view table with more measurement results

Tap to end experiment and export data

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

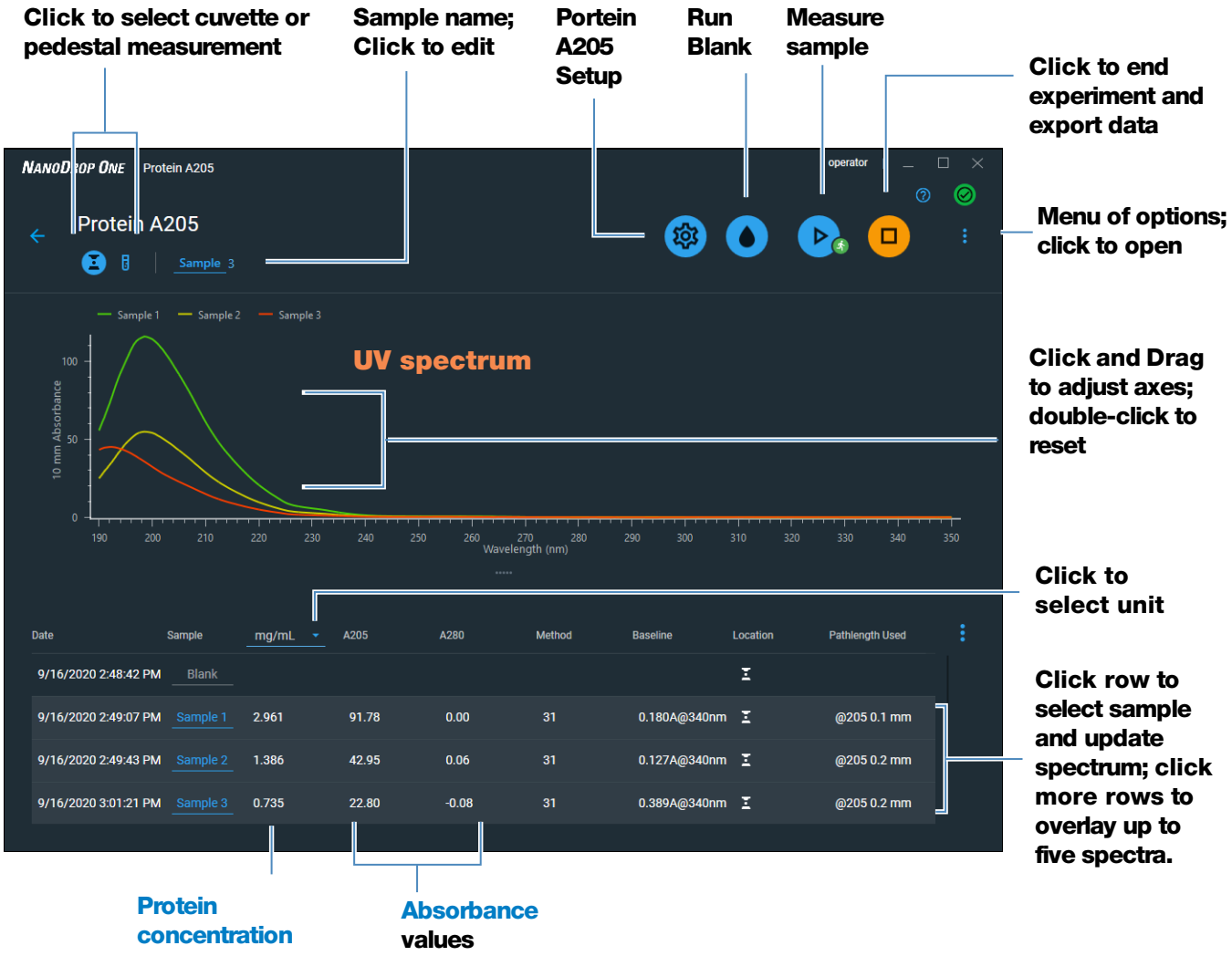
Protein A205 reported values

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:


Application
Sampling method
Sample name; tap to edit
Sample Details
Protein A205
Pedestal
Sample Name
200, 5
Created on
8/24/2015 2:59:24 PM
Protein
0.2287 mg/mL
A205
7.09
A280
0.02
Method
31
Baseline correction
340.00 nm
0.06 absorbance
OK
Date/time measured
Protein conc.
Absorbance at 205 nm
Absorbance at 280 nm
Sample type
Baseline Correction wavelength
Baseline Correction absorbance

Protein A205 measurement screen (PC Control)

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



Related Topics

- Basic Instrument Operations
- Protein A205 Calculations

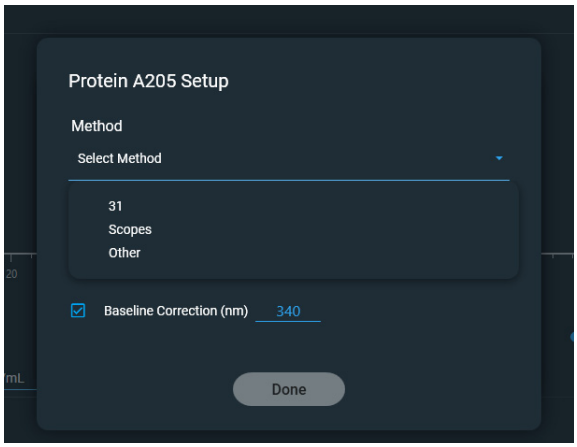
Settings for Protein A205 Measurements

To show the Protein A205 settings, in local instrument control, from the Protein A205 measurement screen, tap > **Protein A205 Setup.**

From the PC control software, from the Protein A205 measurement screen, select the settings icon to view **Protein A205 Setup**.

Protein A205 settings

The Protein A205 application provides a variety of method options for protein analysis.



Setting	Available Options	Mass Ext. Coefficient (L/gm-cm)	Description
Sample type	31	31	Assumes Ɛ0.1% (1 mg/mL) at 205 nm = 31
	Scopes	27 + 120 * (A280/A205)	Assumes Ɛ0.1% (1 mg/mL) at 205 nm = 27 + 120 * (A280/A205)

Setting	Available Options	Mass Ext. Coefficient (L/gm-cm)	Description
	Other protein (ε1%)	User entered mass extinction coefficient	Assumes protein has known mass extinction coefficient (ε). Enter mass extinction coefficient in L/gm-cm for 1 mg/mL (ε0.1%) 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.

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 ε1% (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 ε0.1% (1 mg/mL) at 205 nm = 31
- **Scopes**, assumes ε0.1% (1 mg/mL) at 205 nm = 27 + 120 * (A280/A205)
- **Other protein**, enter mass extinction coefficient in L/gm-cm for 1 mg/mL (ε0.1%) 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.

Measure Proteins and Labels

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

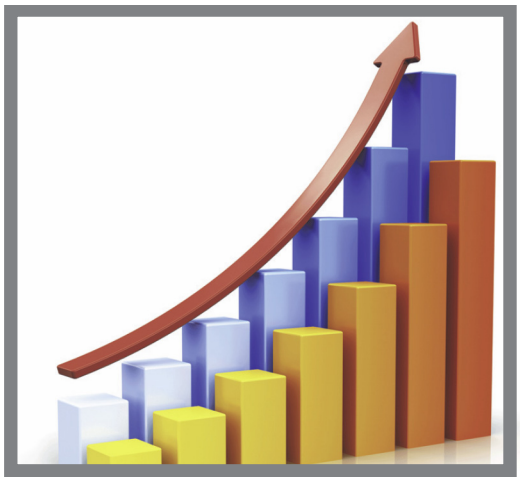
Measure Labeled Proteins

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 select **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:

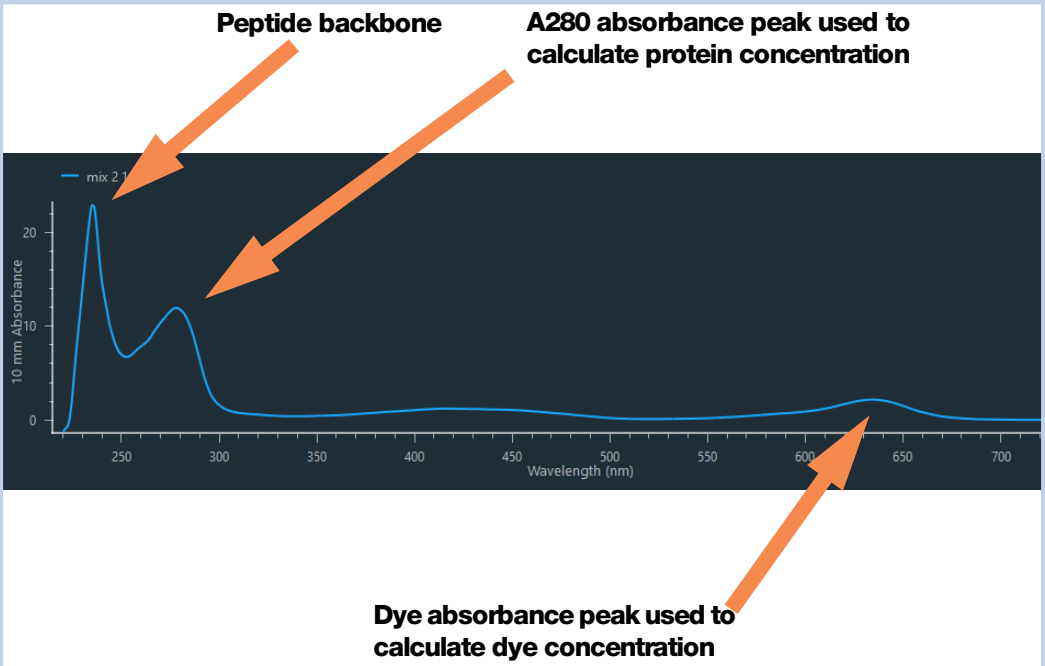
   – Pedestal: If Auto-Measure is On, lower arm; if Auto-Measure is off, lower arm and select **Measure**.

   – Cuvette: select **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, select **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

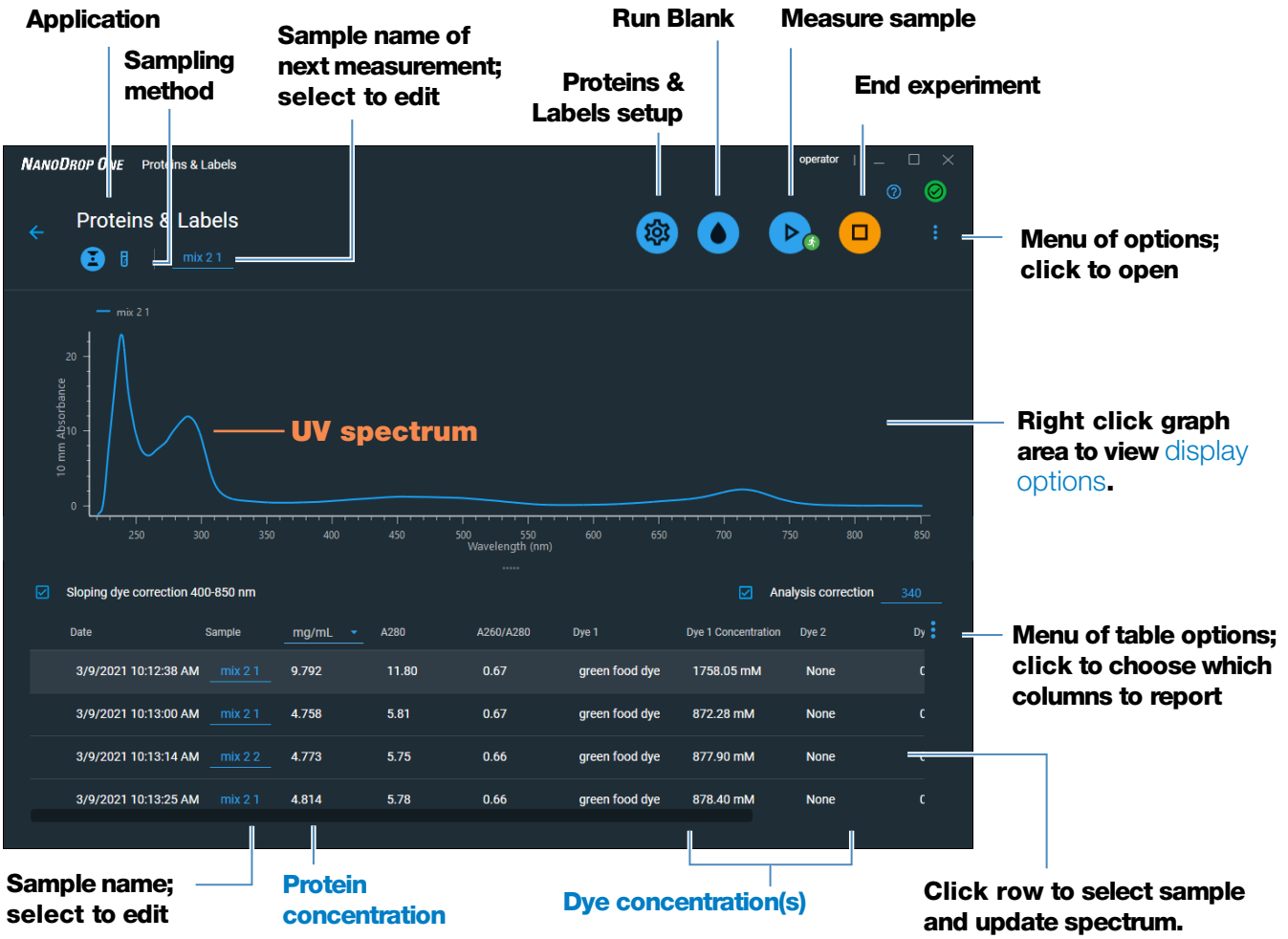
Related Topics

- 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

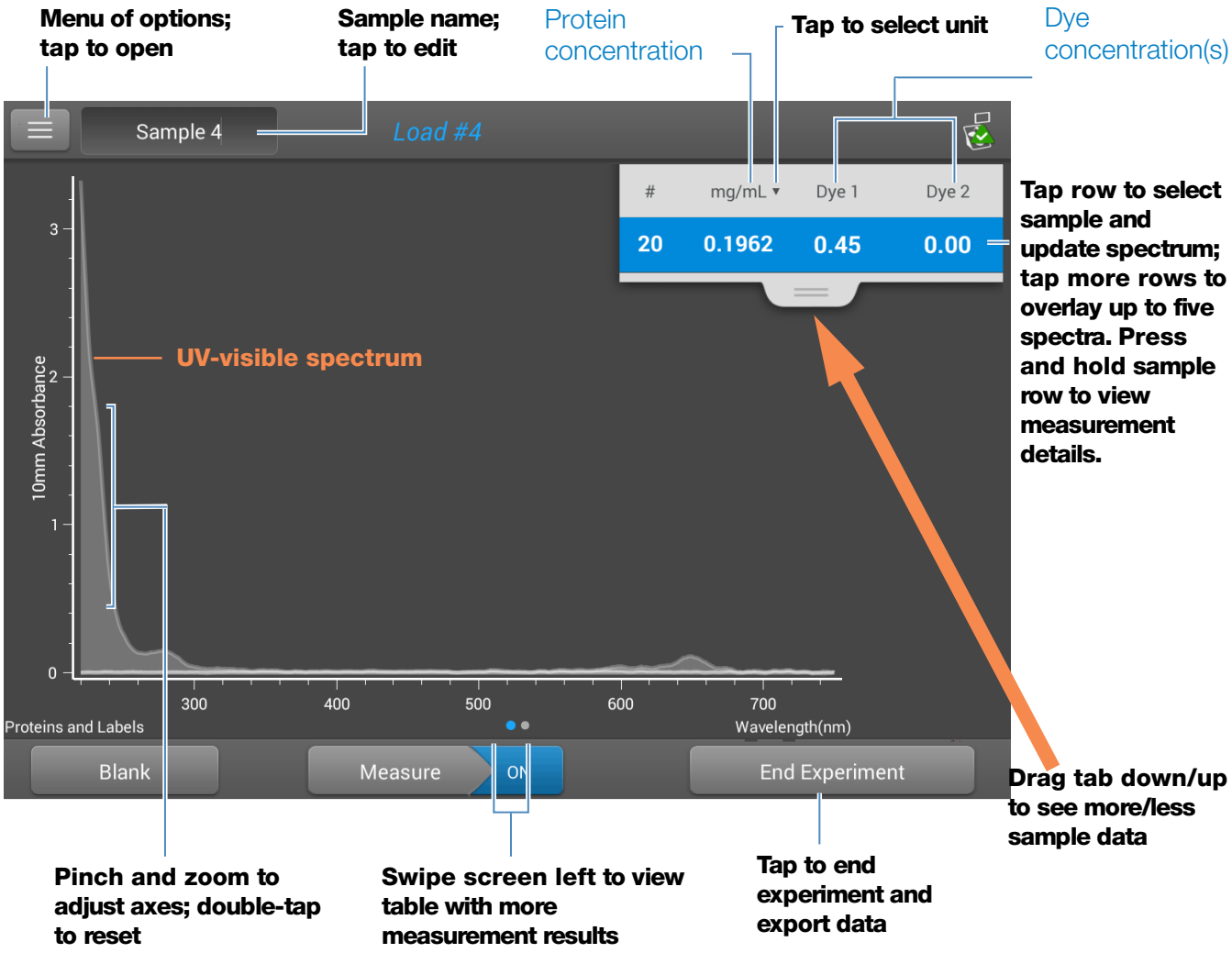
For each measured sample, this application shows the absorbance spectrum and a summary of the results. Below is an example of the measurement screen of the PC control software:



Measurement screen of PC Control software

Note

- A baseline correction is performed at 850 nm (absorbance value at 850 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.



Measurement screen of NanoDrop One local control

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. 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. **Tip**: Ideally, the extinction coefficient should be determined empirically using a solution of the study protein at a known concentration using the same buffer.
	BSA	6.7	
	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	

Setting	Available Options	Mass Ext. Coefficient (L/gm-cm)	Description
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 850 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 mg/mL 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 6.7 for a 1% (10 mg/mL) solution, the equation looks like this:

$$(550 / 6.7) * 10 = 824.6 \text{ (or } \sim 825)$$

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 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.

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, 6.7 L/gm-cm)
- **IgG** (any mammalian antibody, 13.7 L/gm-cm)
- **Lysozyme** (egg white lysozyme, 26.4 L/gm-cm)
- **Other protein** (ε + MW), user-specified molar ext. coefficient
- **Other protein** (ε1%), user-specified mass ext. coefficient

Note: See Sample Type for details.

Measured Values

A280 absorbance

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.

Note: The absorbance value at 850 nm is subtracted from all wavelengths in the spectrum. As a result, the absorbance at 850 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 850 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 850-corrected, normalized and analysis-corrected absorbance value at 280 nm is reported and used to calculate protein concentration.
- If a Dye is used, the 850-corrected, normalized, analysis-corrected and dye-corrected absorbance value at 280 nm is reported and used to calculate protein concentration.

Dye absorbance

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 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 850 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 BCA

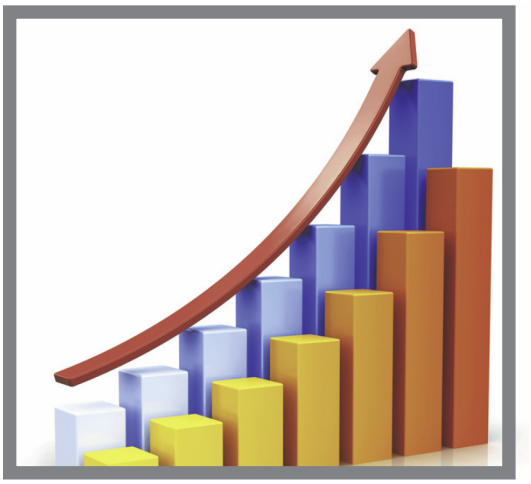
Measures total protein concentration of unpurified protein samples using a bicinchoninic acid colorimetric detection reagent.

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

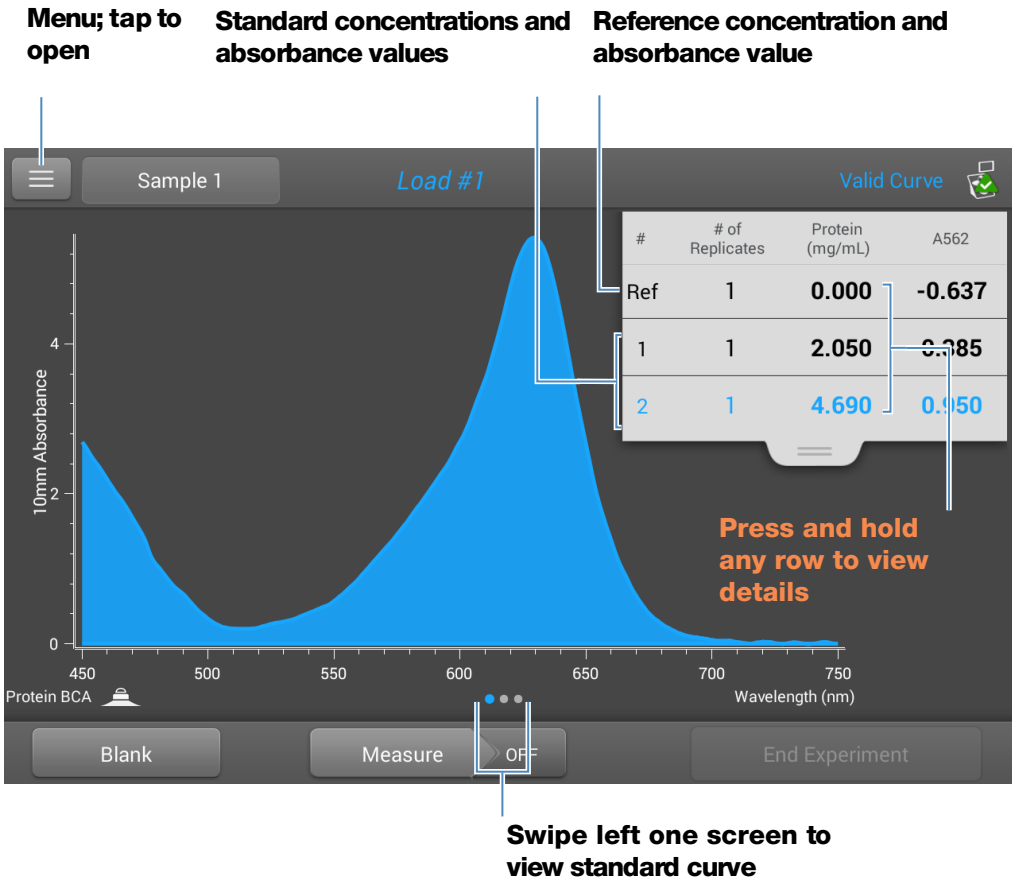
A standard curve is required for colorimetric protein analysis.

- Each experiment requires a standard curve. You can run a new standard curve or import standard from a previously run experiment.
- To import a Standard curve, from the PC control software, select the  Load Standard icon, select a Standard, and click Load.
  From Local control, tap 

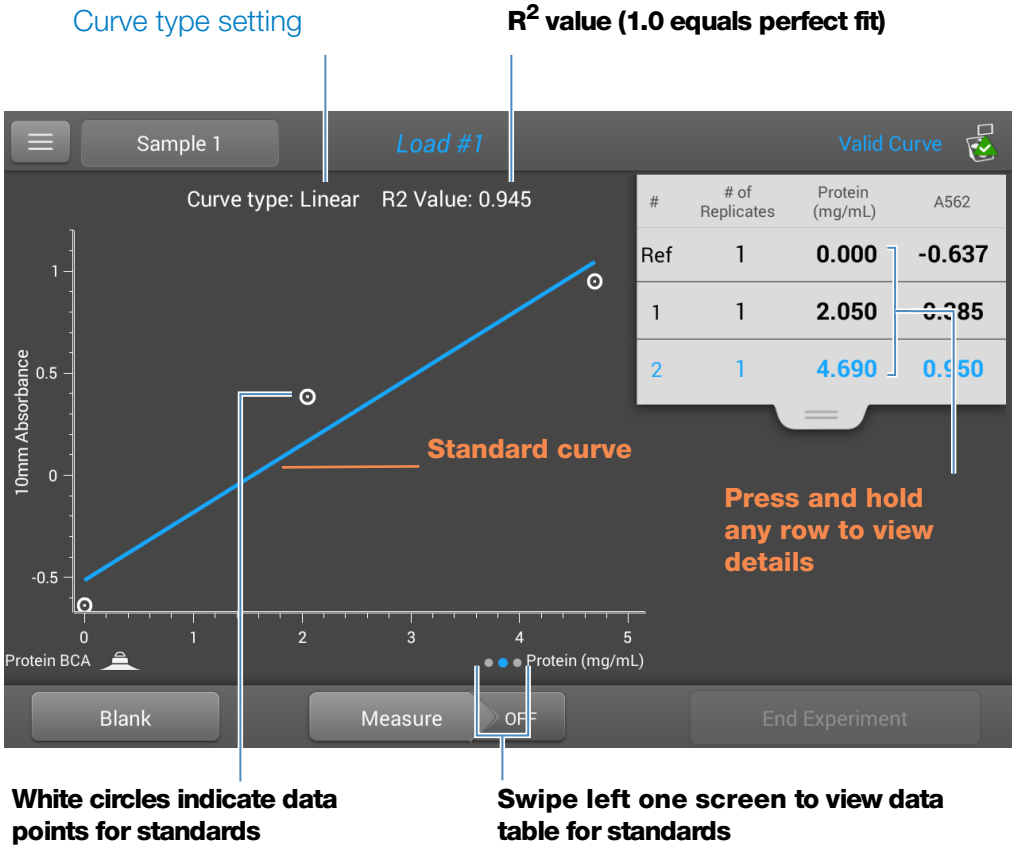
  and select a Standard to import a standard curve.
- Prepare standards and unknown samples the same way. See the kit manufacturer's guidelines and recommendations.
  - **All reference and standards solutions** should be the same buffer used to resuspend the samples plus the same volume of reagent added 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.

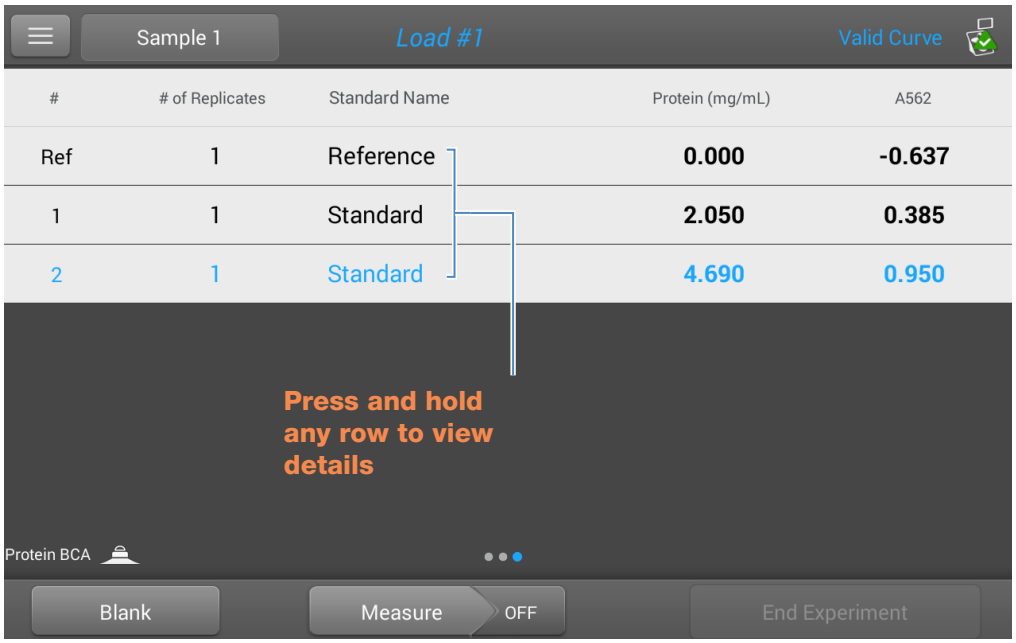


Swipe left one screen to see the standard curve as you build it. Here is an example:

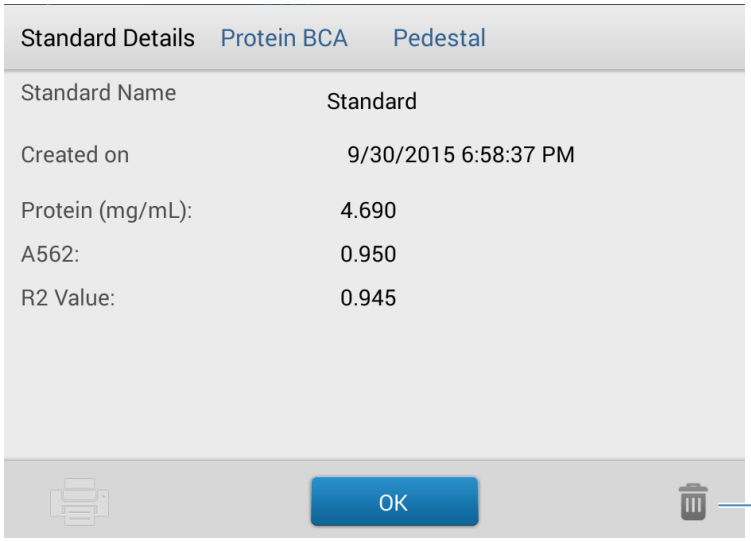


The R^2 value indicates how well the standard curve fits the standard data points (1.0 is a perfect fit; all points lie exactly on the curve).

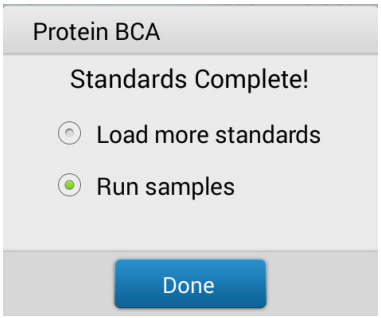
Swipe left one screen to see the data table for the standards. Here is an example:



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.

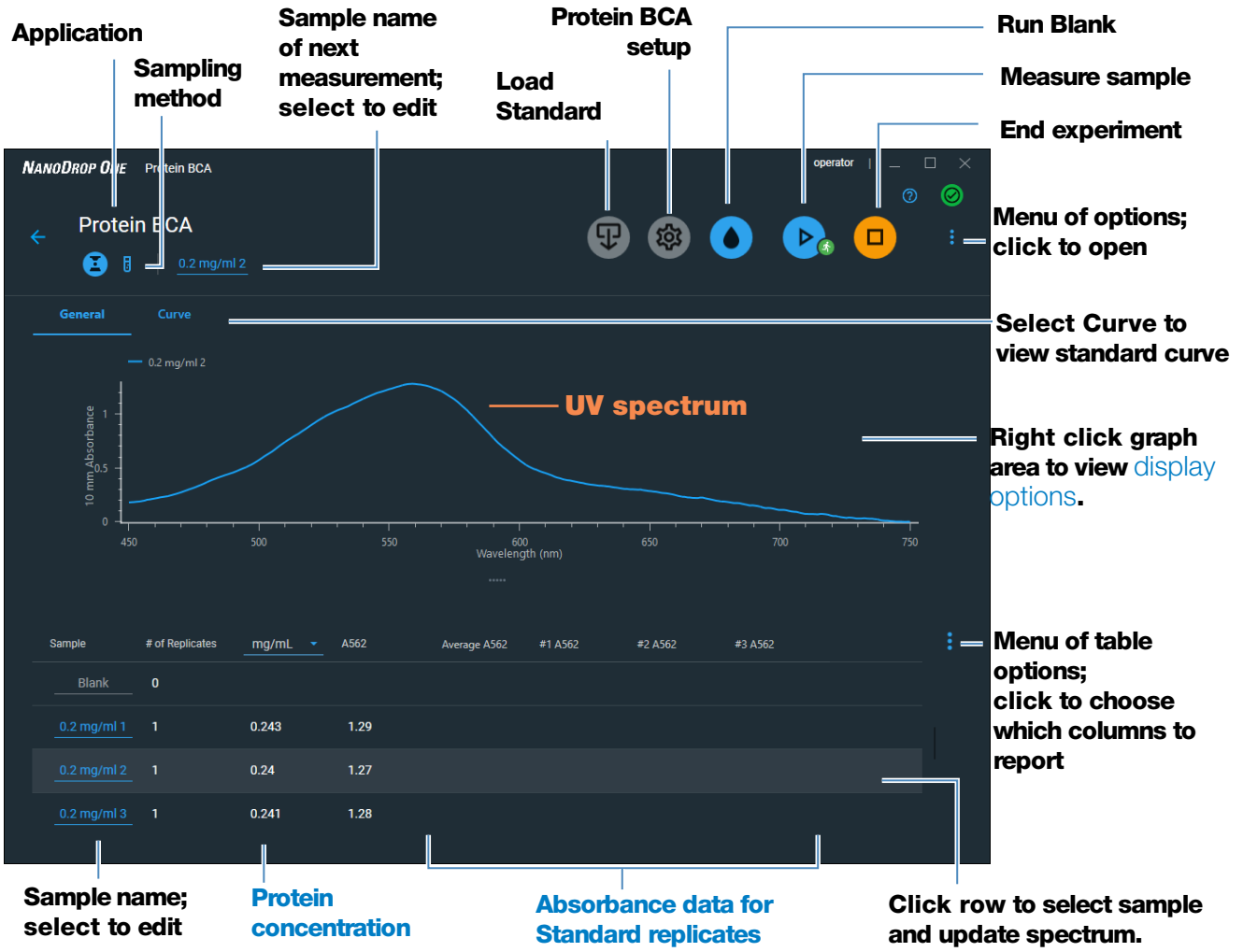
- tap **Blank** and wait for measurement to complete
- lift arm and clean both pedestals with new laboratory wipe, or remove cuvette

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.

Protein BCA Reported Results

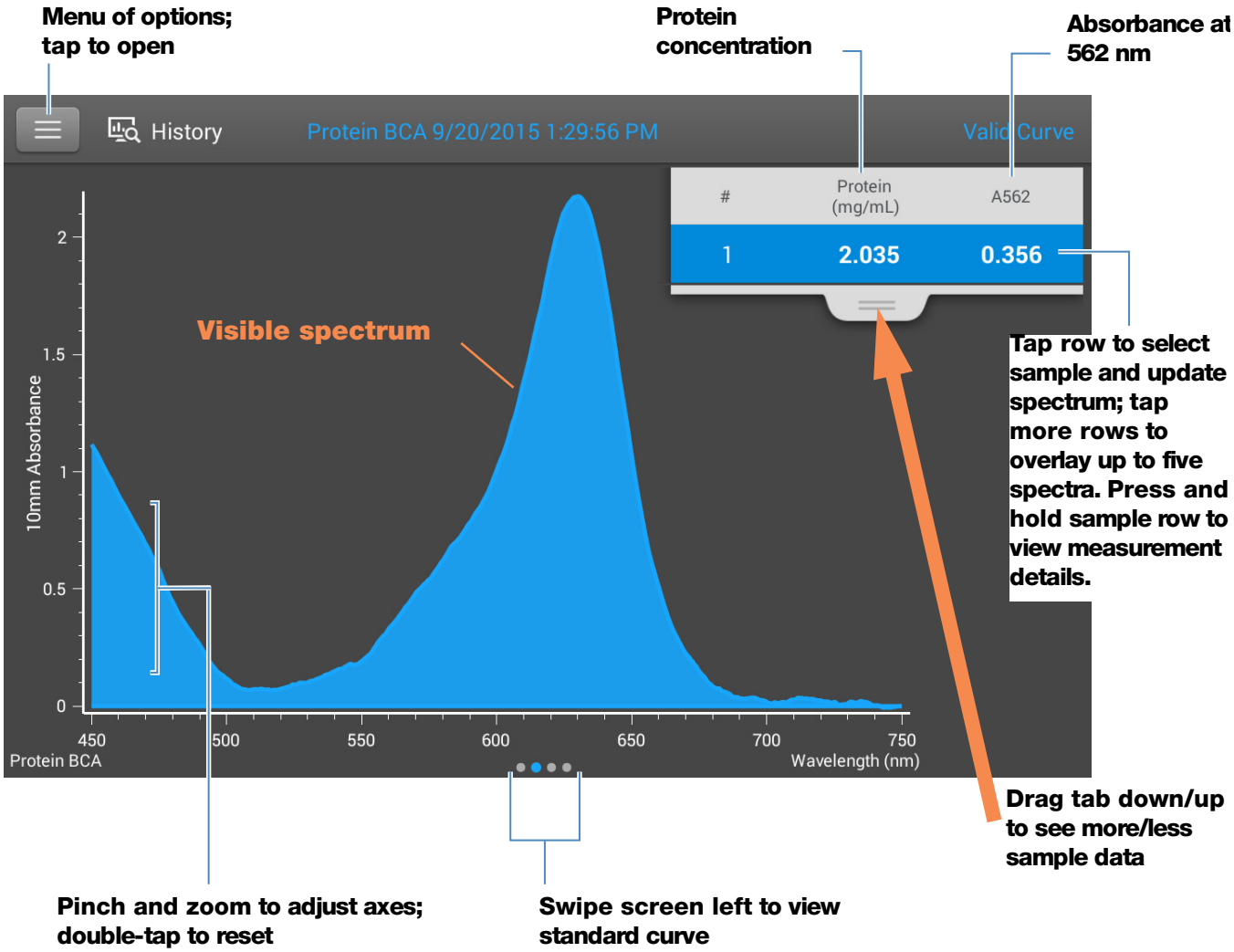
Protein BCA measurement screen

For each measured sample and standard, this application shows the visible absorbance spectrum and a summary of the results. Select “Curve” to view the Standard curve. Below is an example of the measurement screen of the PC control software:



Measurement screen of PC Control software

When using local control, the standard curve is available by swiping left from the measurement screen (or in the History as shown below).



Measurement screen of NanoDrop One local control

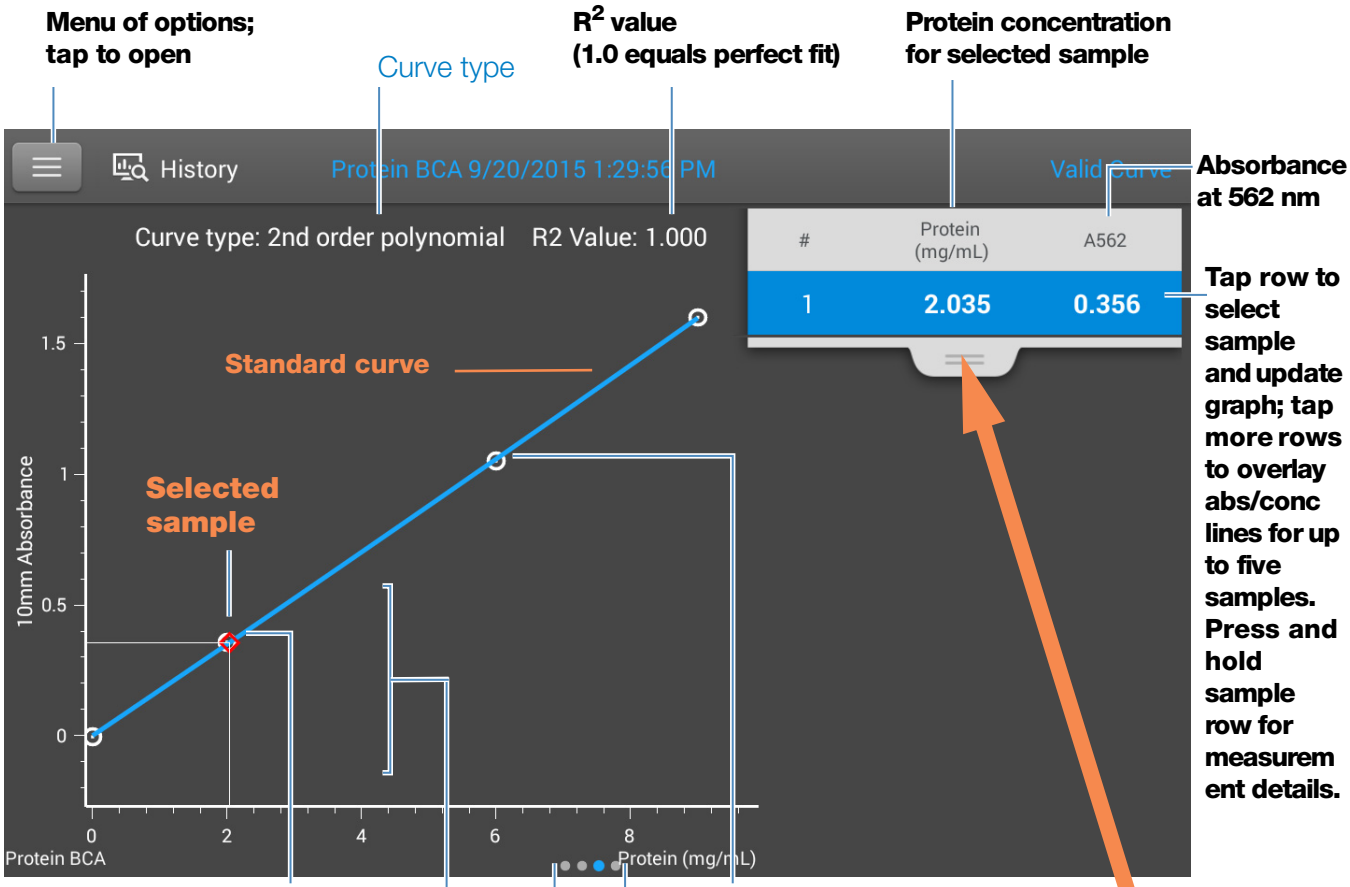
Note

- 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.

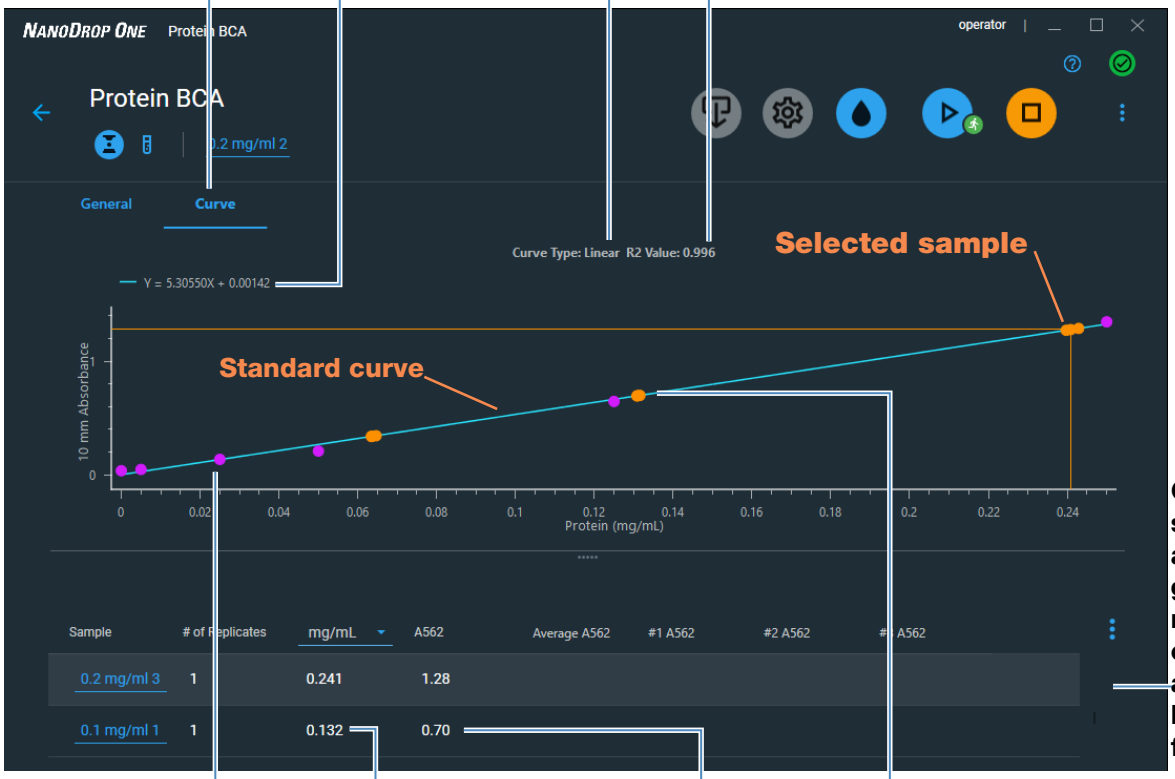
Protein BCA standard curve screen

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).



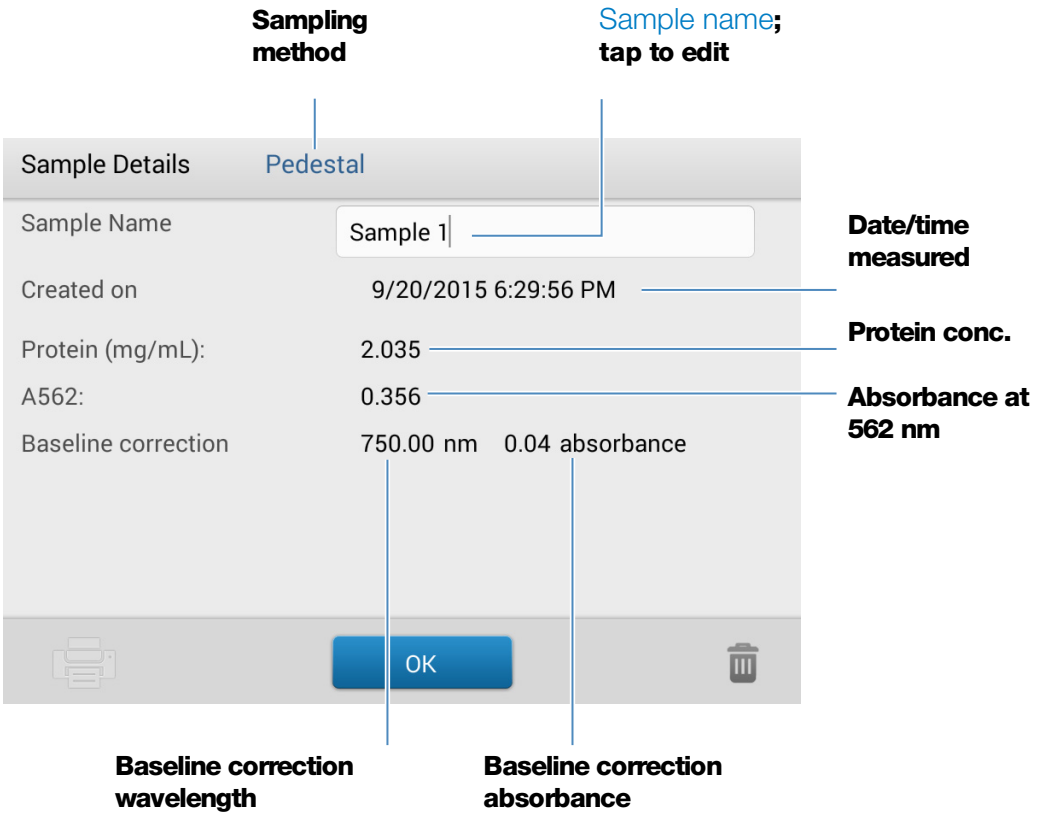
Standard curve screen of NanoDrop One local control software (shown from History)



Standard curve screen of PC Control software

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.**

In the PC control software, click the Protein BCA Setup Icon .

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) This page is intentionally blank.
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.

Measure Protein Bradford

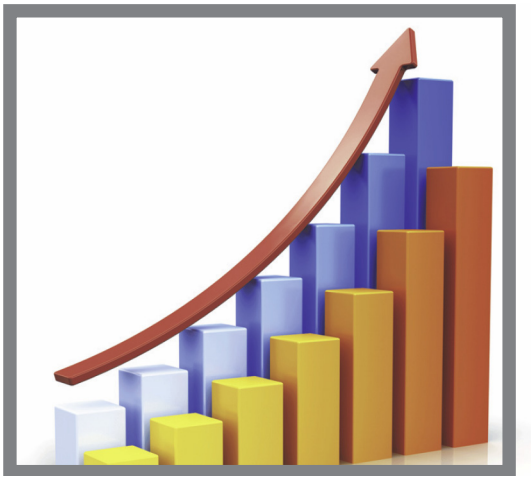
Measures total protein concentration of unpurified protein samples using a Coomassie Blue dye colorimetric detection reagent.

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.

Protein Bradford standard curves

A standard curve is required for colorimetric protein analysis.

- Each experiment requires a standard curve. You can run a new standard curve or import standard from a previously run experiment.
- To import a Standard curve, from Local control, tap 

and select a Standard.

For more information, see Working with Standard Curves.

Note: Importing of standards for the Protein Bradford application is only supported in the Local control software.

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.

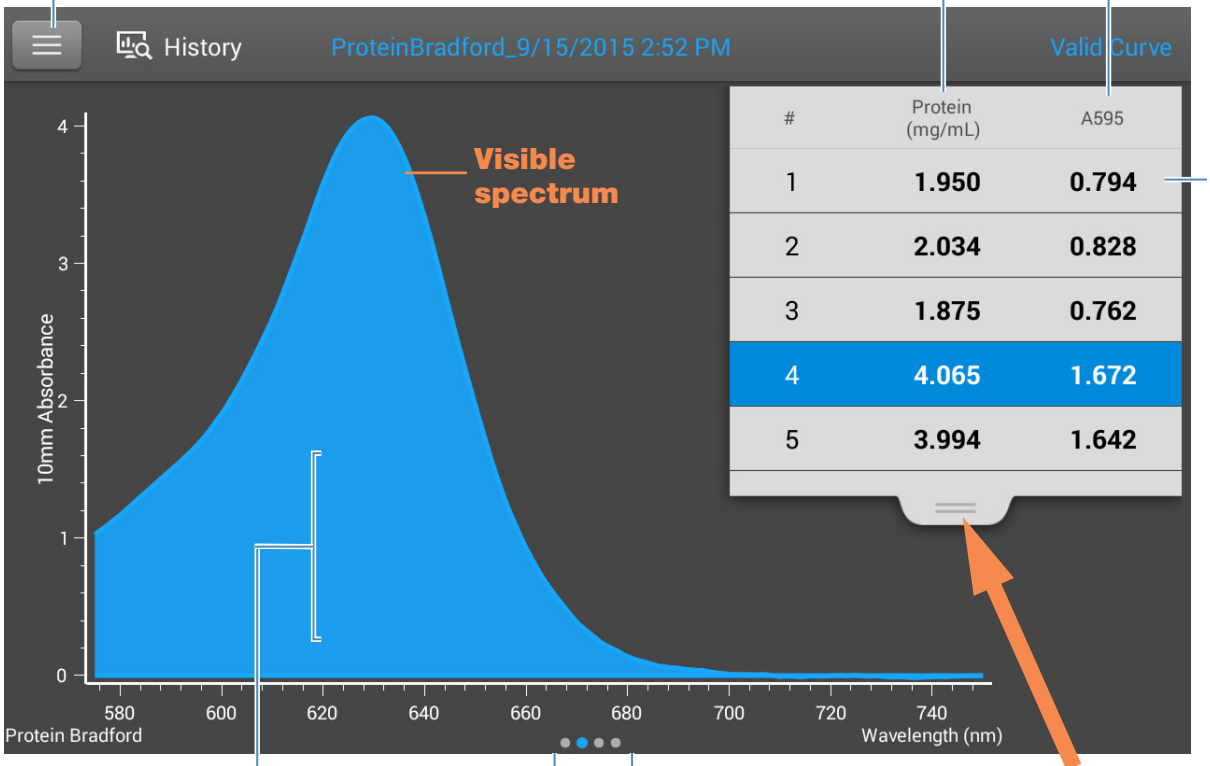
Related Topics

- 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 History)

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 History as shown below).



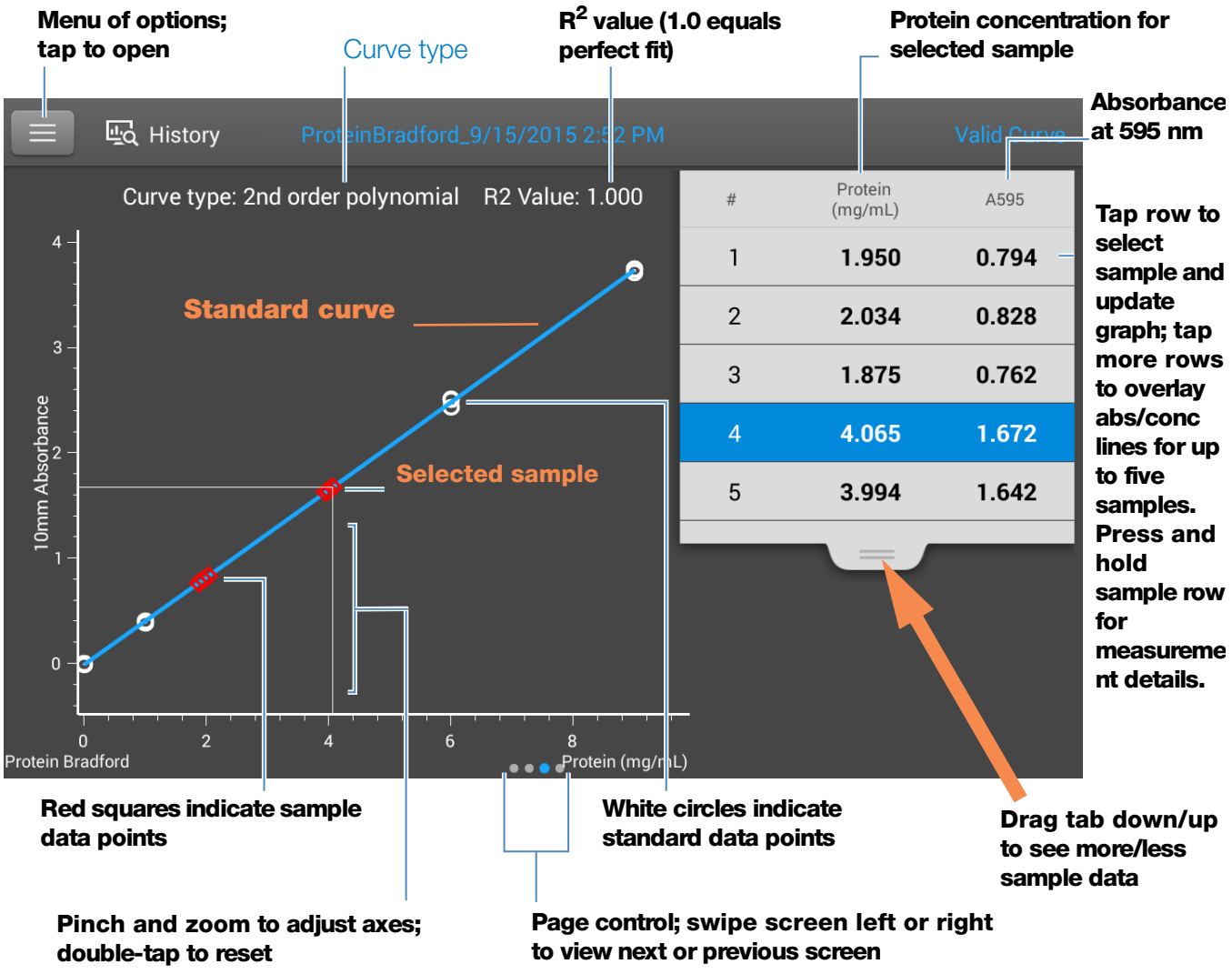
Note

- 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).

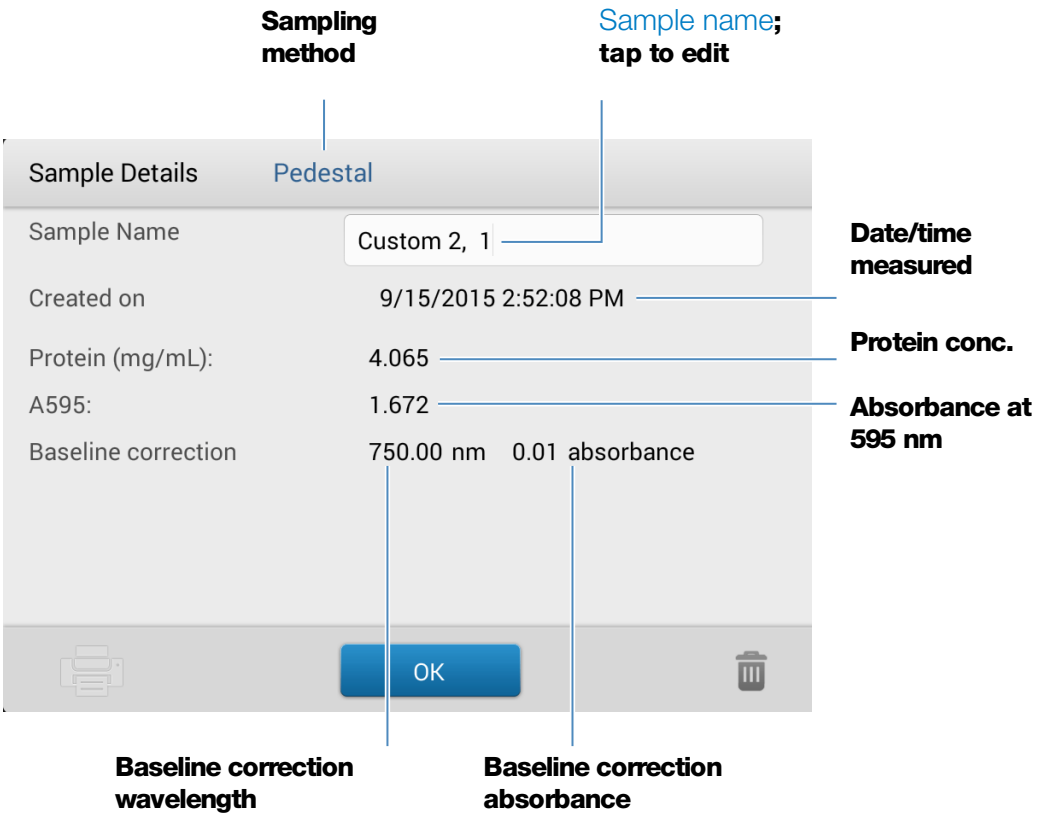


Note

- 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

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Measure Protein Lowry

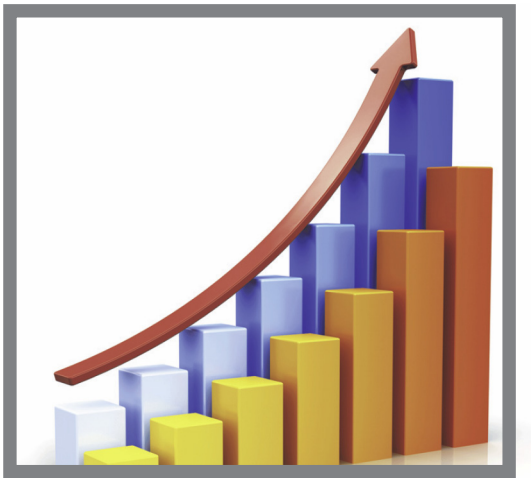
Measures total protein concentration of unpurified protein samples using a Folin-Ciocalteu colorimetric detection reagent.

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.

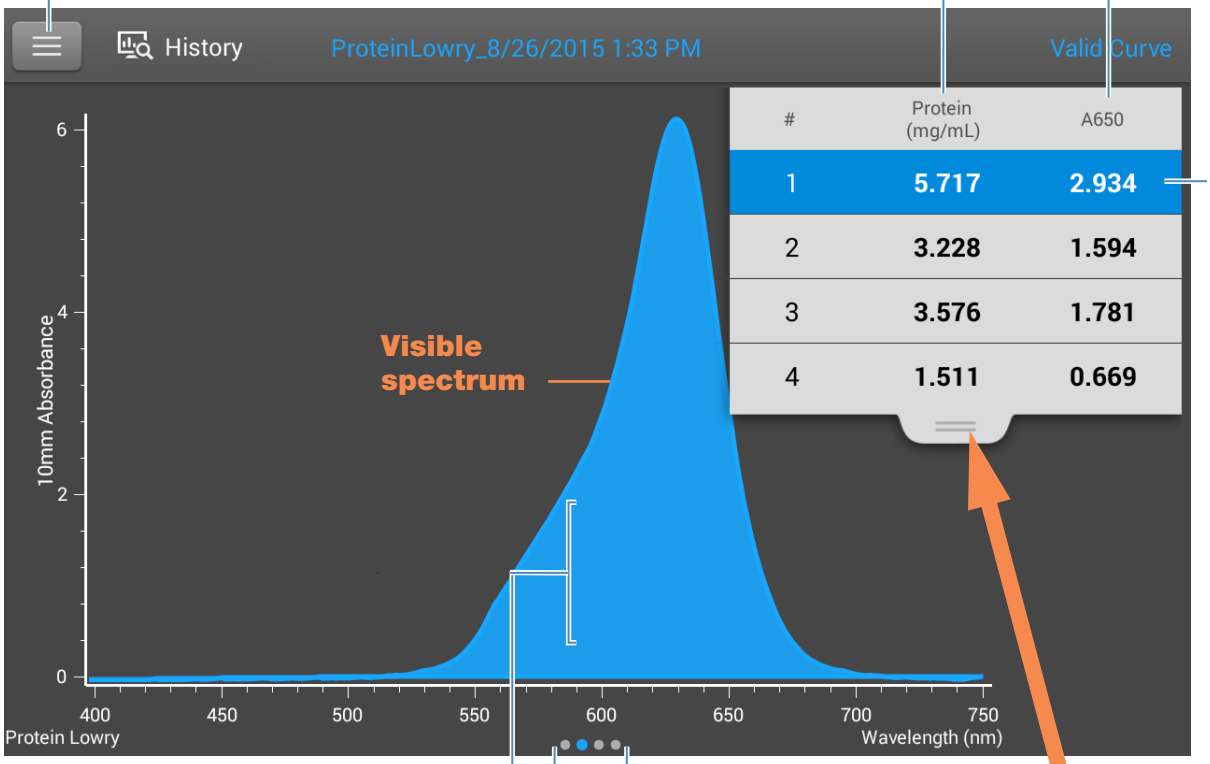
Related Topics

- 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 History)

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 History as shown below).



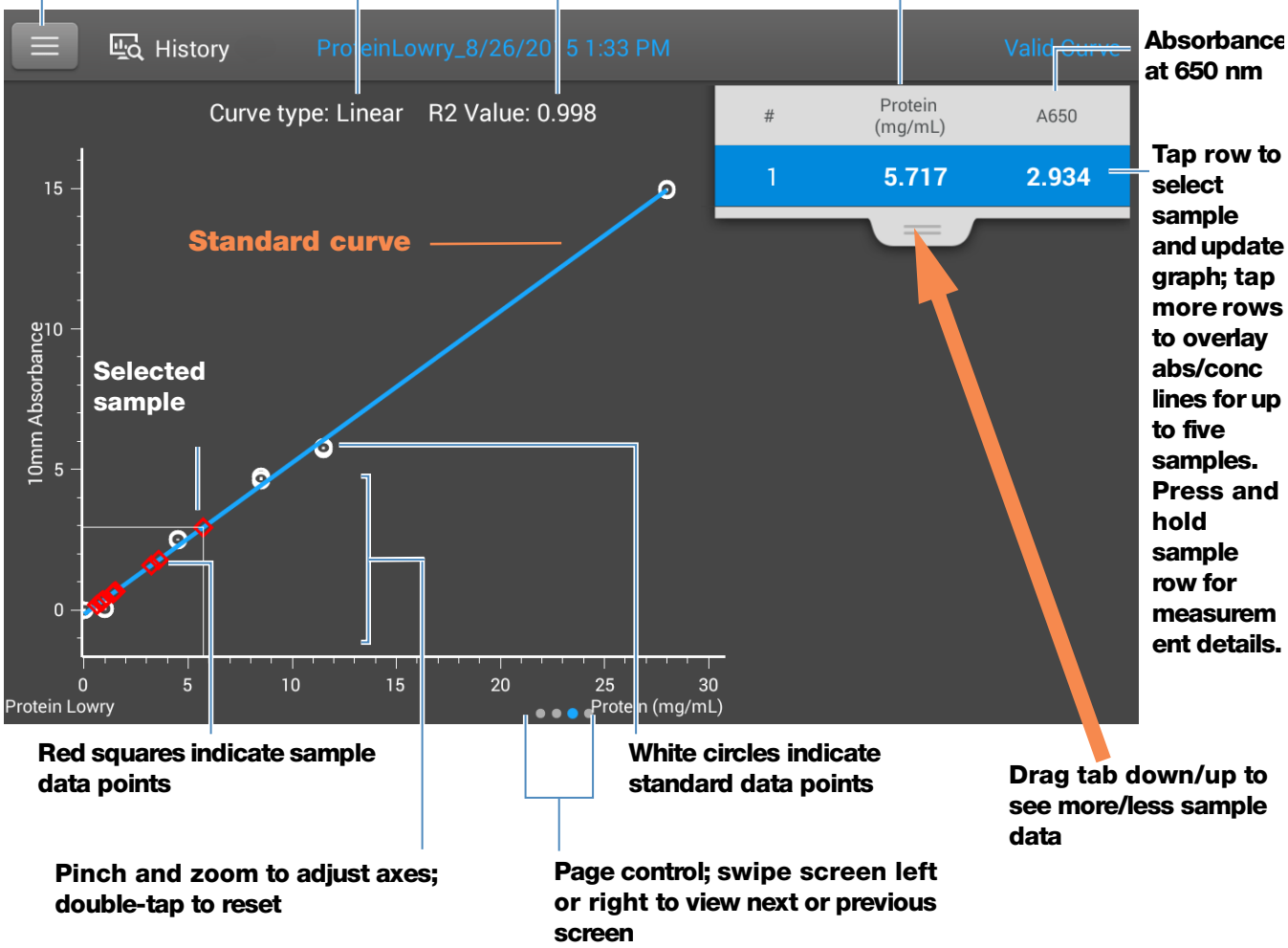
Note

- 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

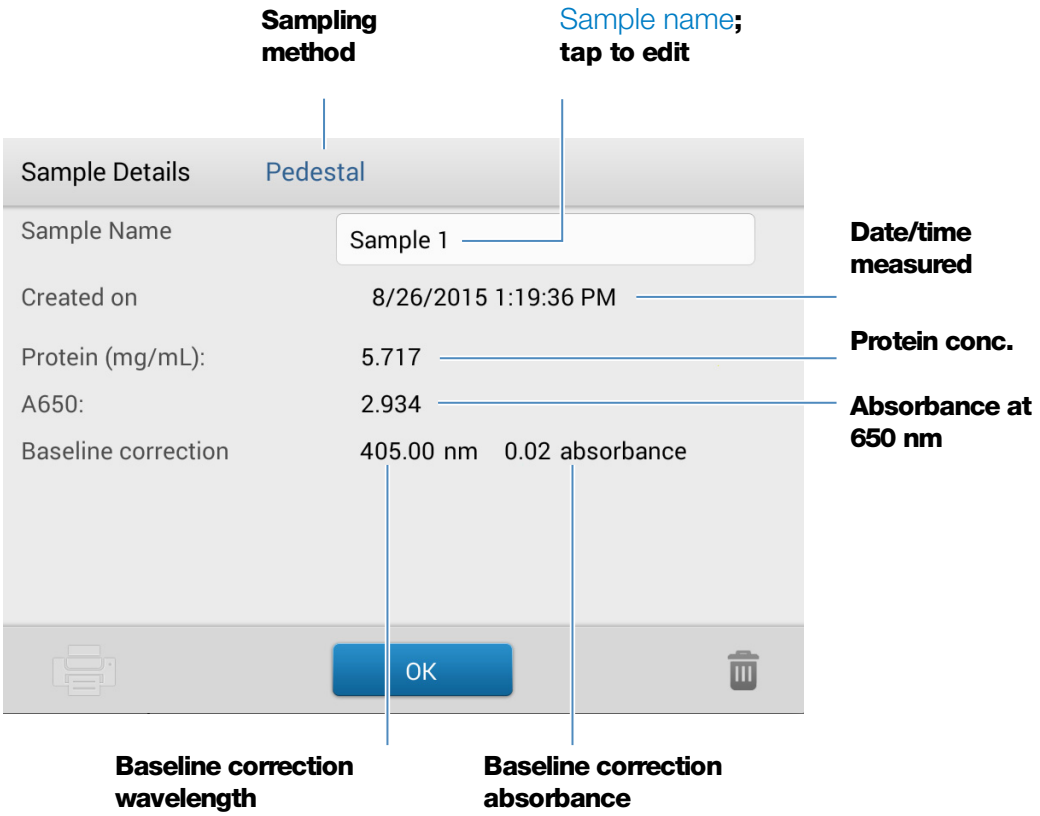
The standard curve screen shows graphically the relationship between the measured standards, the calculated standard curve, and the measured absorbance and calculated concentration for a selected sample. A horizontal line connects the sample absorbance value on the Y-axis to the standard curve. A vertical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



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

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Measure Protein Pierce 660

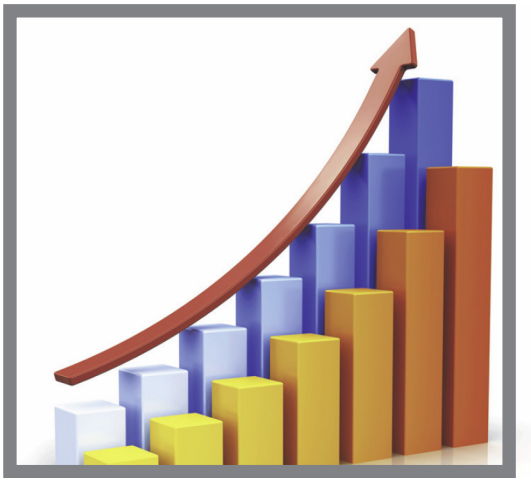
Measures total protein concentration of unpurified protein samples using a proprietary colorimetric detection reagent.

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

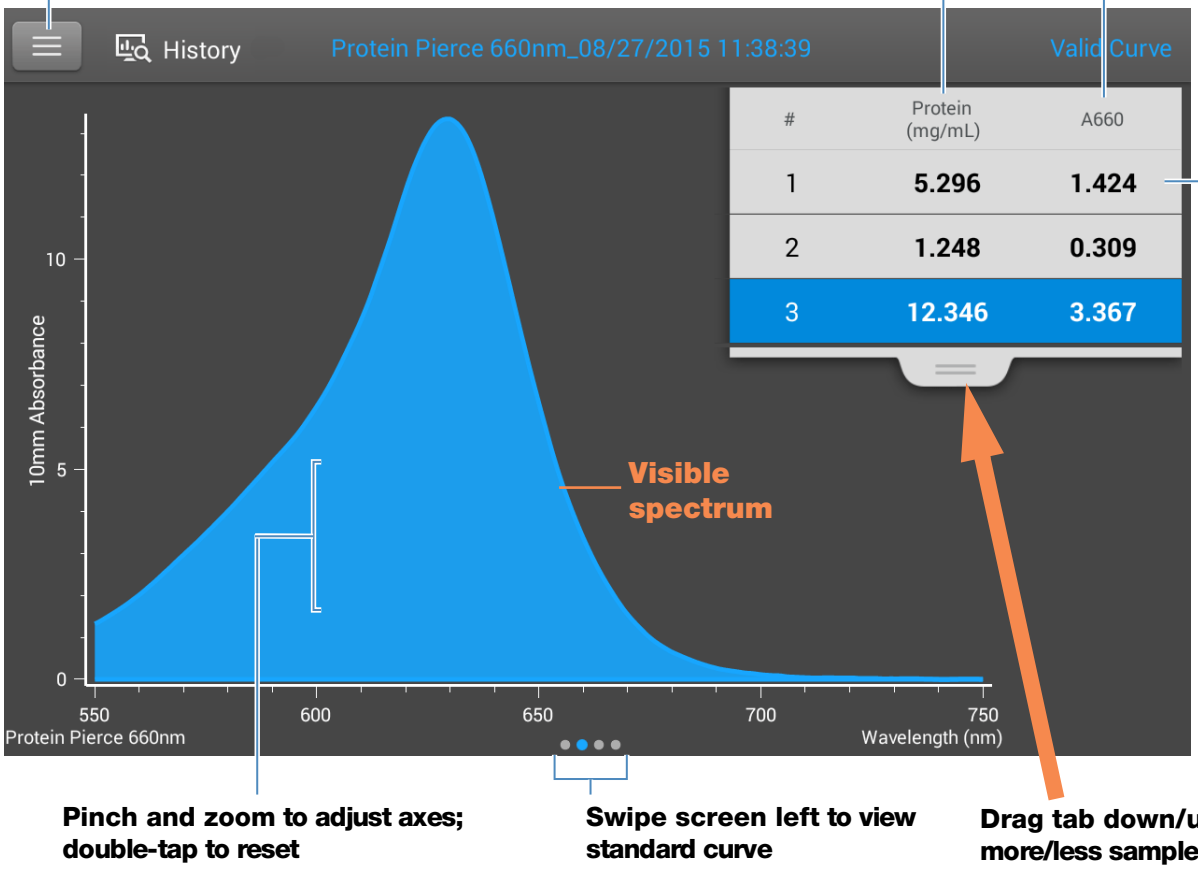
- Working with standard curves
- Best practices for protein measurements

- 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 History)

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 History as shown below).



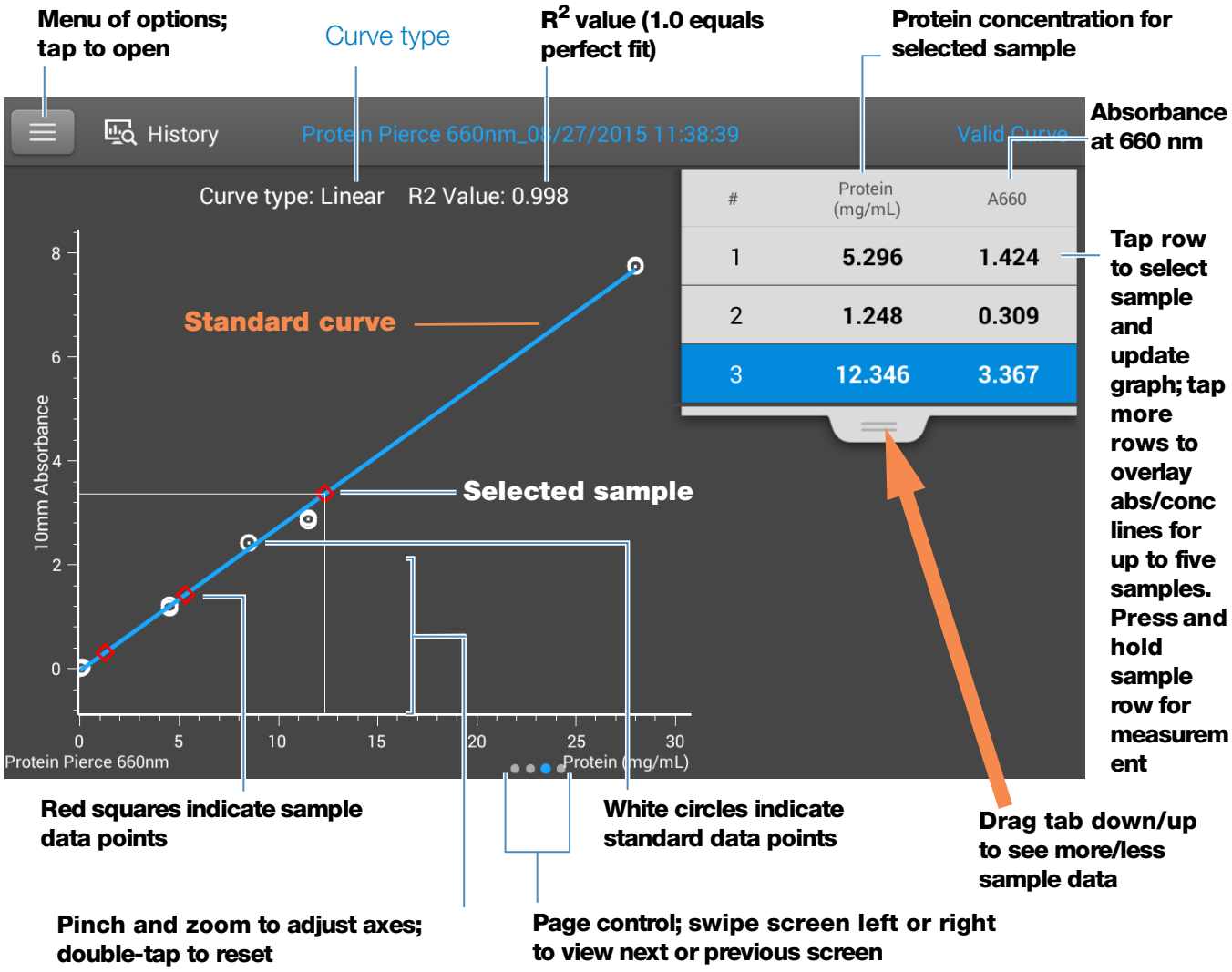
Note

- 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

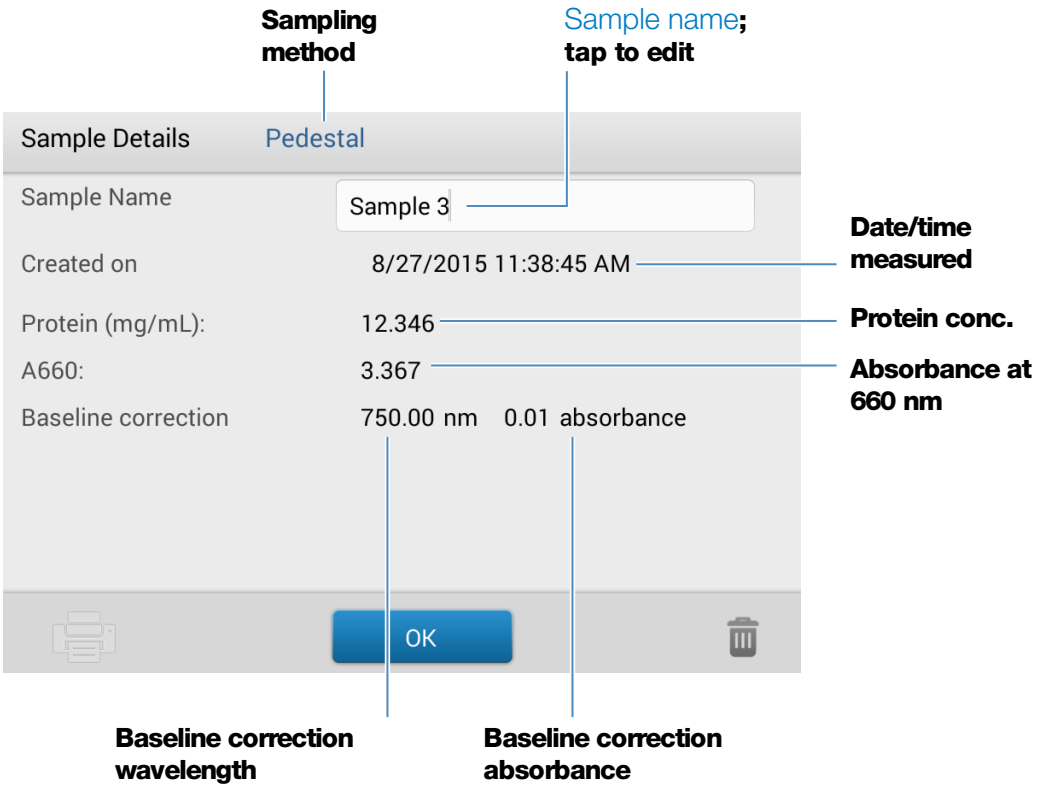
The standard curve screen shows graphically the relationship between the measured standards, the calculated standard curve, and the measured absorbance and calculated concentration for a selected sample. A horizontal line connects the sample absorbance value on the Y-axis to the standard curve. A vertical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



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: ☑ Absorbance data for standards can be either measured or entered manually. Uncheck this box to measure absorbance data. Check the box to manually enter the absorbance values. and then enter absorbance values for all the standards.

Related Topics

- Instrument Settings

Measure OD600

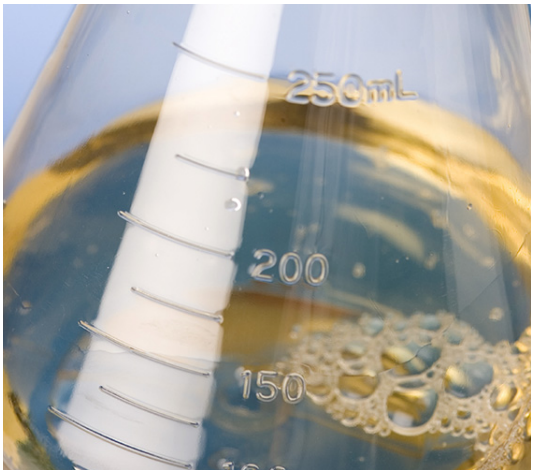
Measures the concentration of microbial cell cultures in solution by measuring scattered light at 600 nm.

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

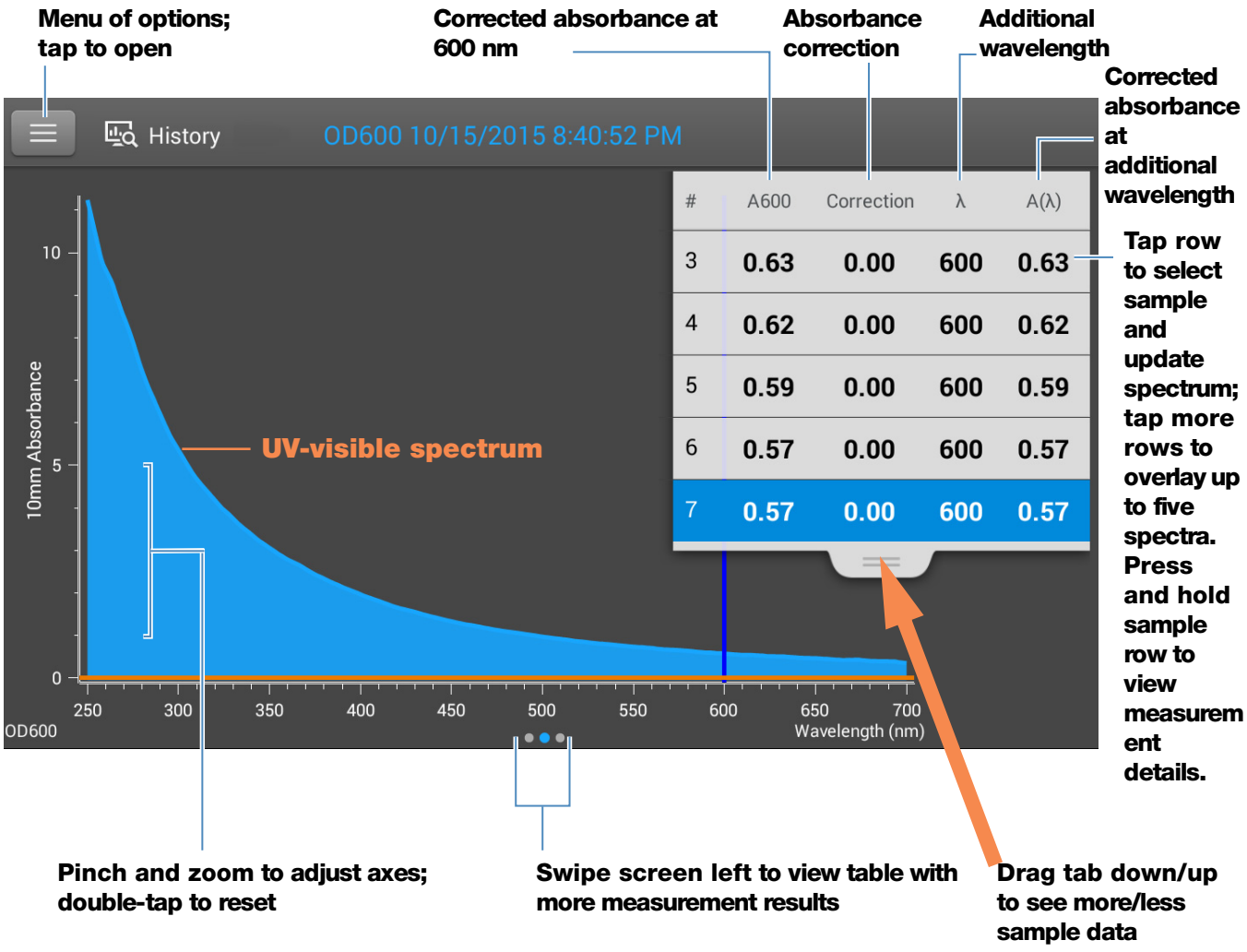
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette

- Prepare Samples and Blanks
- Basic Instrument Operations

OD600 Reported Results

OD600 measurement screen (shown from History)

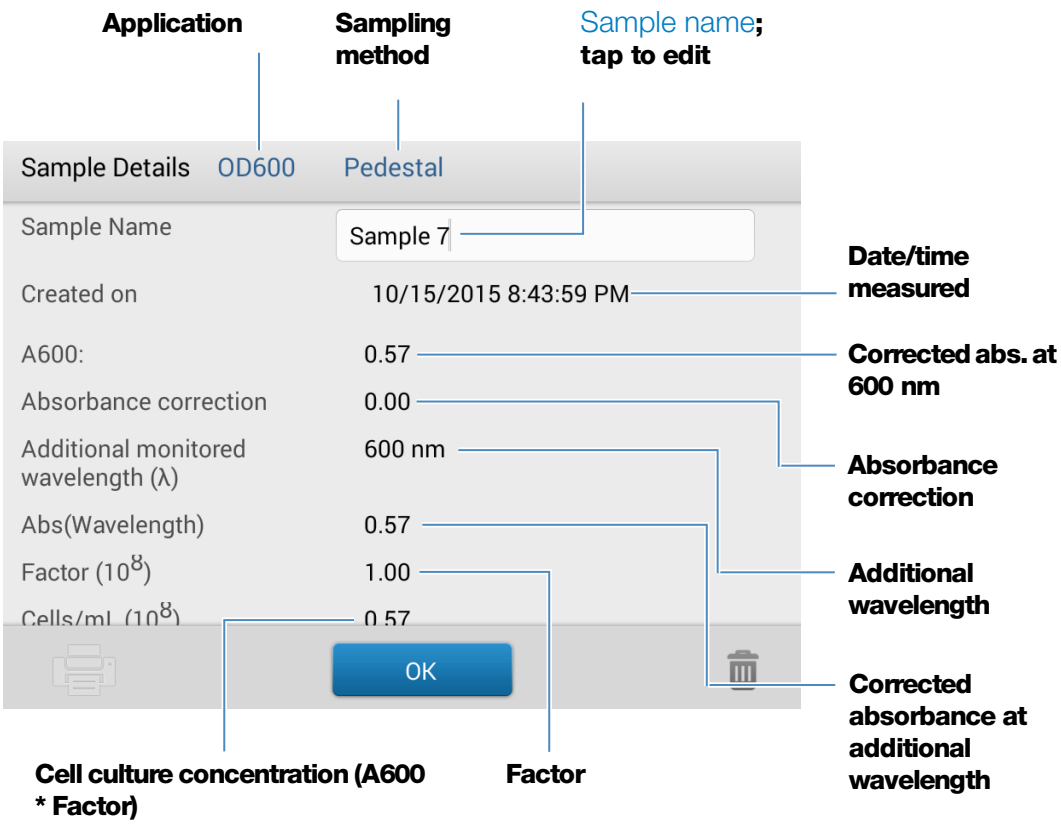
For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example:



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 (λ)	Any wavelength between 250 nm and 700 nm	User-defined wavelength. Enter an additional wavelength to measure if desired (useful for dilute samples that exhibit low absorbance at 600 nm). If an alternative wavelength is specified, use this equation to calculate cell concentration: $c = A(\lambda) * factor(\lambda)$ where: c = analyte concentration in cells/mL A(λ) = UV-visible absorbance at specified wavelength in absorbance units (A) factor(λ) = 1/(ε(λ) * b) in mL/cell-cm where: ε(λ) = molar absorption coefficient (or extinction coefficient) at specified wavelength b = pathlength in cm (1.0 cm for the NanoDrop 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(λ) absorbance

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

Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1.0 mm and 0.03 mm) based on sample absorbance at the analysis wavelength.
- For cuvette measurements, 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.

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7

Custom Applications

Use the NanoDrop One to perform UV-Vis or your own Custom measurements.

The UV-Vis application can be set up directly from the touchscreen and allows the instrument to function as a conventional spectrophotometer. Up to 40 wavelengths from 190 nm to 850 nm can be monitored and reported.

The Custom application provides additional flexibility for the method that you use with the instrument. See the NanoDrop PC Control software to learn about custom method features that are supported.

- Measure UV-Vis ..... page 154
- Measure Custom ..... page 161

Measure UV-Vis

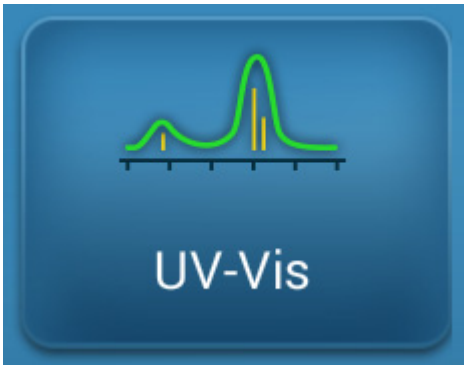
Measures the absorbance of any sample at up to 40 wavelengths across the ultra-violet (UV) and visible regions of the spectrum.

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, from the **Custom** tab, select **UV-Vis**.
2. Specify up to 40 wavelengths to monitor (or you can specify them later if desired) and whether automated pathlength adjustment, analysis wavelength, 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 re-suspend 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.

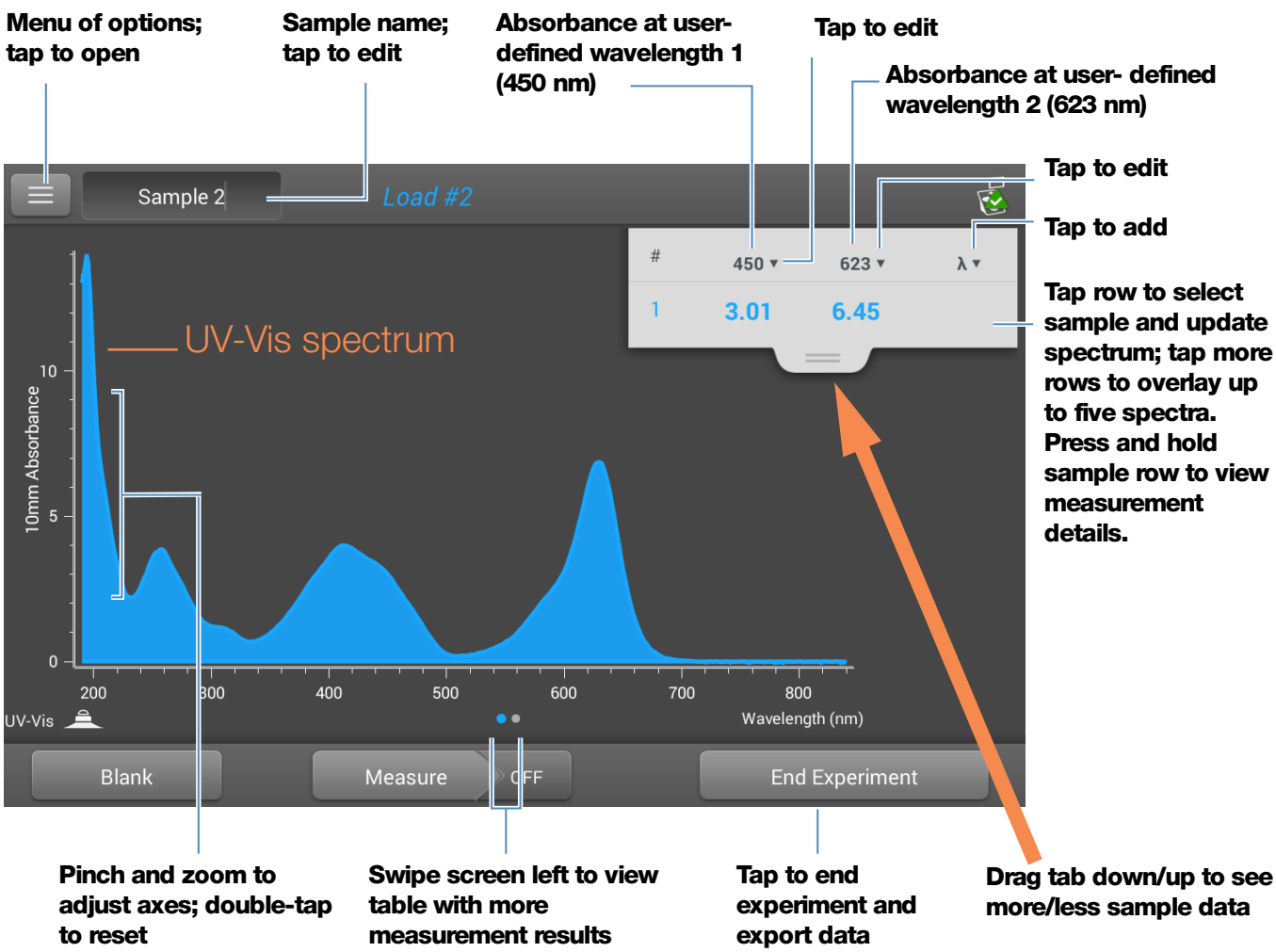
– Use a 1-2 µL sample volume. See Recommended Sample Volumes for more information.

- For cuvette measurements (NanoDrop One^{C} instruments only), use compatible cuvettes and follow best practices for cuvette measurements.

UV-Vis Reported Results

UV-Vis measurement screen

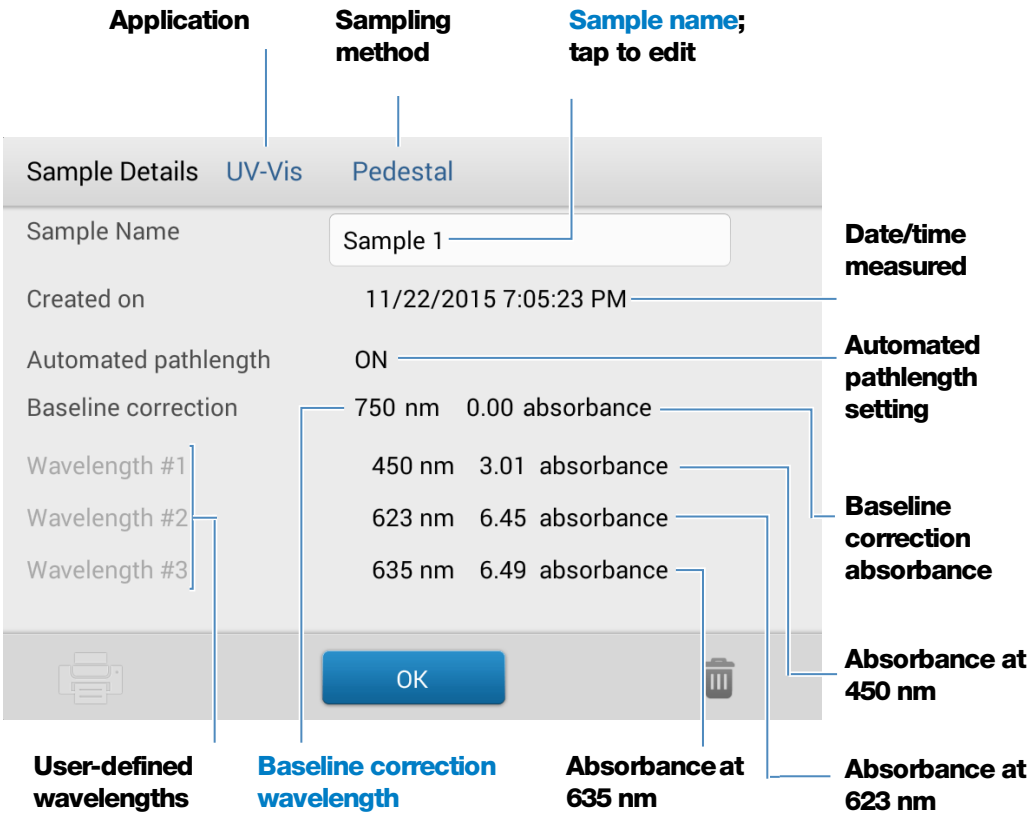
For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example as it appears on the local instrument display:



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

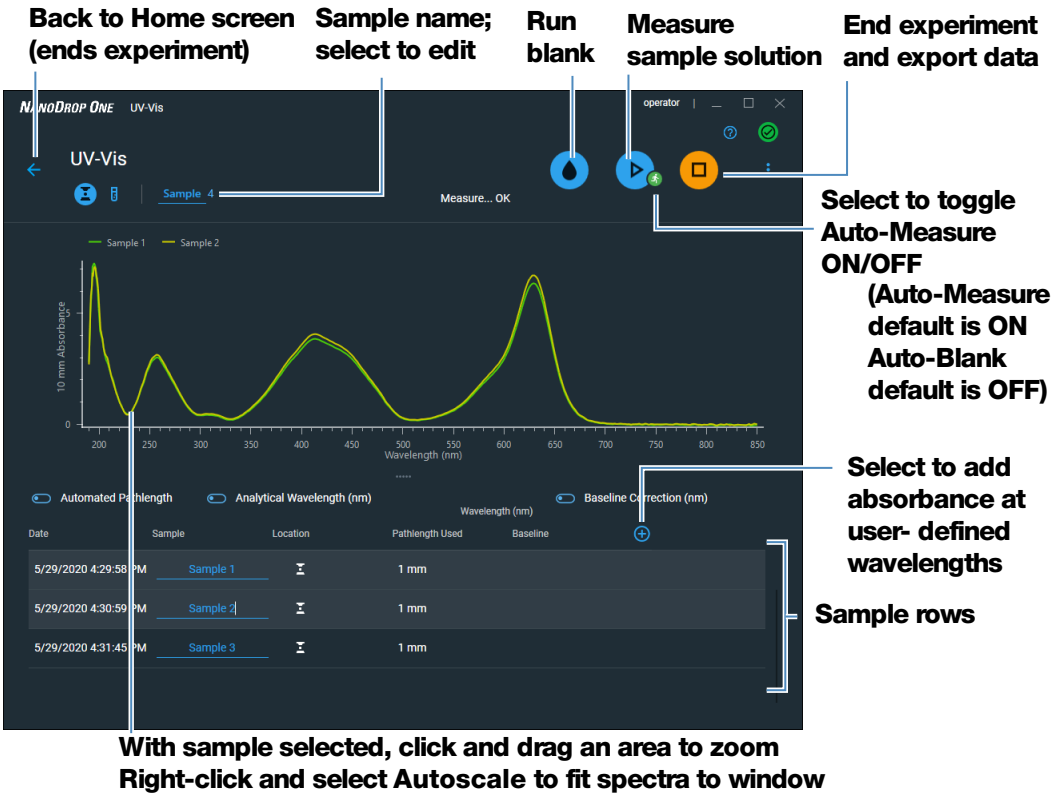
UV-Vis reported values

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.

Below is an example of the measurement screen with reported values as it appears in the NanoDrop PC software:



Tips:

Click sample row to select sample and update spectrum
Shift-click multiple sample rows to overlay up to five spectra
Click a sample and hover locations on spectra to view measurement values

Settings for UV-Vis Measurements

To show the UV-Vis settings, from the Home screen, from the **Custom** tab, select **UV-Vis**.

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.
Analytical Wavelength	Any wavelength between 190 nm and 850 nm	This is the wavelength the software will use to determine the pathlength selection.
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 1 mm pathlength equivalent.

Setting	Available Options	Description
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.

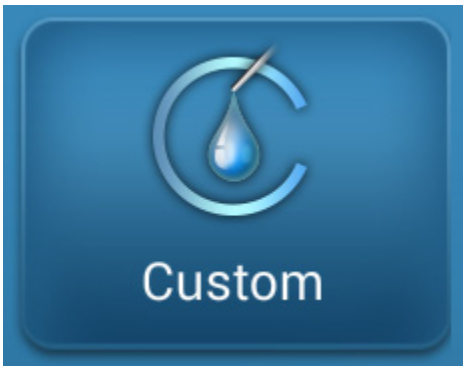
Measure Custom

Runs a custom measurement method created using NanoDrop One 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 software running on a personal computer. For more information, see "Create Custom Method" on page 167.

To load a custom method

Custom methods can only be created on a personal computer running the NanoDrop One 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.)

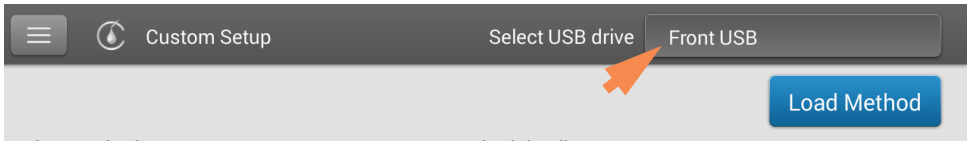
Load custom methods onto the instrument

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.

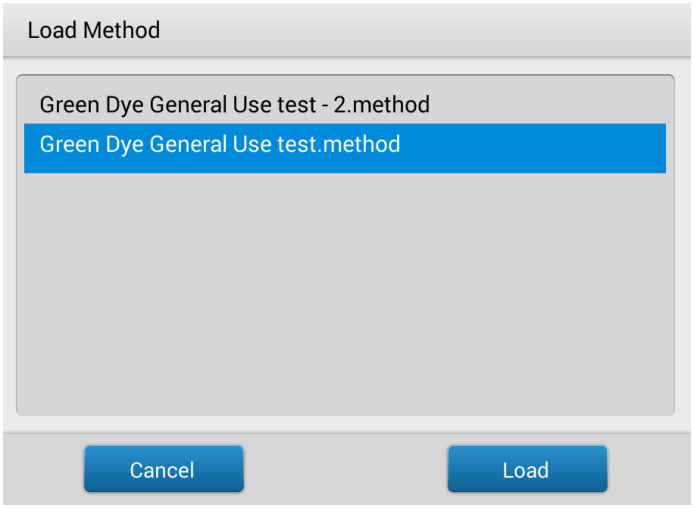
   Note Custom methods downloaded from the NanoDrop One website have a .zip filename extension and must be extracted using a third-party file decompression program before the software will recognize them as custom methods.

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



5. Select **Load Method**.

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



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

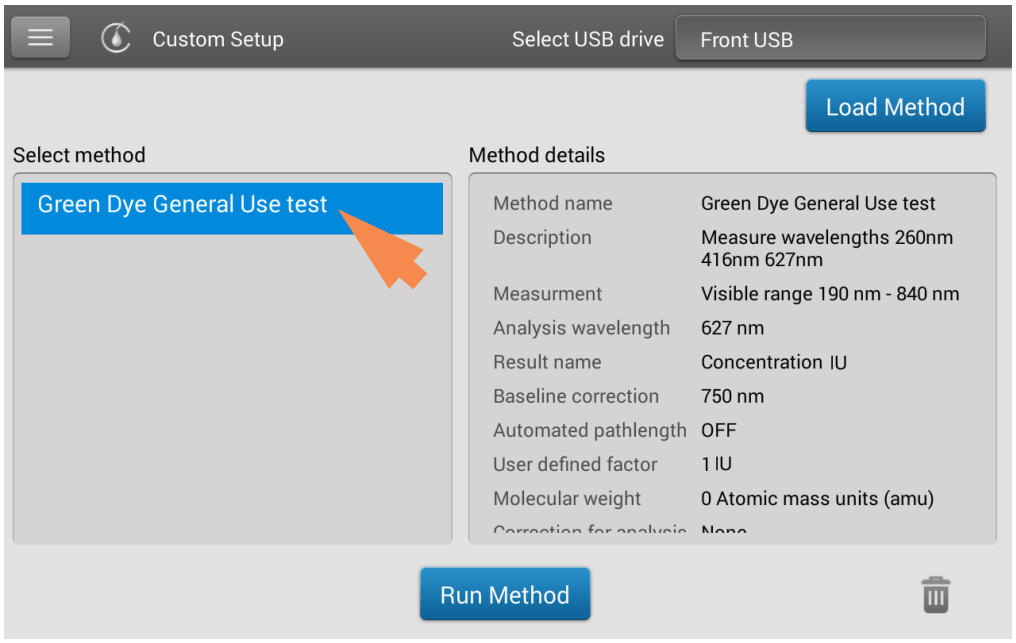
Before you begin...

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 using the local instrument interface

1. Make sure the method resides on the NanoDrop One instrument (see To Load a Custom Method for details).
2. From the Home screen, select the **Custom** tab and select **Custom Method**.

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

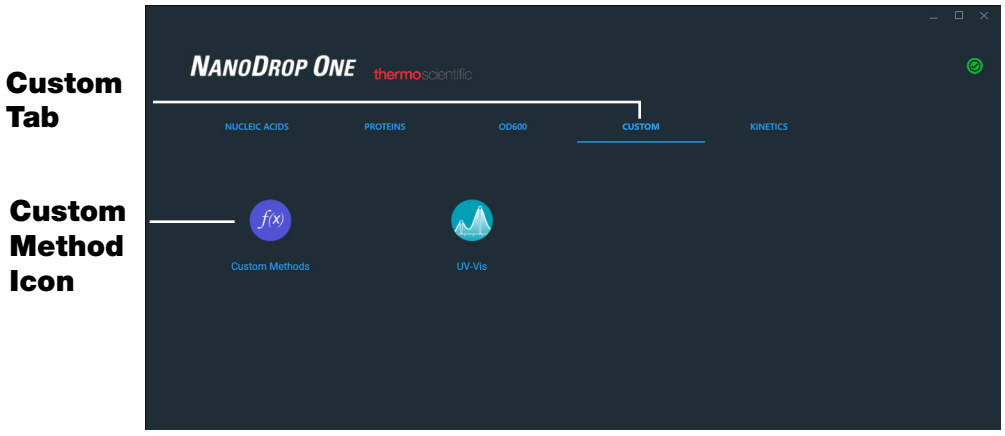


Information about the selected method appears in the Method Details box.

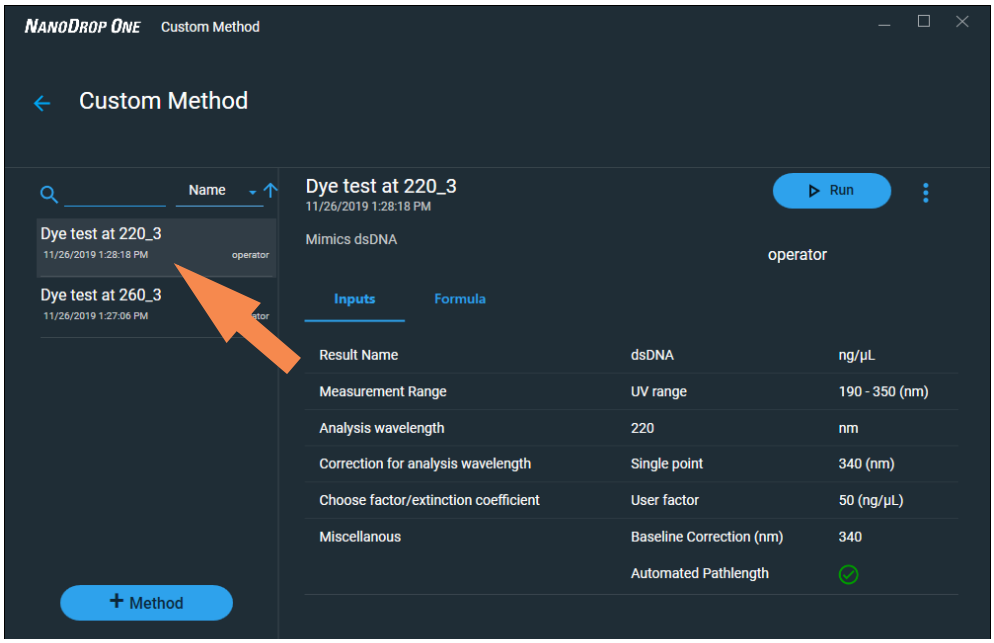
4. Select **Run Method**.
5. Follow the on-screen instructions to measure a sample.

To measure a sample using a custom method using the PC software

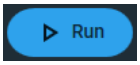
1. From the Home screen, from the **Custom** tab, select **Custom Methods**.



2. In the method selection pane, select to select the method to run.



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

3. Select  .

4. Follow the on-screen instructions to measure a sample.

Delete Custom Method

– From Home screen, from the **Custom** tab, select **Custom Methods**.

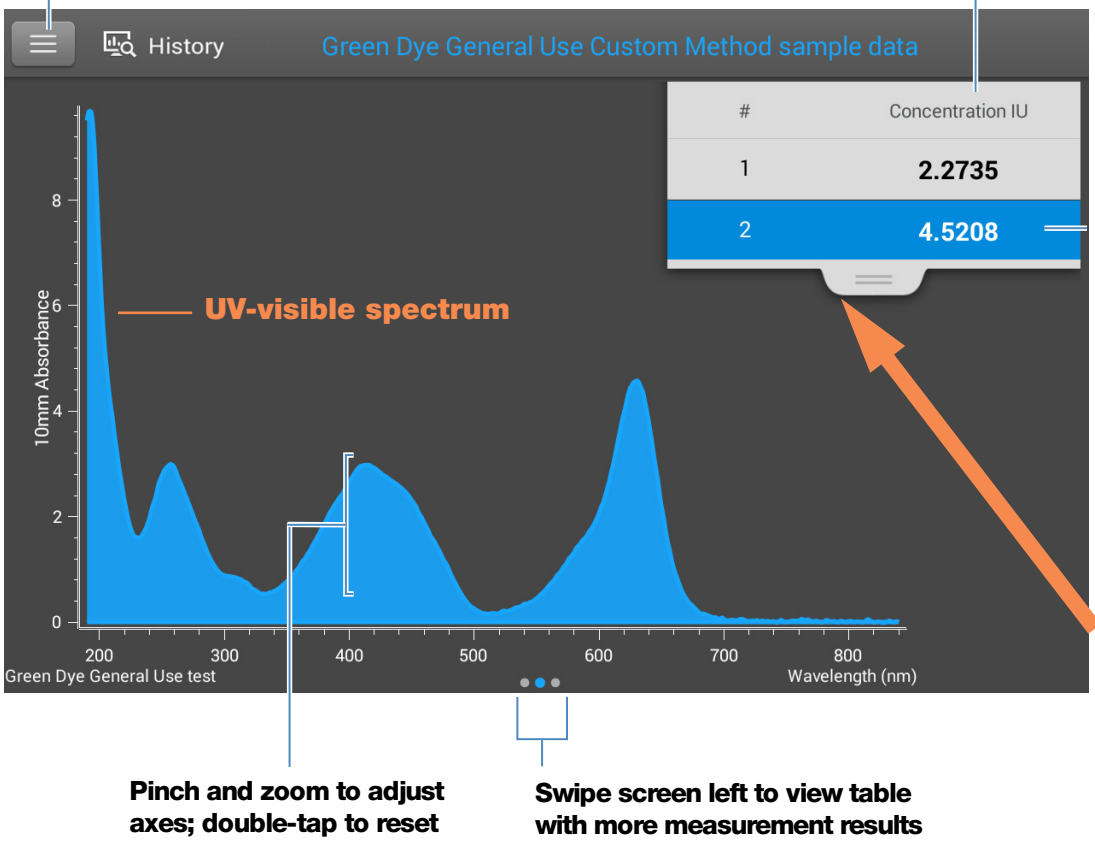
– In Select Method box, select a method to delete

– Select 

Custom Method Reported Results

Custom method measurement screen (shown from History)

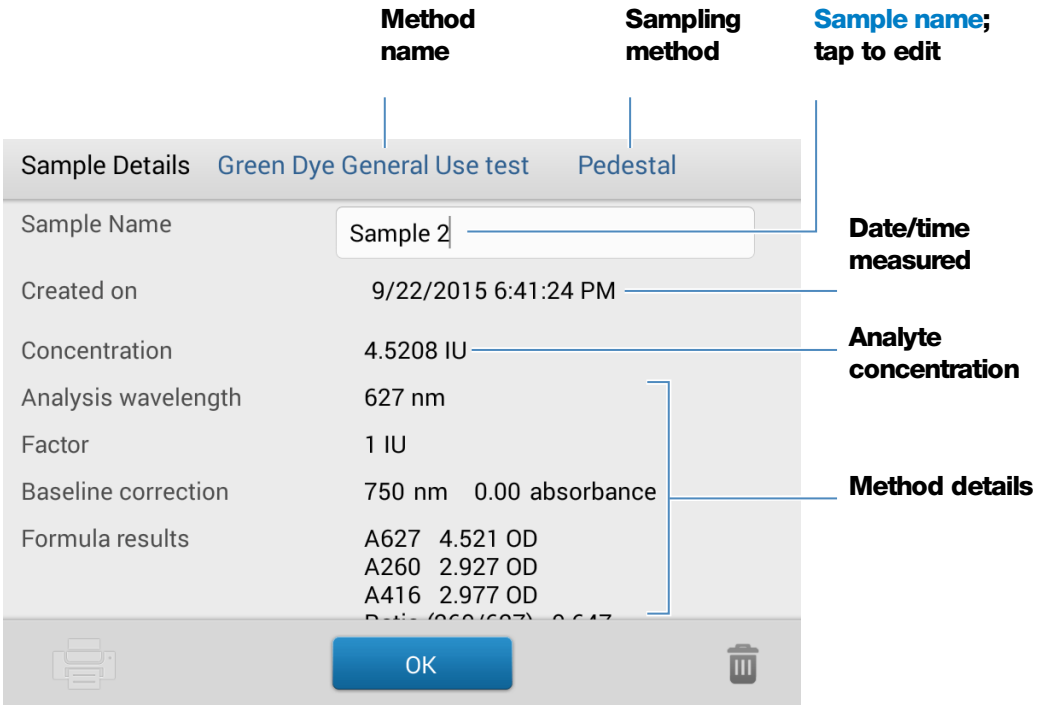
For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example:



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:


Method name
Sampling method
Sample name; tap to edit
Sample Details
Green Dye General Use test
Pedestal
Sample Name
Sample 2
Created on
9/22/2015 6:41:24 PM
Concentration
4.5208 IU
Analysis wavelength
627 nm
Factor
1 IU
Baseline correction
750 nm 0.00 absorbance
Formula results
A627 4.521 OD
A260 2.927 OD
A416 2.977 OD
OK
Date/time measured
Analyte concentration
Method details

Manage Custom Methods

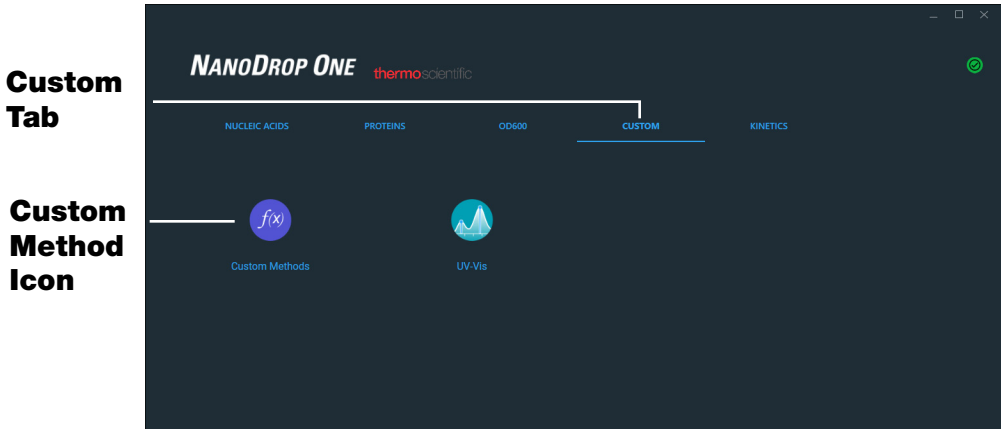
The NanoDrop One PC Control software is your tool for creating and managing custom methods, which contain user-defined settings that can be used to acquire data with the instrument. Custom methods can be made with or without standards.

Create Custom Method

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

Create new custom method

– From the NanoDrop Home screen, select the **Custom** tab and select **Custom Methods**



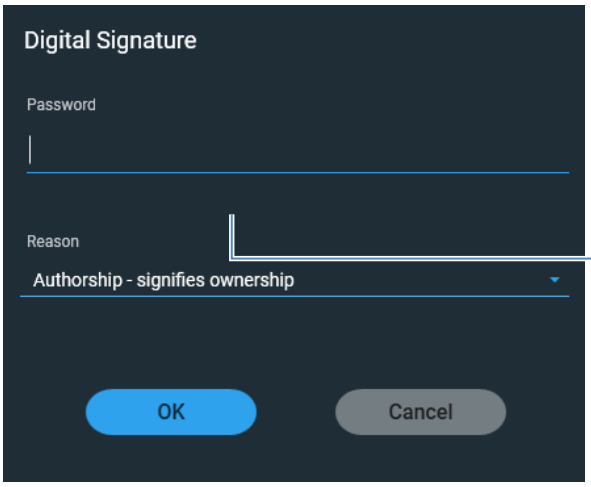
– In the Manage Custom Methods screen, select + NEW METHOD and choose one of the following:
  – **Formula** (if your method will not have standards)
  – **Standard Curve** (if your method will have standards)
– In the setup window, enter **Method Name** (this name appears in the Custom Setup box on the instrument after the method has been transferred there)
– Enter detailed **Description** of method, if desired
– Specify how to calculate and report the method results:
  – if method does not have standards, specify factor or extinction coefficient of analyte (enter “1” to report absorbance measurements only)
  – if method has standards, enter name and concentration of each standard and select the curve fit type

– Enter or choose remaining custom settings as needed

– Choose **Save**

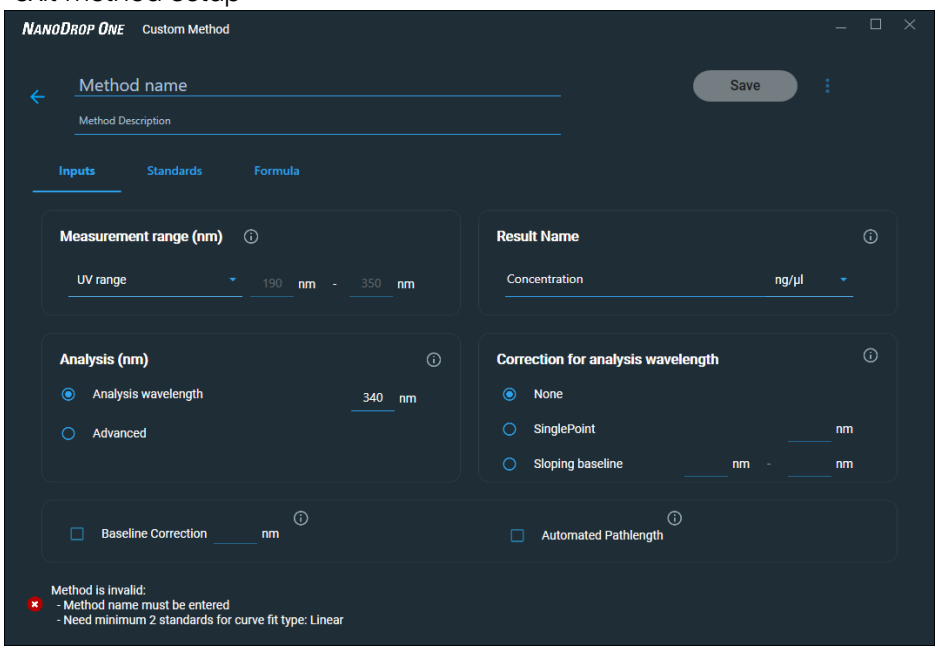
> **Note** If ⊗ appears at the bottom left of the screen instead of a green check mark icon, the method is invalid because it contains an error.

– Enter your password to sign the changes if prompted.



Enter your password to sign the changes

If the method has a green check mark icon at the bottom, select **Close** to exit method setup

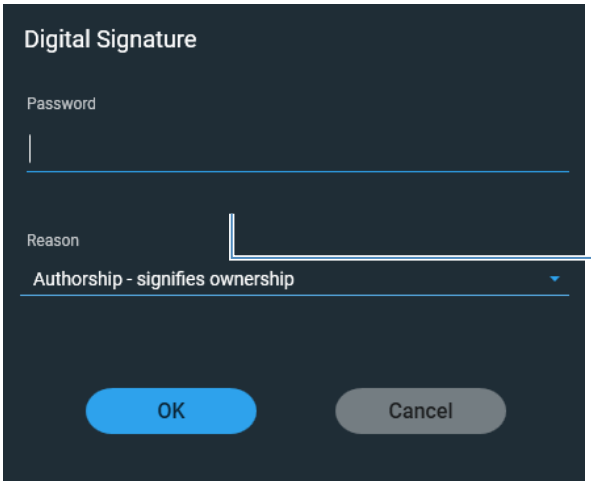


View or edit custom method

– Select **Custom Method** (existing methods are listed in Select Method box along with their type (formula or standards) and Description

– From the Custom Method Management screen, select the method you would like to edit from the list of loaded methods.

– From the drop-down menu  select **Edit**
– View and adjust the method settings as desired
– Select **Save**
– Enter your password to sign the changes if prompted.



Enter your password to sign the changes

Custom method settings

These settings are available for creating custom methods.

Setting	Available Options
Result name	Enter descriptive name for calculated concentration result (for example, “MTT Assay”) and use adjacent drop down list to select appropriate unit. Result name appears as column heading for reported concentration value.
Measurement range	Select spectral range in which method will acquire data. Available options: • Ultra-violet only (190 nm - 350 nm) • Visible only (350 nm - 850 nm) • Ultra-violet and visible (190 nm - 850 nm) • Custom (specify starting and ending point in nanometers) **Notes**: • If a Baseline correction and/or Analysis wavelength correction are used, make sure your selected spectral range includes your specified baseline correction and/or analysis correction wavelength. • For micro-volume absorbance measurements and measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent.

Setting	Available Options
Analysis wavelength correction	Use this option to specify absorbance correction at analysis wavelength only. Available options: • **None.** No correction at analysis wavelength. • **Single point**. Enter wavelength for analysis correction. (Absorbance value at specified analysis correction wavelength is subtracted from absorbance value at analysis wavelength. Corrected value is used to calculate sample concentration.) • **Sloping baseline**. Enter two wavelengths that define sloping baseline for analysis correction. (Absorbance value of sloping baseline at analysis wavelength is subtracted from absorbance value at analysis wavelength. Corrected value is used to calculate sample concentration.)
Factor or Extinction coefficient at 1 cm pathlength (Formula methods only)	Specify whether to use factor or extinction coefficient to calculate concentration result: • **User-defined factor**. Enter **factor** for 1 cm pathlength and use adjacent drop down list to select appropriate **unit**. Equation below shows how factor is used to calculate sample concentration: $c = (A * f) / b$ where: c = analyte concentration A = absorbance in absorbance units (A) **f** = factor (typically $1/\varepsilon$, where ε = wavelength-dependent molar absorptivity coefficient, or extinction coefficient) b = pathlength in cm (determined at measurement time, then normalized to 10 mm (1 cm) pathlength equivalent) • **Extinction coefficient and molecular weight**. Enter **extinction coefficient** for 1 cm pathlength and use adjacent drop down list to select appropriate **unit**. Equation below shows how extinction coefficient is used to calculate sample concentration: $c = A / (\varepsilon * b)$ where: c = analyte concentration A = absorbance in absorbance units (A) ε = wavelength-dependent molar absorptivity coefficient (or extinction coefficient) b = pathlength in cm (determined at measurement time, then normalized to 10 mm (1 cm) pathlength equivalent)

Setting	Available Options
	Notes: • Refer to product literature for information about factors and extinction coefficients for specific materials. • To set up a method that reports absorbance measurements only, select Factor or Extinction Coefficient with the factor or extinction coefficient set to “1”. • If specified unit for factor or extinction coefficient is based on mass (such as mg/mL) and specified unit for calculated result is based on molarity (such as pmol/µL) or vice versa, enter **molecular weight** and use adjacent drop down list to select appropriate **unit**.
Standards (Standard curve methods only)	Define the standards: • Enter name and analyte concentration of each standard and a reference, if desired: – Depending on the Curve Type setting, a standard curve can be generated using two or more standards. (The software **allows a reference and up to 7 standards**.) – **All reference and standards solutions** should be in the same buffer used to resuspend the samples plus the same volume of reagent added to the samples. – **First standard** can be a reference measurement. The reference solution should contain none of the analyte of interest. (The reference measurement is not the same as a blank measurement.) – **Concentration values for standards** can be entered in any order but the standards must be measured in the order in which they were entered; however, best practice dictates that standards be measured from the lowest concentration of the standard analyte stock to the highest. – **Concentration range of the standards** must cover the dynamic range of the assay and the expected range of the unknown samples. Sample analyte concentrations are not extrapolated beyond the concentration of the highest standard.

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

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

Setting	Available Options
Formula table (optional)	Use the Formula table to specify additional reported results, such as a purity ratio, for each sample. Available options: • **Predefined**. Select from a list of predefined formulas, which can be used as is or edited, and choose **Add**. The predefined formula is listed in the Formula Table. • **Add**. Create formula for current method. Available options: • **Formula Name**. Enter a name for the formula. After a measurement, the name is reported in Data Table and Sample Details screens. • **Formula**. Enter valid formula (see below for rules and examples). After a measurement, the measured or calculated value is reported in Data Table and Sample Details screens. • **Unit**. Enter unit for reported result. After a measurement, the unit is reported in Data Table and Sample Details screens. • **Edit**. Edit selected formula for current method. • **Delete**. Delete selected formula from current method.
Formula rules	Custom formulas can include the following operators and functions: • **Path()**. Returns sample pathlength in cm. • **A(nm)**. Returns sample absorbance at specified wavelength (for example, enter A(650) to add the measured absorbance at 650 nm to your equation). • **Operators**: + (add), - (subtract), * (multiply), / (divide). • **Functions**: Log(x), Pow(x,y). **Notes**: Follow these additional rules for all languages: • Use period “.” decimal separators for floating point and double-floating point numbers. • Use comma “,” list separators (for example, “POW(2,8)”). • Do not use comma “,” group separators for large numbers (for example, enter 1000 rather than 1,000).

Copy Custom Method

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

Copy custom method

- From the Custom Methods screen, select a custom method
- From the drop-down menu  choose **Edit**
- Enter new **Method name** and **Description**
- Select **Save As**
- Enter a filename for the method and click **Save**

You can now select the saved method and edit the **Description** and settings.

Run Custom Method

If you want to run a custom method and store the measurement results on the instrument, the method must also reside on the instrument (see Load a Custom Method for details).

Export Custom Method

Export a custom method in order to run it and store the measurement results on the NanoDrop One instrument.

- From the Custom Methods screen, select a custom method
- From the drop-down menu  , choose **Export** (if method is invalid, an error message is displayed; errors must be fixed before method can be exported)
- Choose **Save** (method is exported to method file (*.method filename extension) in proprietary format)

  To transfer the method to the NanoDrop One instrument, copy the method file to a USB memory device and then load the method (see Load a Custom Method for details)

Import custom method

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

- From the Custom Methods screen, choose **Import**
- Locate and select ".method" file
- Choose **Open** (imported method is added to end of Select Method list)

Edit custom method

Edit a custom method in order to change the method settings.

- From Custom Methods screen, select a custom method from the list of available methods
- From the drop-down menu  , choose **Edit**
- Edit method settings as desired
- Choose **Save**

Delete custom method

- From Custom Methods screen, select a custom method from the list of available methods
- From the drop-down menu  , choose **Delete**
- After the confirmation message, choose **Yes**

Measure Kinetics

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

Measure Kinetics

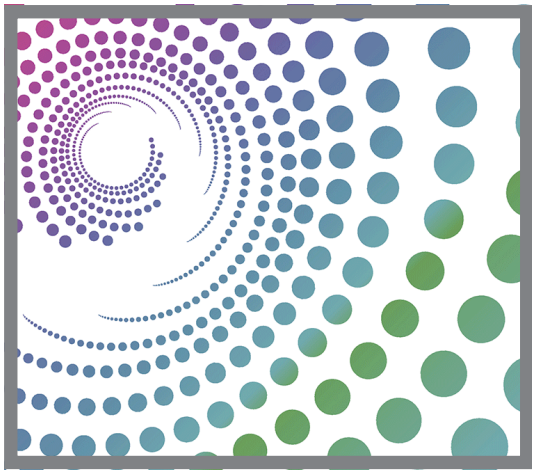
Create Kinetics Method

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

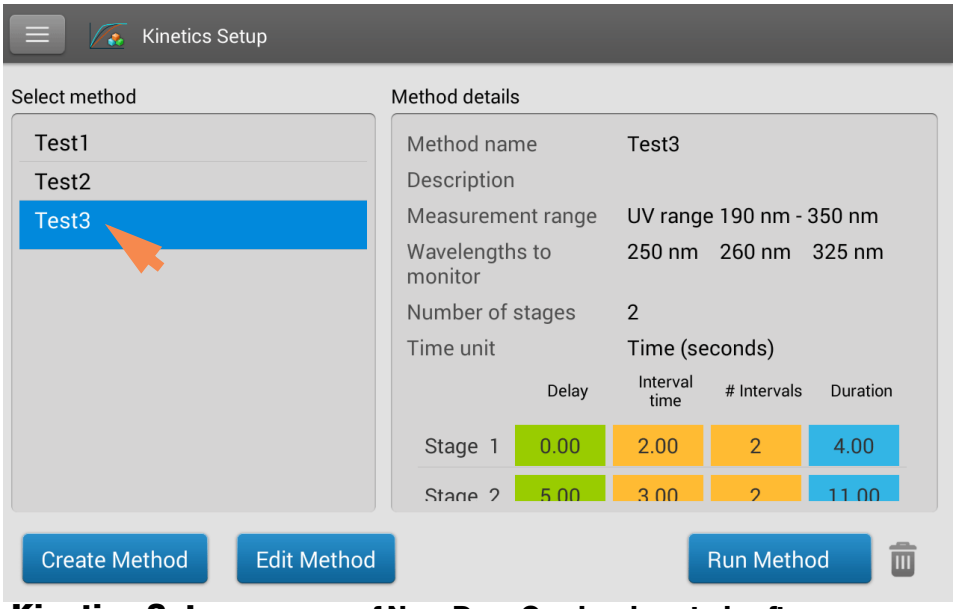
NOTICE

- 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.

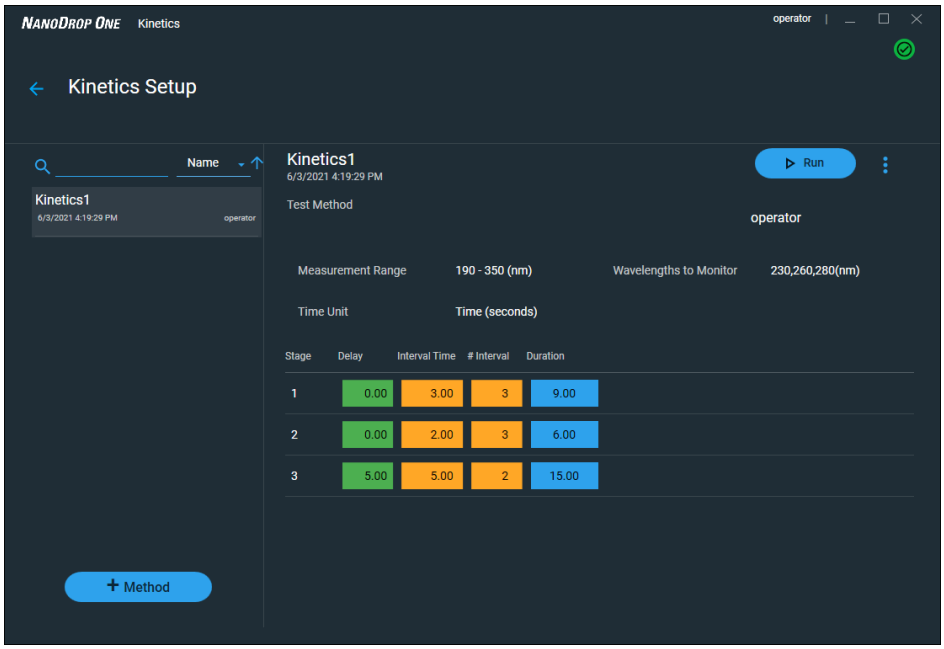
To measure a sample using the Kinetics application

1. From the Home screen, select the **Kinetics** tab and select 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 or the methods list on the left. A description of the selected method appears in the Method Details box on the right.



Kinetics Setup screen of NanoDrop One local control software

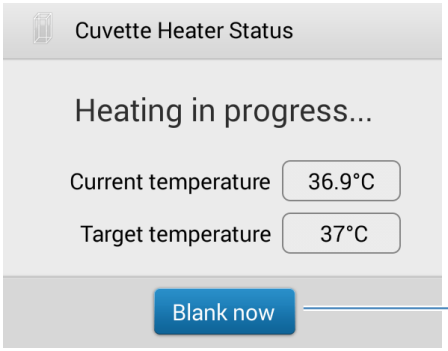


Kinetics Setup screen of PC Control software

2. Select a method:
   - select an existing method by clicking or tapping the **method name** in the Select Method box
   - create a new method by selecting **Create Method** or **+Method**, specifying the method settings and choosing **Save Method**
   - edit an existing method by selecting the **method name** and choosing **Edit Method.** In PC Control, select the method you want to edit and choose **Edit** from the drop down menu next to the run button.
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. Select **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
   - Select **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
   – Select **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, select **Stop**)



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

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

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

Related Topics

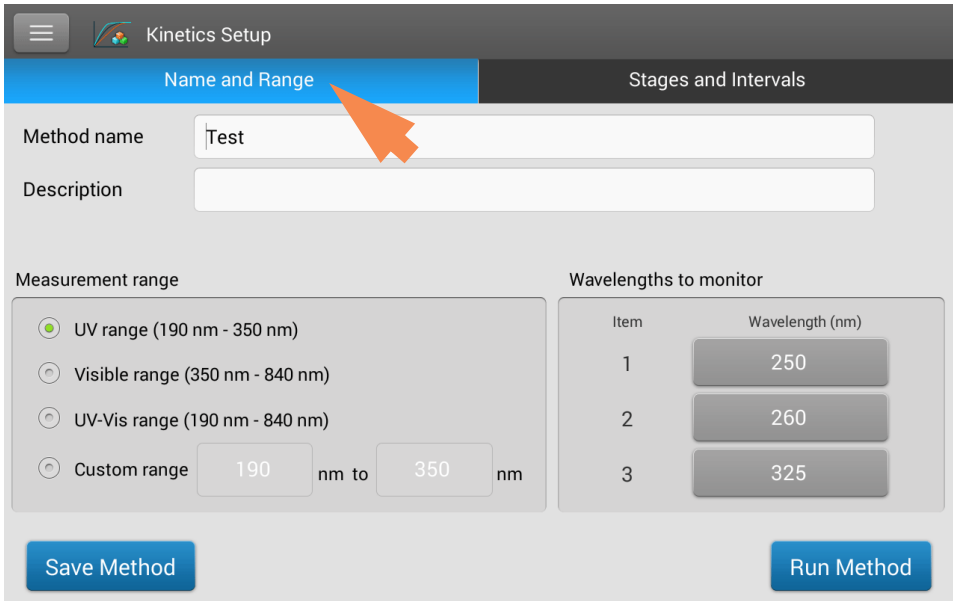
- Measure a Sample Using a Cuvette
- Best Practices for Cuvette Measurements
- Prepare Samples and Blanks
- Basic Instrument Operations

Create Kinetics Method

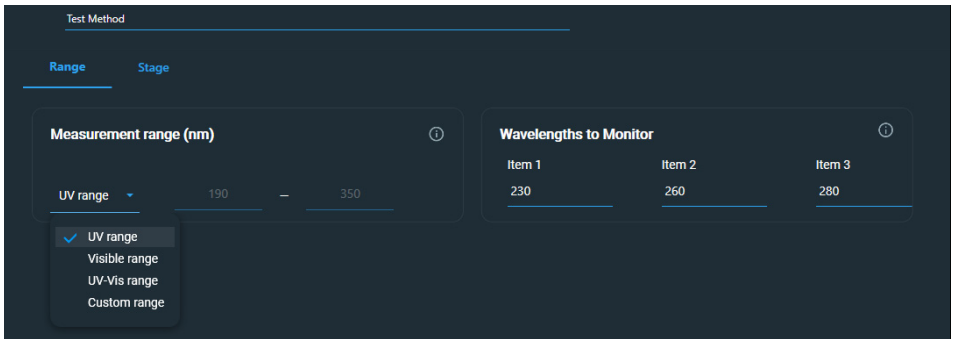
To create a new kinetics method:

– From the Home screen, select **Kinetics** tab > **Kinetics** application

– Select **Create Method**. The method settings are displayed with Name and Range tab selected in local control (**Range** selected in PC control software)



– Enter **Method Name** and **Description** (if desired), select **Measurement range** (use the drop down in PC Control to select ranges, shown below) and specify up to three **Wavelengths to monitor.**



– Select the Stages and Intervals tab in the Local Control, or the **Stage** tab in PC Control. 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, select **Save Method**

– To run the method, select **Run Method**

Edit Kinetics Method

To edit an existing kinetics method:

– From Home screen, select **Kinetics** tab > **Kinetics** application

– Select a method by selecting the method name from the Select Method list

– Select **Edit Method.** In PC Control, select **Edit** form the drop down list next to the **Run** button.

– Edit method settings as desired

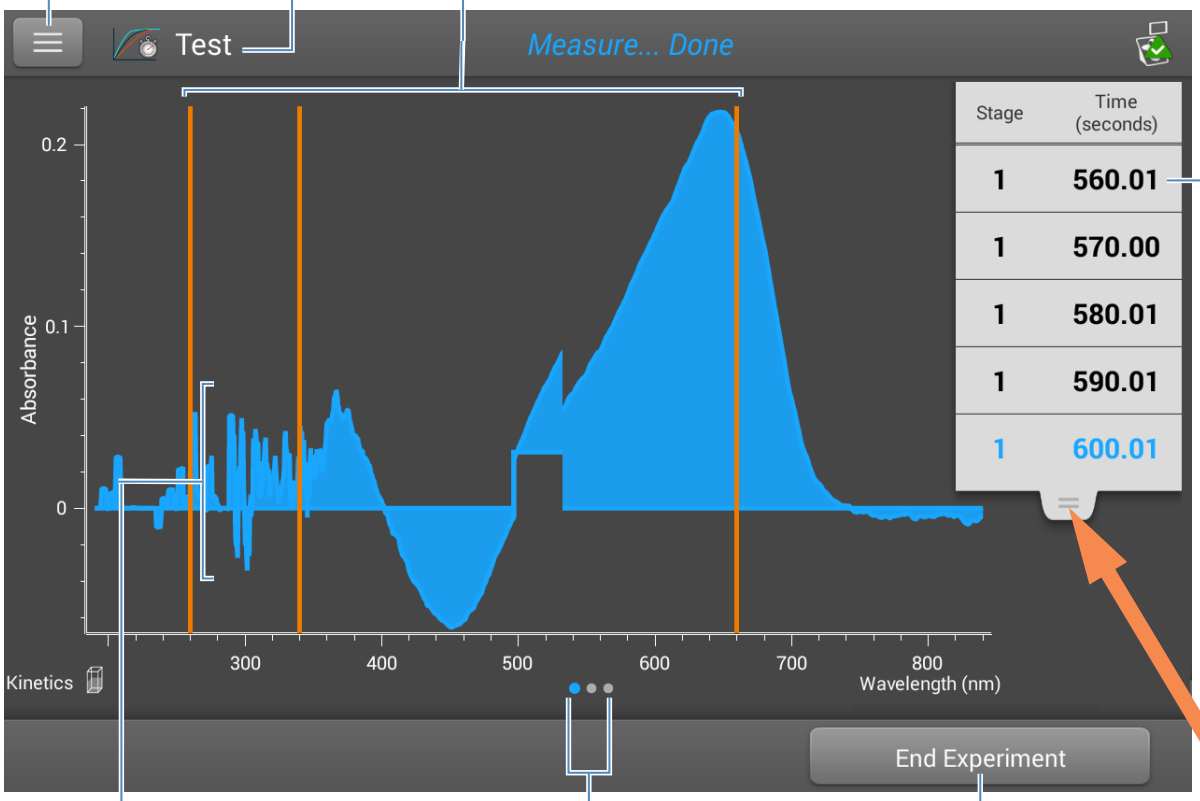
– Select **Save Method** to save your changes

– Select **Run Method**, or **Run** from PC Control, to run the updated method

Kinetics Reported Results

Absorbance measurement screen

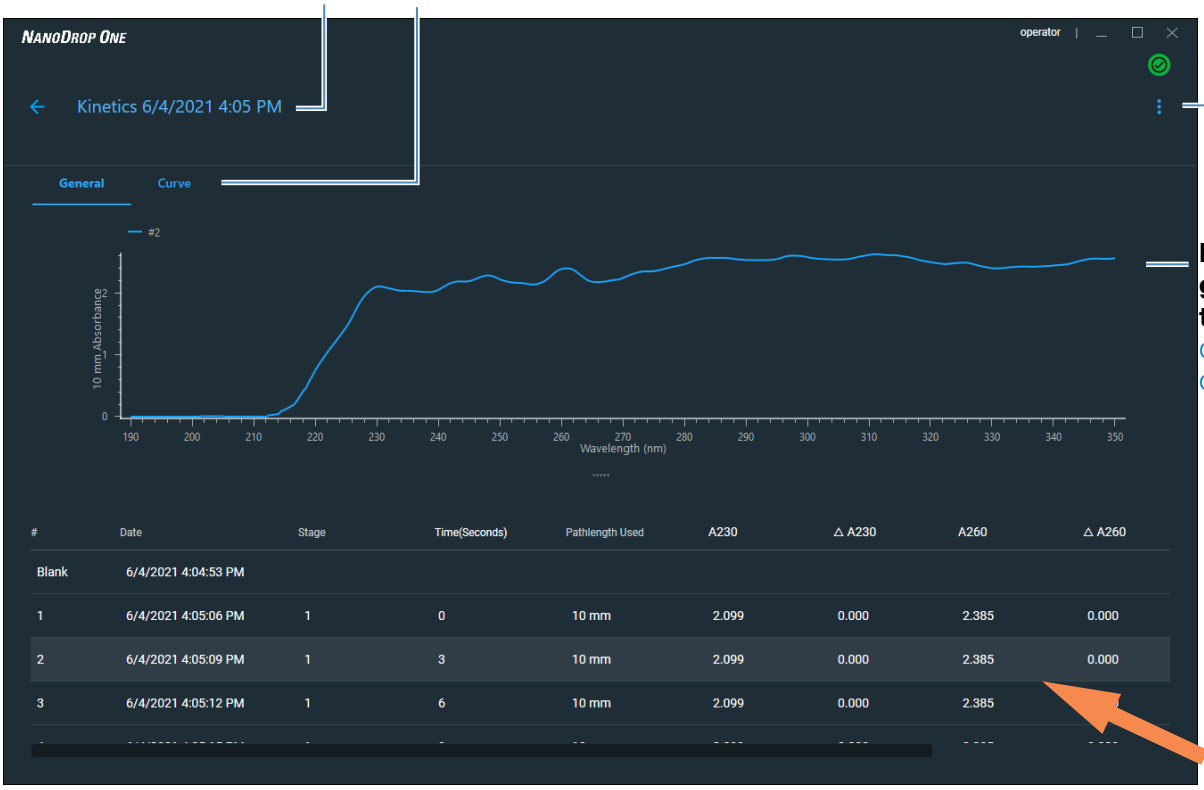
The absorbance measurement screen appears immediately after you tap Measure in the kinetics experiment. This screen shows the absorbance spectrum for each measurement, with wavelength on the X-axis and absorbance on the Y-axis. Vertical lines indicate the specified wavelengths to monitor. The list at 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.



Measurement screen of NanoDrop One local control

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

The following is an example of the measurement screen of the PC Control software:

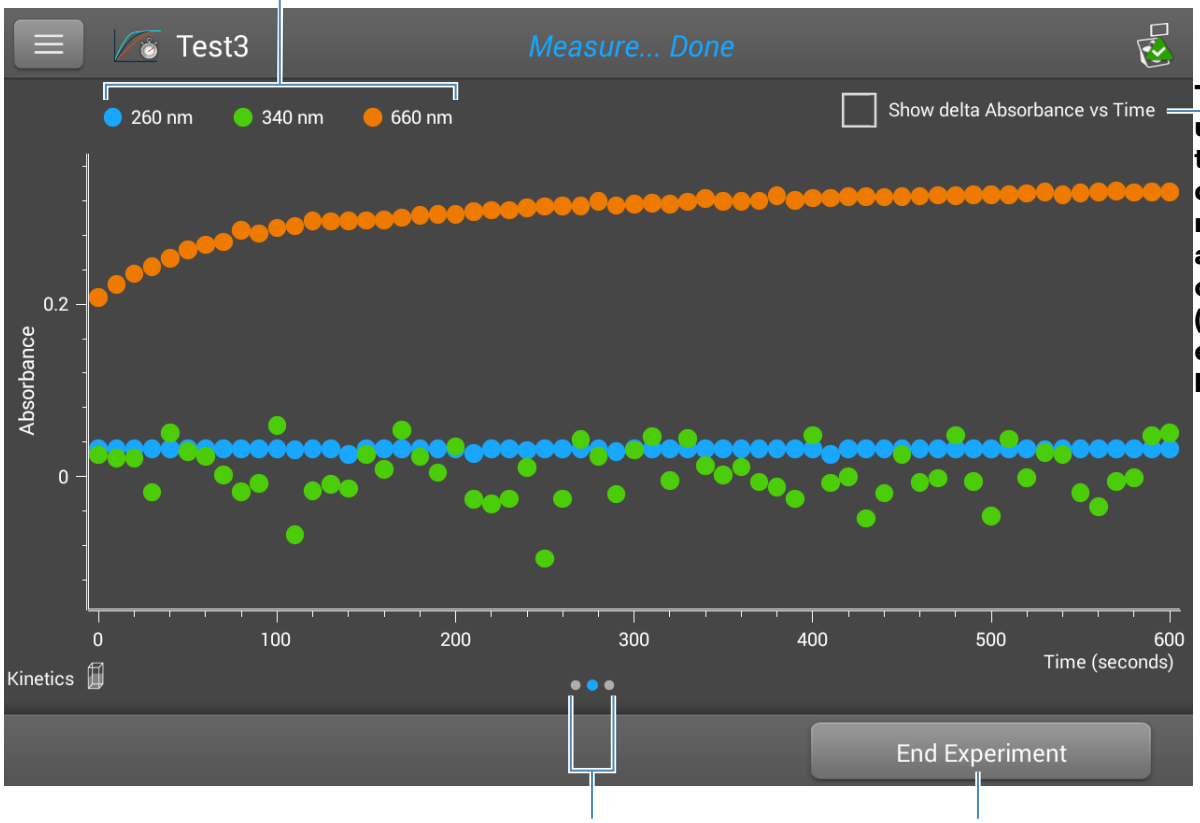


Absorbance screen shown from History screen of PC Control software

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)

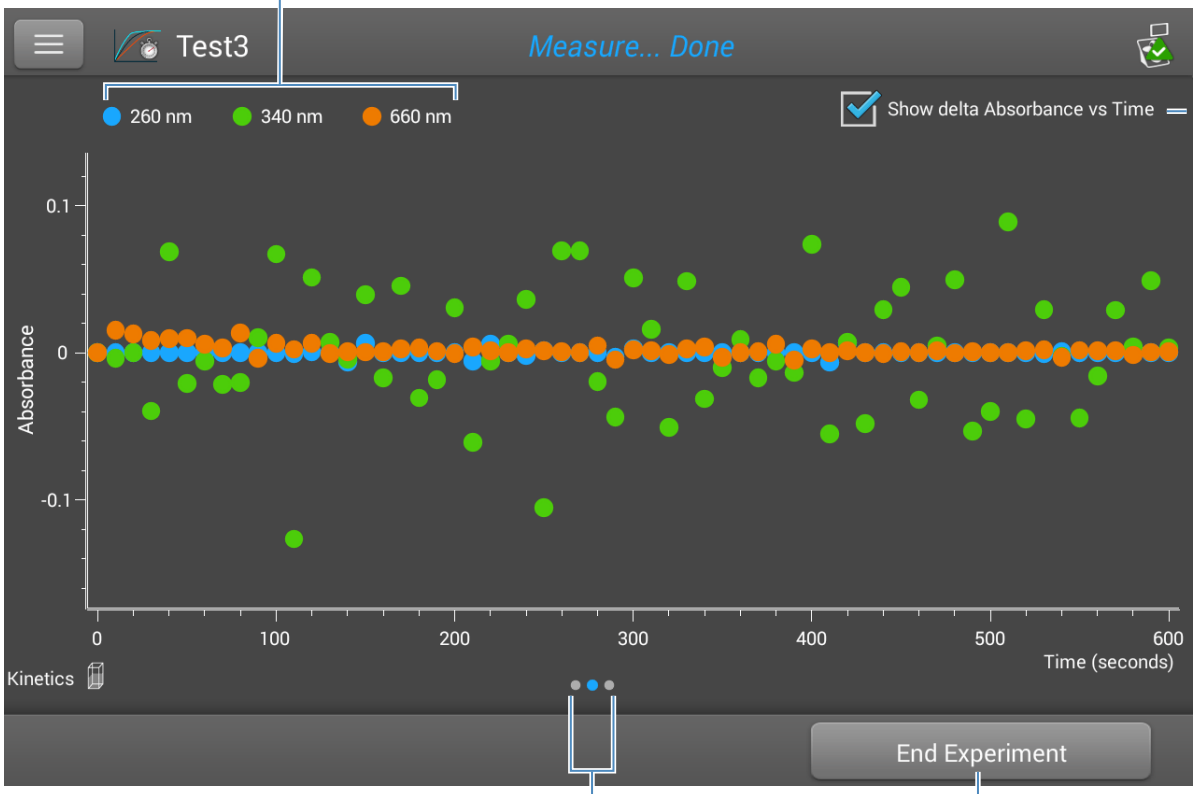
Swipe screen left to view data table; swipe right to return to absorbance measurement screen

Tap to end experiment and export data

Rate Measurement screen of NanoDrop One local control

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



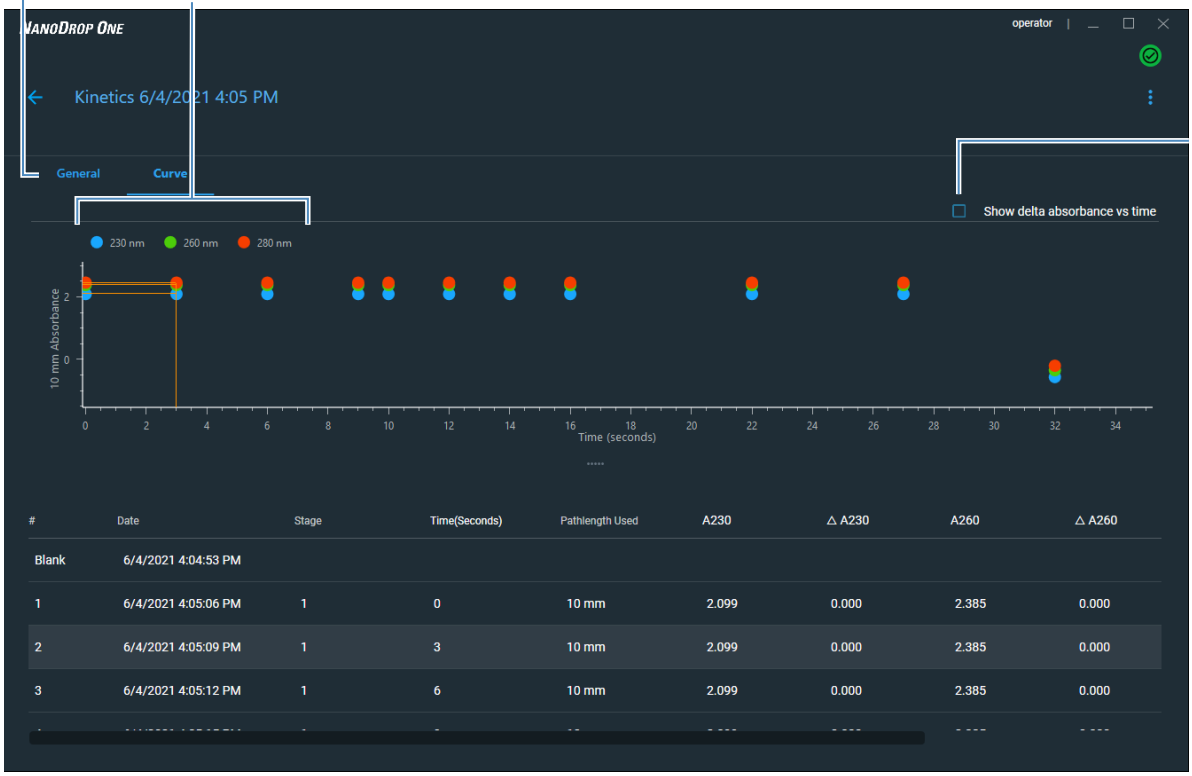
Tap to update plot to show measured absorbance over time (see example above)

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

Tap to end experiment and export data

Click General to return to absorbance measurement screen

User-defined wavelengths

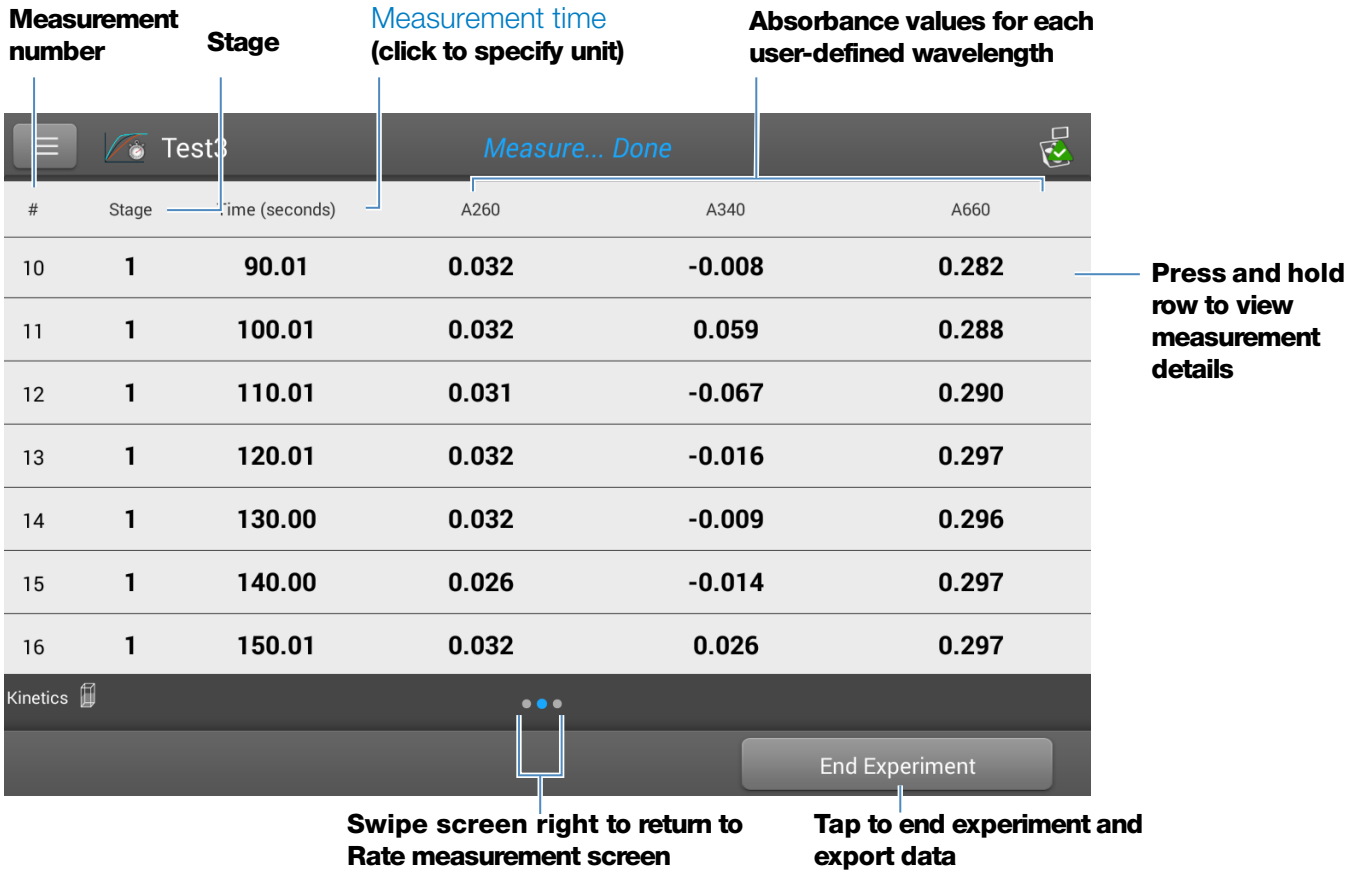


Click to update plot to show change in measured absorbance over time

Rate Measurement screen as shown from History screen of PC Control software

Data Table

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



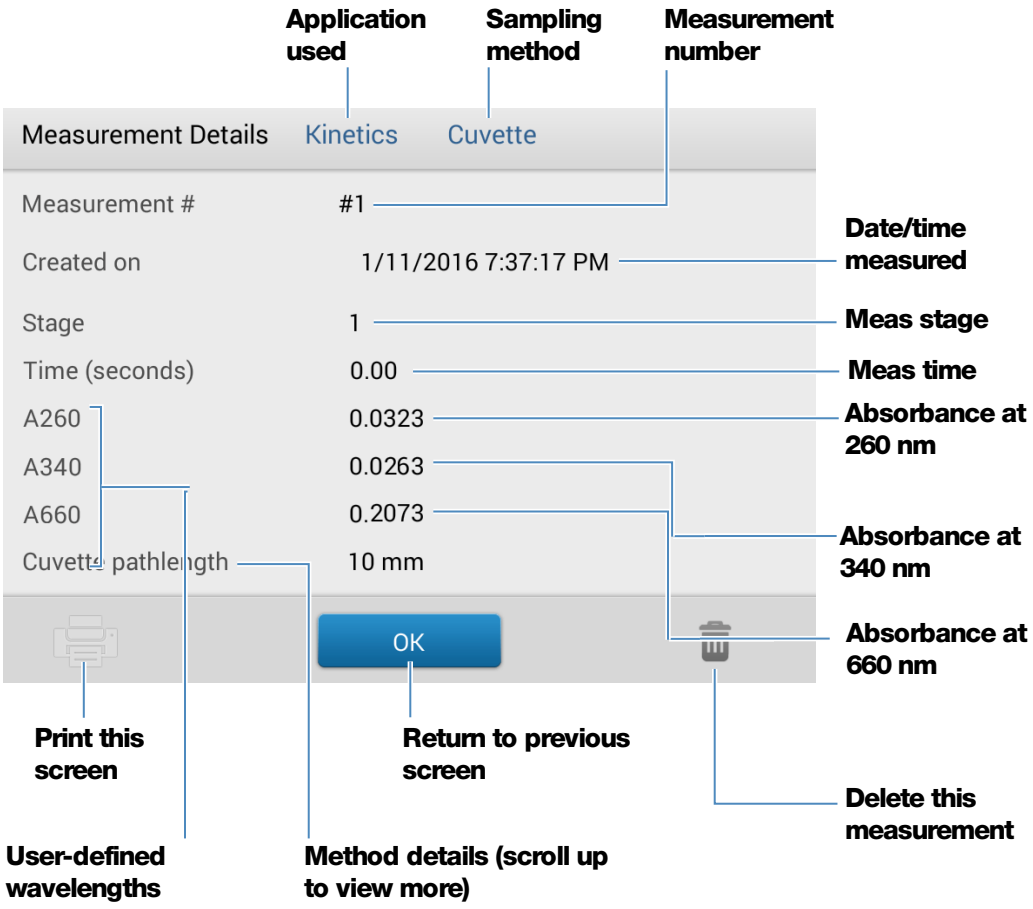
Data table as shown in NanoDrop One local control



Data table as shown in PC Control software

Measurement Details

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



Related Topics

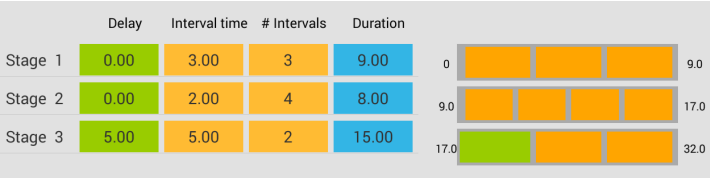
- Basic Instrument Operations

Settings for Kinetic Measurements

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

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.
	Time Unit	Select the **unit** for time-based measurements (seconds or minutes).

Tab	Setting	Description
	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

9

Learning Center

Contents

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- Operating Specifications 205
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- Measure a Sample Using a Cuvette 211
- Prepare Samples and Blanks 214
- Basic Instrument Operations 219
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- Instrument Settings 255
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Micro-Volume Sampling—How it Works

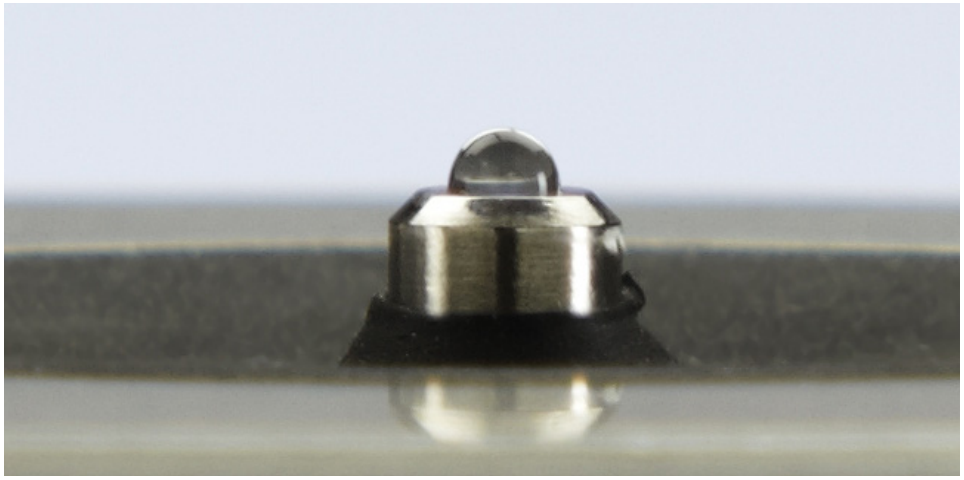
Surface Tension

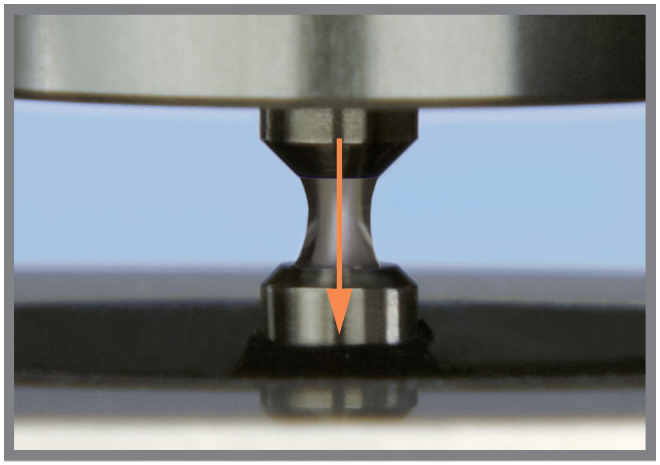
Absorbance Spectrum

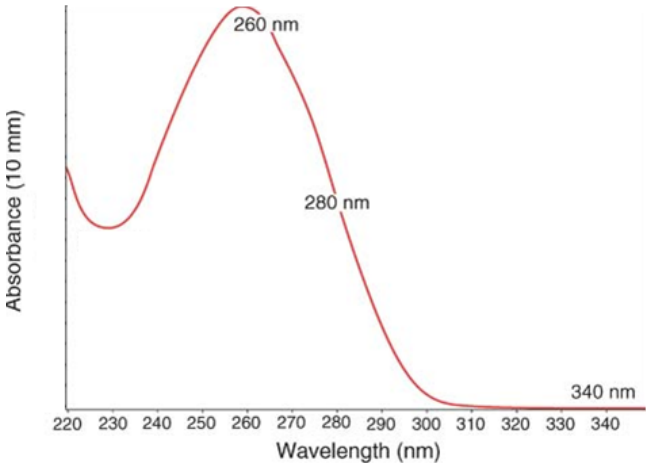
Sample Absorbance

Sample Concentration

Baseline Correction







Surface Tension

The NanoDrop One^{C} 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.

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

Sample Absorbance

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

Beer-Lambert equation

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

where:

A = absorbance in absorbance units (A)

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

b = pathlength in cm

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

Sample Concentration

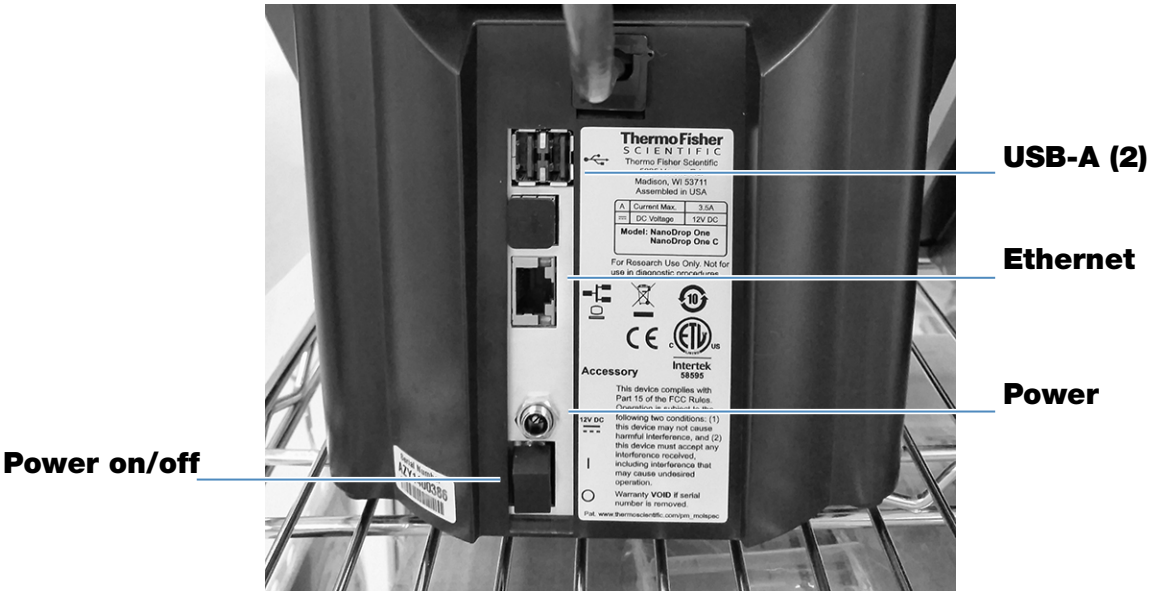
The Beer-Lambert equation (Beer's law) shown at the left is used to correlate sample absorbance with concentration.

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

Baseline Correction

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

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. See “Power Cords” on page 292 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

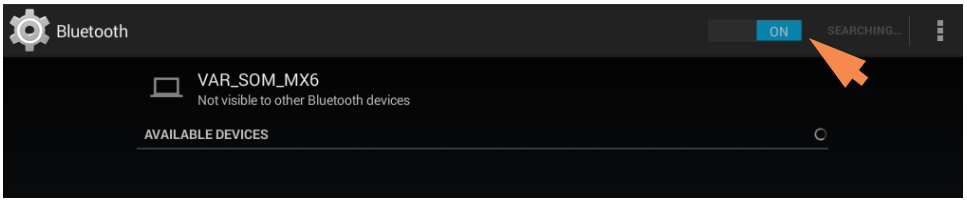
– from instrument Home screen, tap  (**Settings**)

– tap **System** tab

– tap **Bluetooth (**if Bluetooth is disabled, button in upper right is set to “Off” and no Bluetooth input devices are listed)

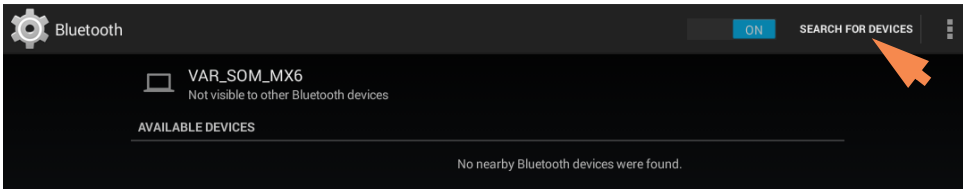


– tap **Off** button to enable Bluetooth connectivity (button turns blue, changes to “On” and software automatically searches for any available Bluetooth input devices)

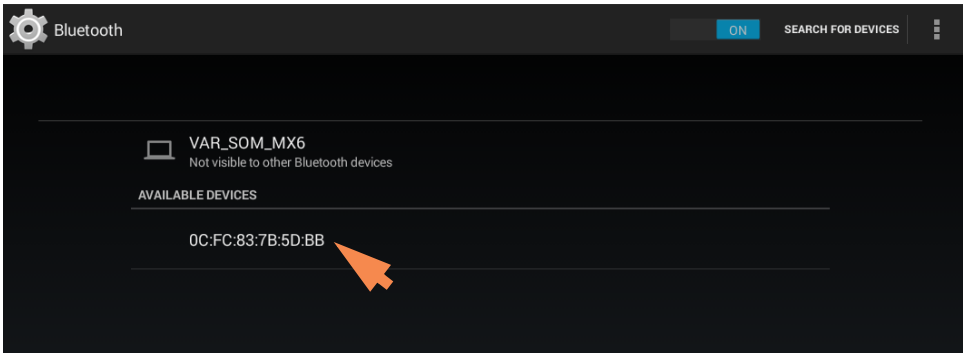


If no Bluetooth devices are found, after a few seconds the message “No nearby Bluetooth devices were found” is displayed

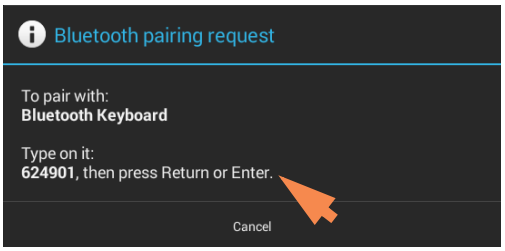
– to **add a Bluetooth device**, follow manufacturer instructions to pair the device (for example, you may need to hold down a button) and tap **Search For Devices** on instrument)



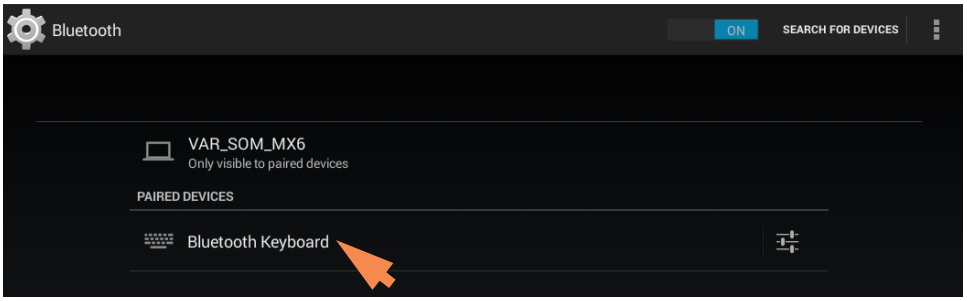
device name should appear in Available Devices list



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

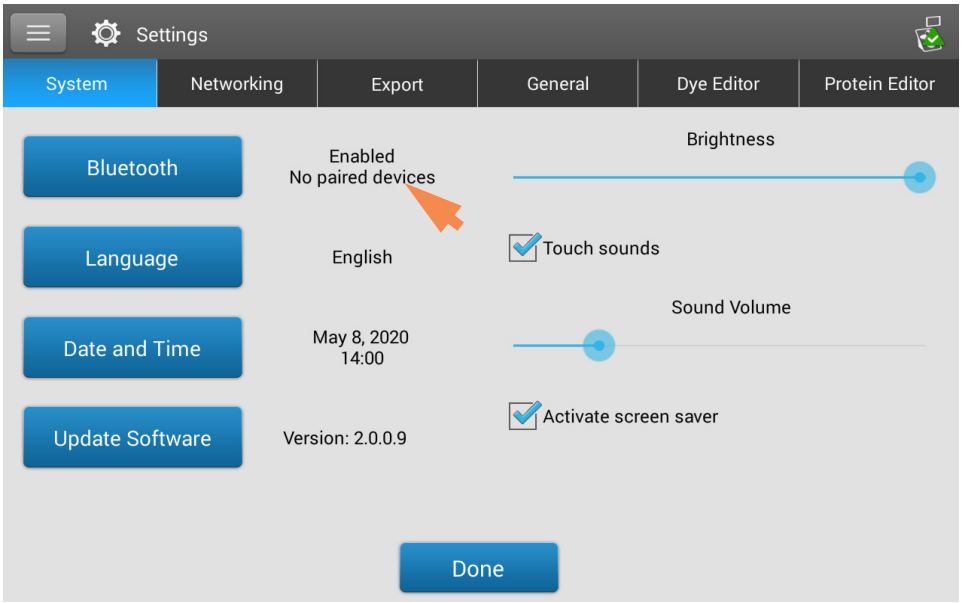


– **complete any instructions** to pair the device



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)

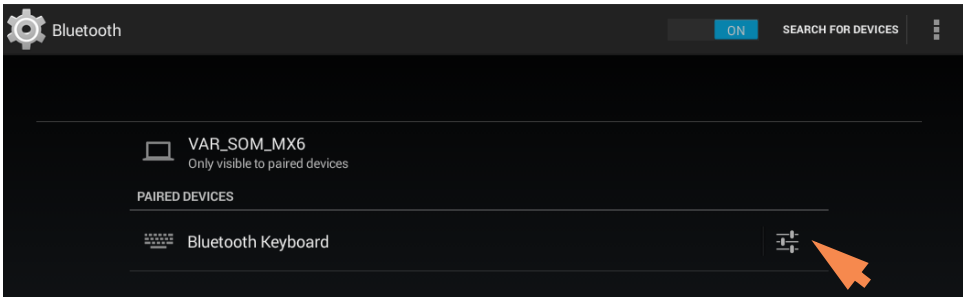


– repeat steps above to add another Bluetooth device or tap **Done** to close Settings

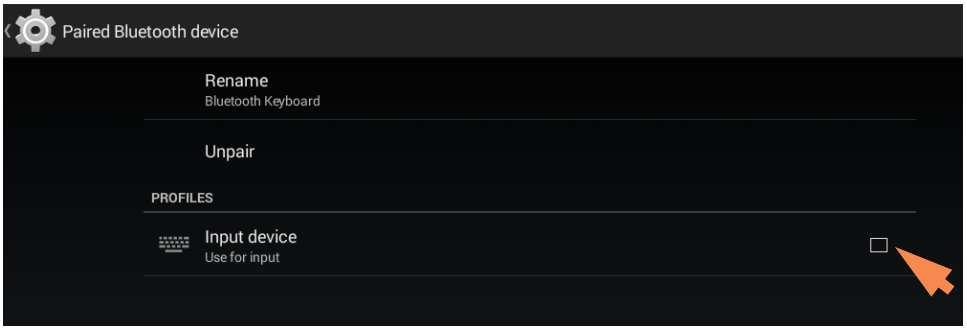
Deselect Bluetooth input device

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



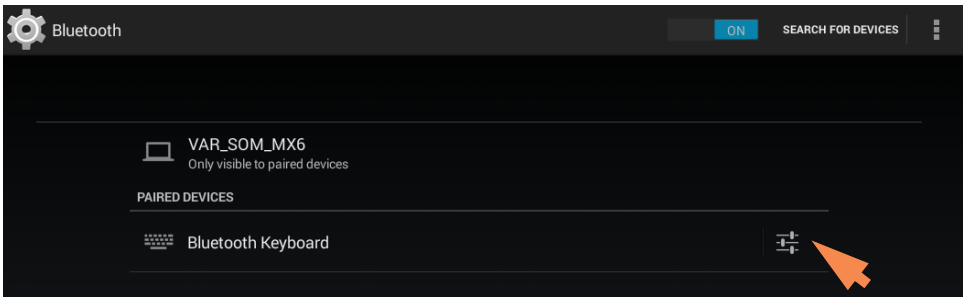
- tap **Paired Bluetooth Device** in upper left to return to previous screen
- tap **Back** to return to System settings
- tap **Done** to close Settings

Note

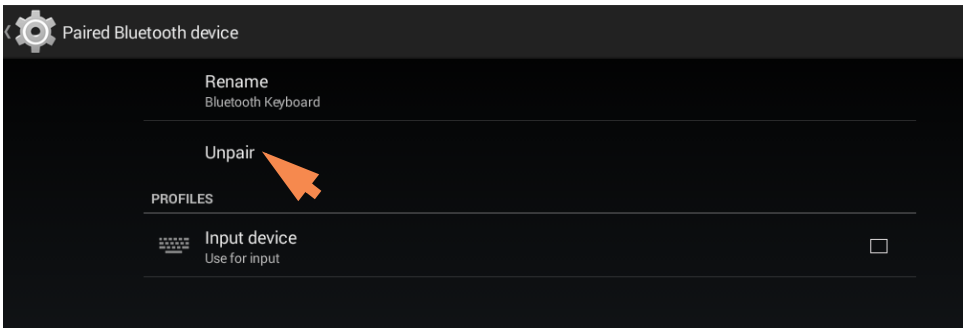
- If no Bluetooth device is selected for input, the instrument relies on the integrated touchscreen keyboard for input.
- To select the device again, follow the steps above and select the device's Use for Input checkbox.

Disconnect Bluetooth device

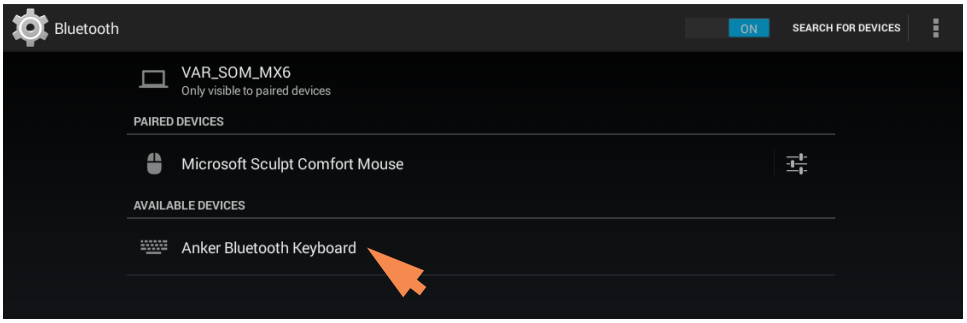
- from instrument Home screen, tap 
- tap **System** tab
- tap **Bluetooth**
- to disconnect paired Bluetooth device, tap its Profiles button 



- 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 either a personal computer (PC) or an active network wall jack.

If the instrument is connected to a network wall jack, you can export data files to a network location, for example, in order to transfer them to another computer. You can define multiple network paths that the operator can select when exporting data. See Export Settings for details.

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

– from instrument Home screen, tap 

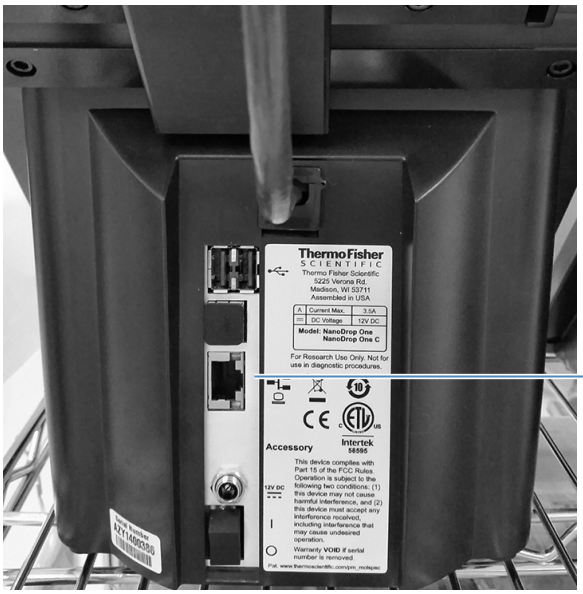
– tap **Networking** tab

– tap **Ethernet**

– select an Ethernet option and choose **OK**.

  - **Direct connection to a PC**. Select if you plan to connect an Ethernet cable between the NanoDrop One instrument and a personal computer.
  - **Connection to a network jack**. Select if you plan to connect an Ethernet cable between the NanoDrop One instrument and a network wall jack.

– connect one end of Ethernet cable to Ethernet port on instrument back panel



– connect other end of Ethernet cable to either the computer Ethernet port or an active network wall jack

Set up Wireless Connections

Select Wi-Fi network on the instrument

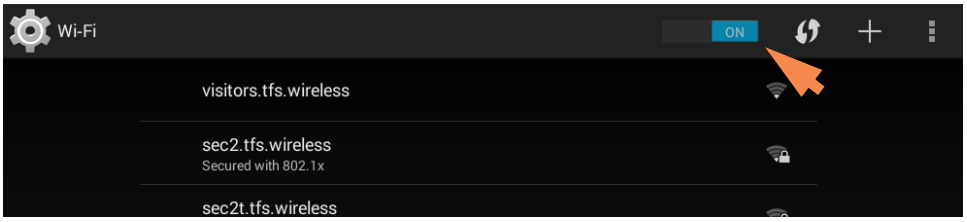
– from instrument Home screen, tap (Settings)

– tap **Networking** tab

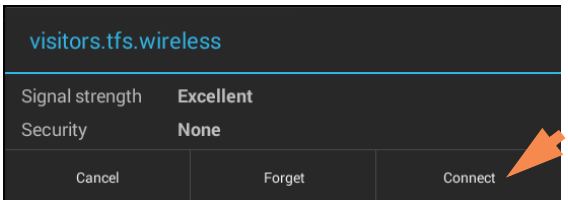
– tap **Wi-Fi (**if Wi-Fi is disabled, button in upper right is set to “OFF” and no wireless networks are listed)



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

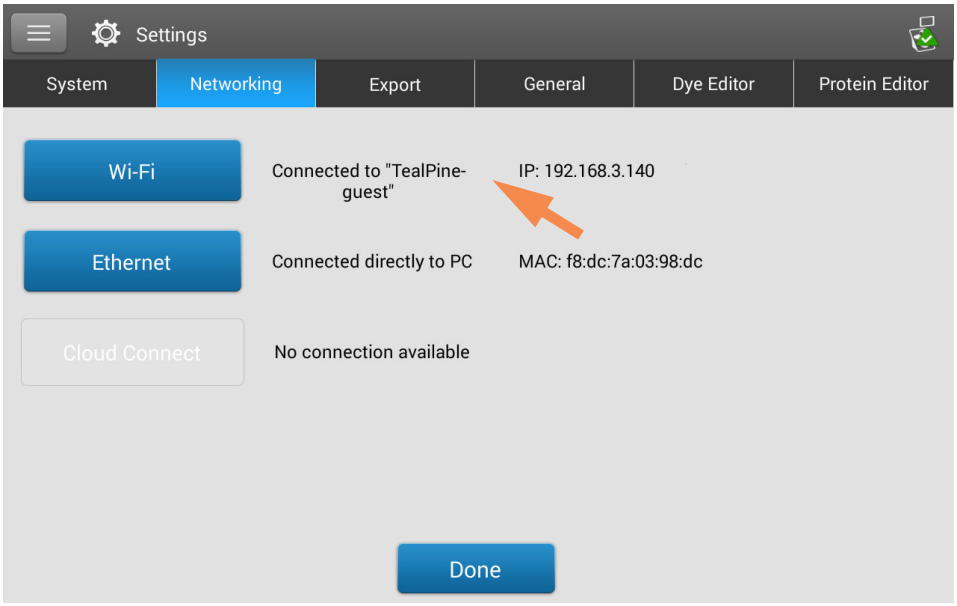


– select remote computer's Wi-Fi network host and tap **Connect** (here is an example)



– 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)

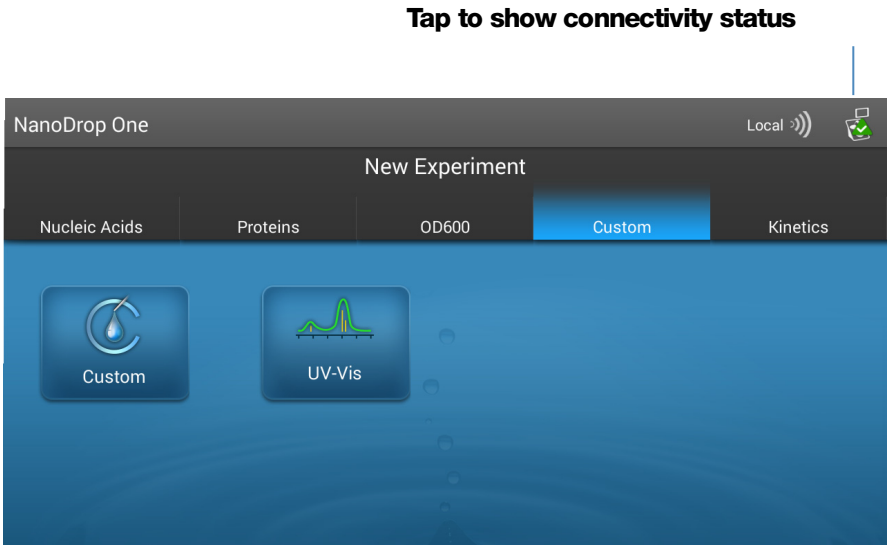
Note Some Wi-Fi networks may require an identity, password or other information before you can connect to them, or they may be anonymous (that is, you may have to search for them by name). For more information, see the system administrator at your work site.



– tap **Done** to exit Settings

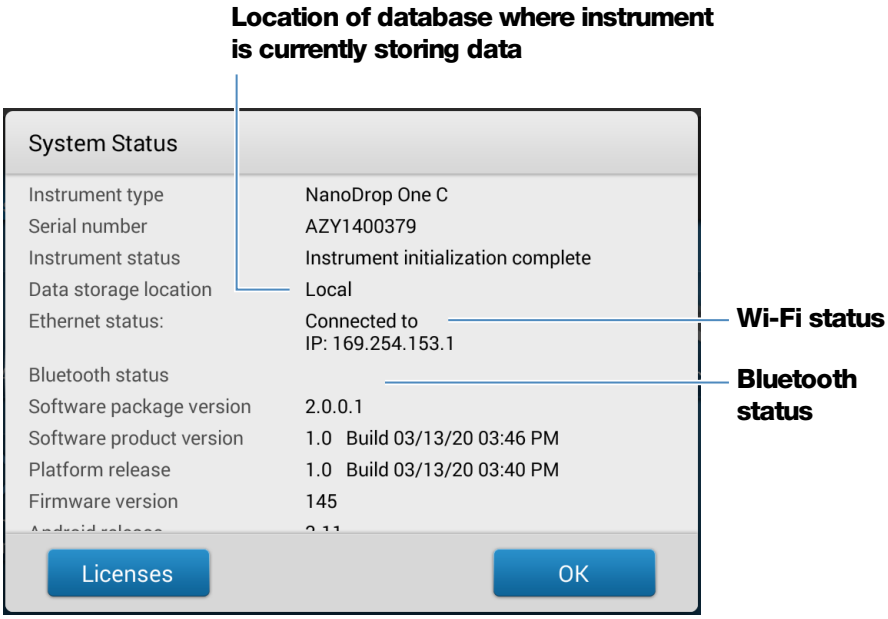
Assess Instrument Connectivity

Use the System Status icon at the top right of the instrument Home screen to quickly assess the instrument's connectivity status including Bluetooth 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%

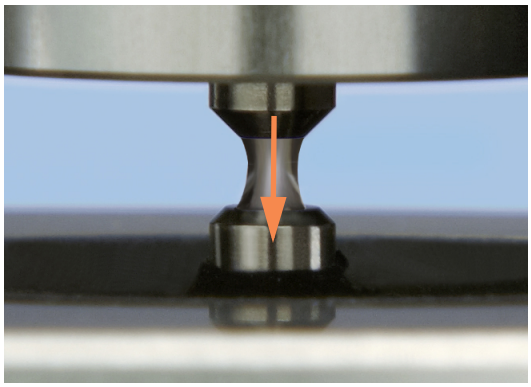
Locate the instrument away from air vents and exhaust fans to minimize evaporation.

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.

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

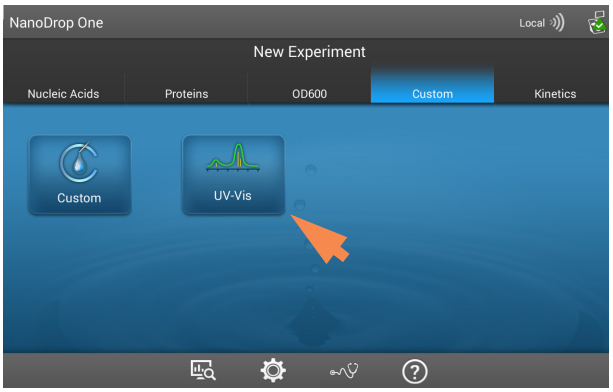
Application	Sample Volume
Nucleic acid (aqueous solution)	1 µL[a]
Purified protein	2 µL
Other protein applications such as Bradford or BCA	2 µL
Microbial cell suspensions	2 µL

[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. From the instrument Home screen, select an application from one of the application categories, such as **UV-Vis**, or **Custom** Methods.

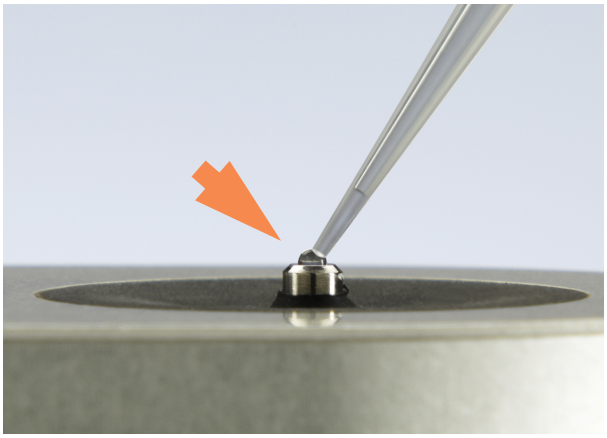


2. Lift the instrument arm and clean the upper and lower pedestals with new laboratory wipe.

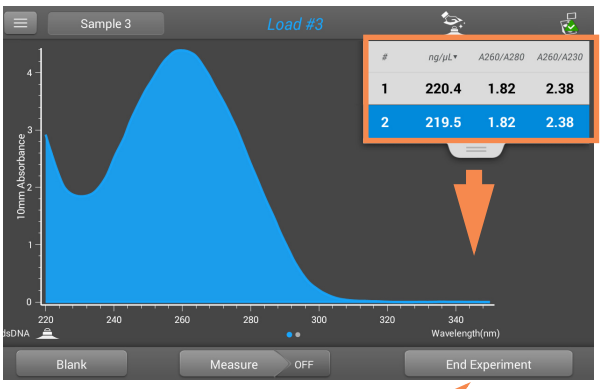


3. Measure a blank:
   - Pipette 1–2 µL blanking solution onto the lower pedestal and quickly lower the arm
   - Tap **Blank** and wait for the measurement to complete

     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



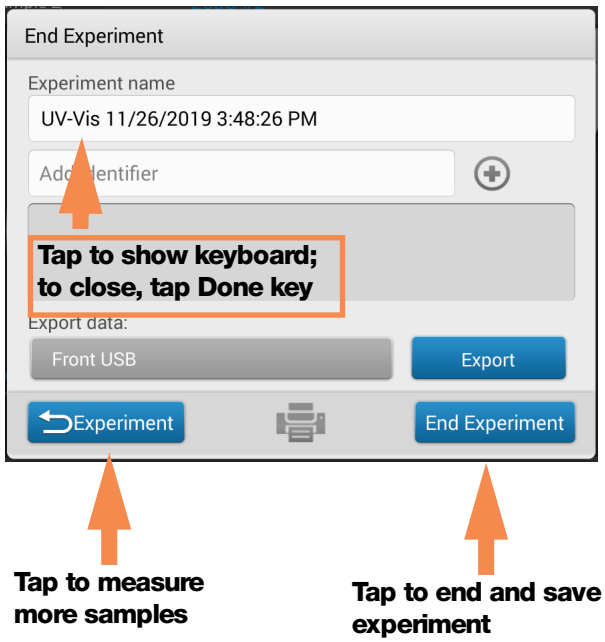
4. Measure the first sample:
   - Pipette 1-2 µL sample solution onto the pedestal and quickly lower the arm (see Recommended Sample Volumes for more information)
   - Start the sample measurement:
     - if Auto-Measure is On, lower arm
     - if Auto-Measure is off, lower arm and tap **Measure**
 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



Tap to end experiment

5. 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.)



6. When you are finished measuring samples:
   - Tap End Experiment (see previous image)
   - Enter an experiment name (tap **Experiment Name** box to display keyboard), 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).

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.

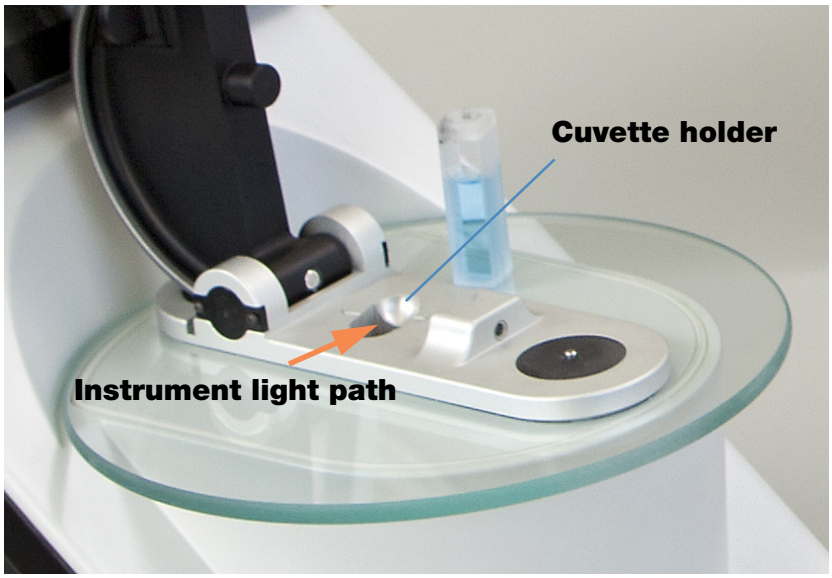


Supplies needed

- 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.
- 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.



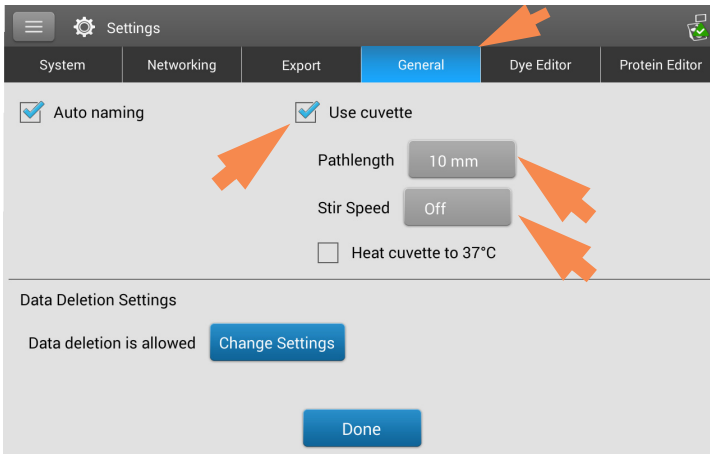
To measure a sample using a cuvette

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 ans may cause permanent damage.

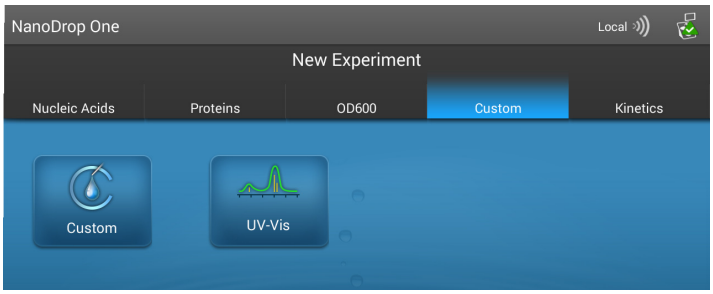


1. From the Home screen, select (Settings)

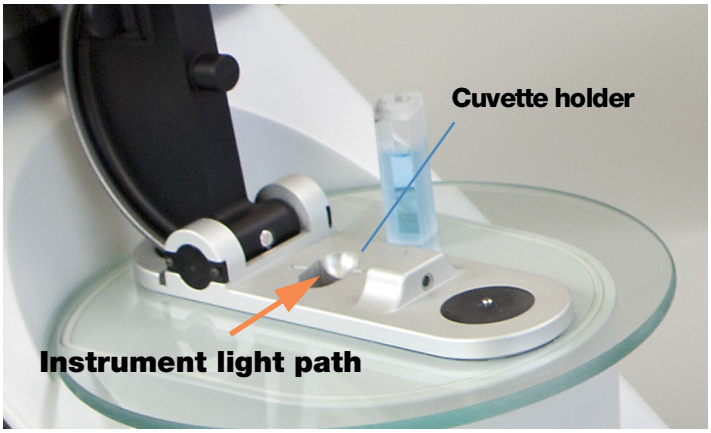


2. Specify the cuvette options:
   - Select **General**
   - Select **Use Cuvette**
   - Set **Pathlength** to pathlength (width) of cuvette (see cuvette manufacturer for specifications)
   - Set stirrer and heater if desired
   - Select **Done**

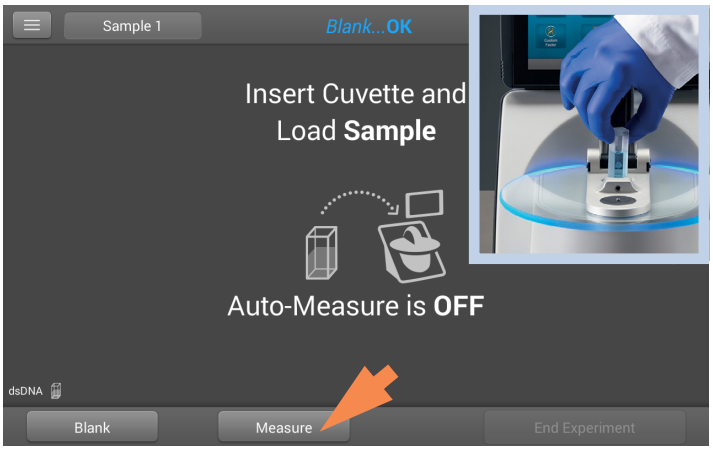
   See General settings for details.



3. From the Home screen, select an application



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



5. 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

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.

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

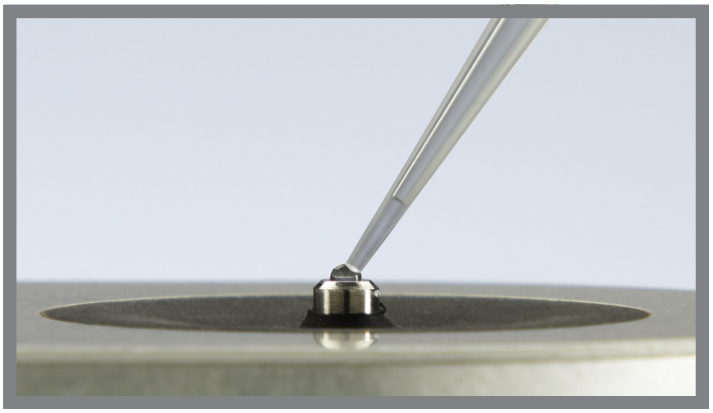
The buffer used to resuspend a sample analyte can contribute absorbance. Blanking minimizes any absorbance contribution due to the buffer components from the sample measurement. The resulting sample spectrum represents the absorbance of only the analyte of interest. For more information, watch the multimedia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

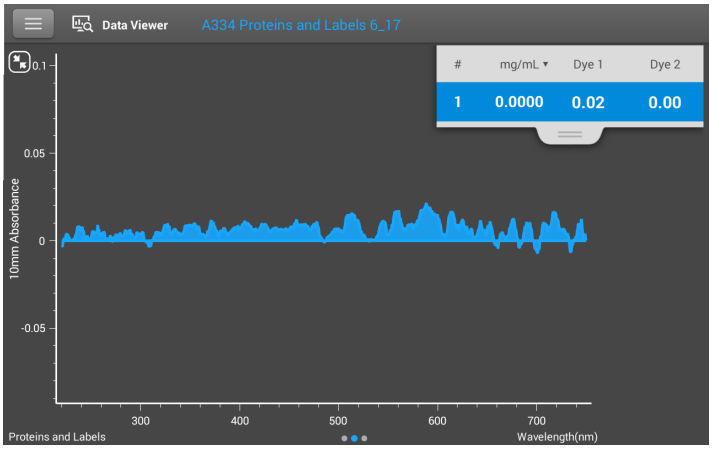
For best results:

- 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.





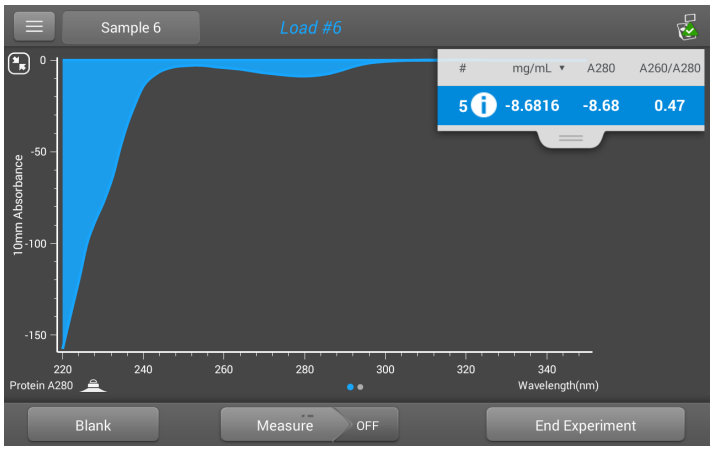
Good blanking buffer (measured abs < 0.04)

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.)

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.



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

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

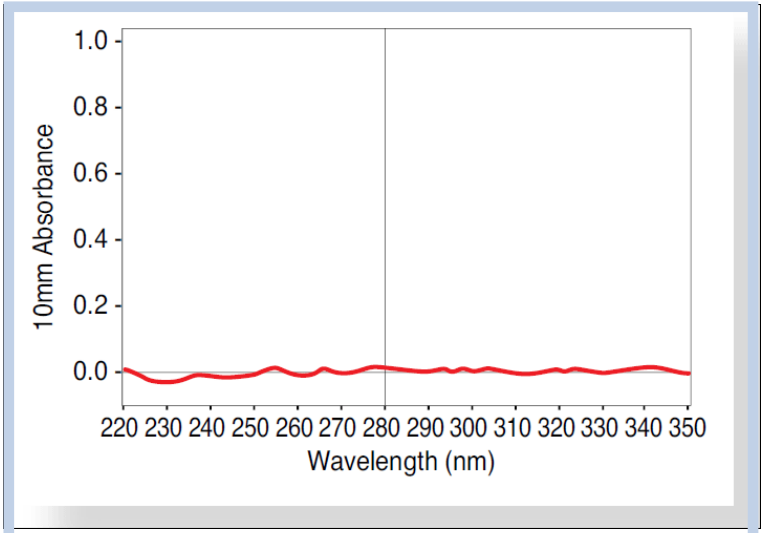
- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.

1. From the Home screen, select an application name.
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.

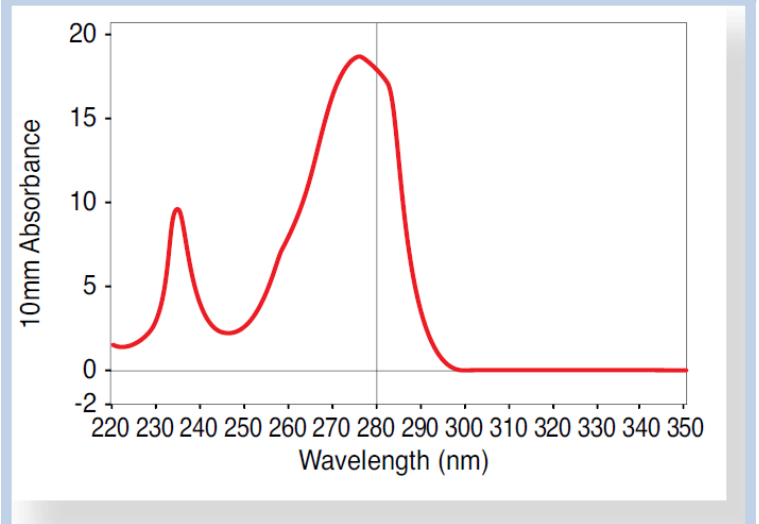
   The resulting spectrum should vary no more than 0.04 A from the baseline at the analysis wavelength.

   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

Basic Instrument Operations

- NanoDrop One Home Screen
- NanoDrop One Measurement Screens
- View History
- NanoDrop One General Operations

NanoDrop One Home Screen

These operations are available from the NanoDrop One Home screen.

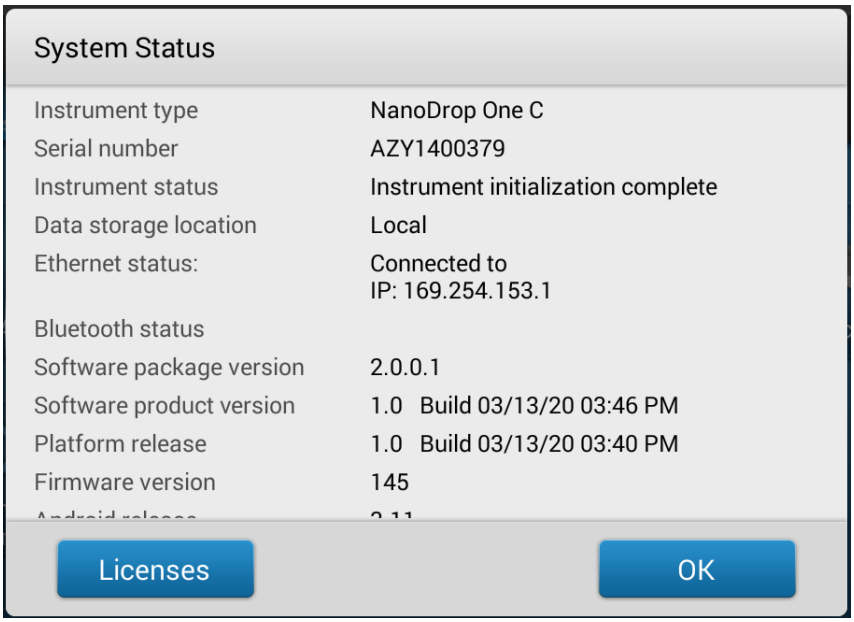


Applications

The NanoDrop One software offers several configurable applications, which gives users full control of the measurement. See"Custom Applications" on page 153 for detailed information about each available application.

System Status

Tap  on the instrument Home screen to open the system status box.



The available information is described below.

Instrument type	Instrument model (NanoDrop One^{C})
Serial number	Instrument serial number
Instrument status	Current status of the instrument
Data storage location	Indicates location of database set where instrument is currently storing data.
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 package version	Version of instrument operating software installed
Platform release	Version of instrument platform software installed

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

History

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. See "View History" on page 230 for more information about the History feature on the instrument.

Instrument Settings

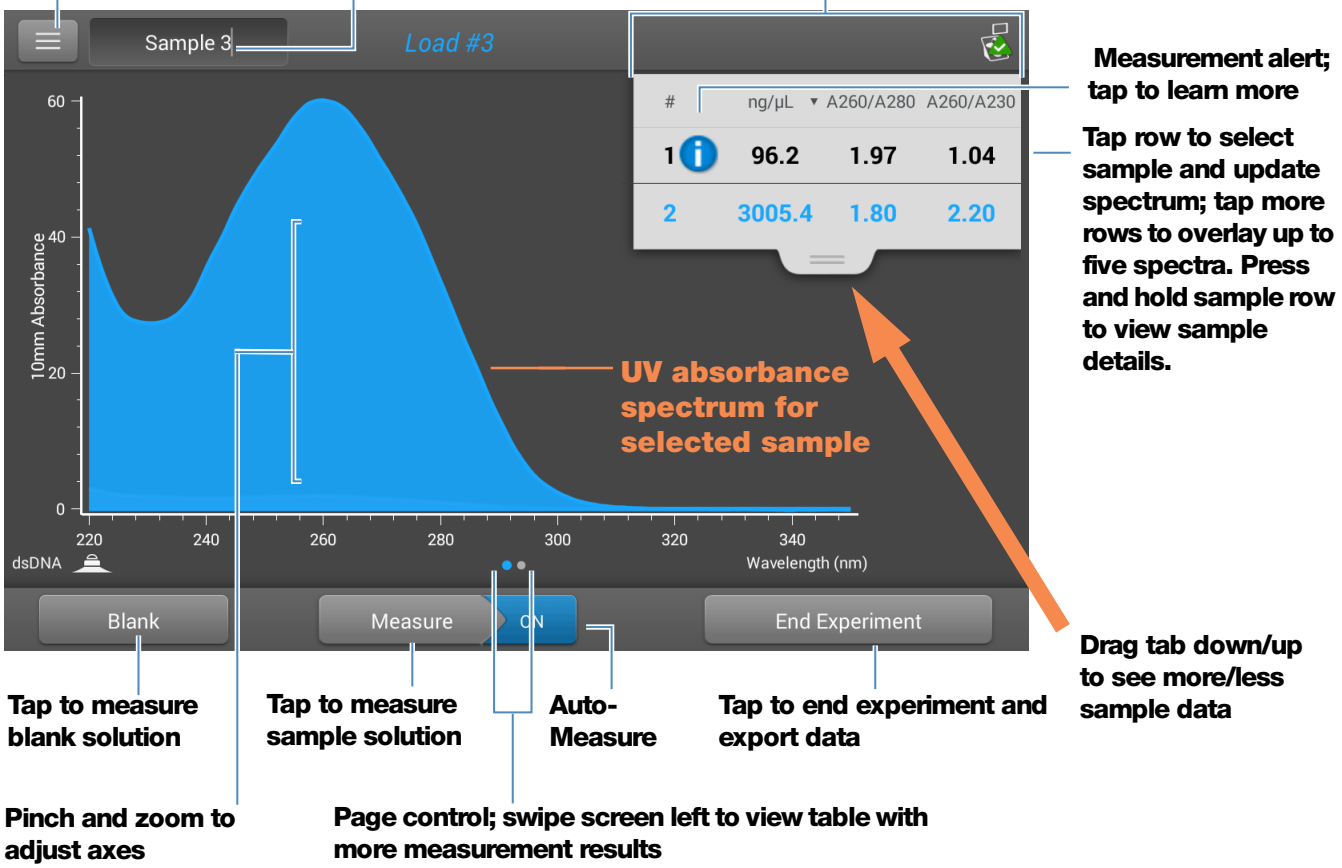
Tap  on the Home screen to access instrument settings for software updates, cuvette sampling, networking and more. See "Instrument Settings" on page 255 for detailed information about all available instrument settings.

Instrument Diagnostics

Tap  on the Home screen to verify instrument operation. Instrument diagnostics should be run periodically according to the recommended maintenance schedule. See "Instrument Diagnostics" on page 278 for information about how to run the available instrument diagnostics.

NanoDrop One Measurement Screens

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



Menu

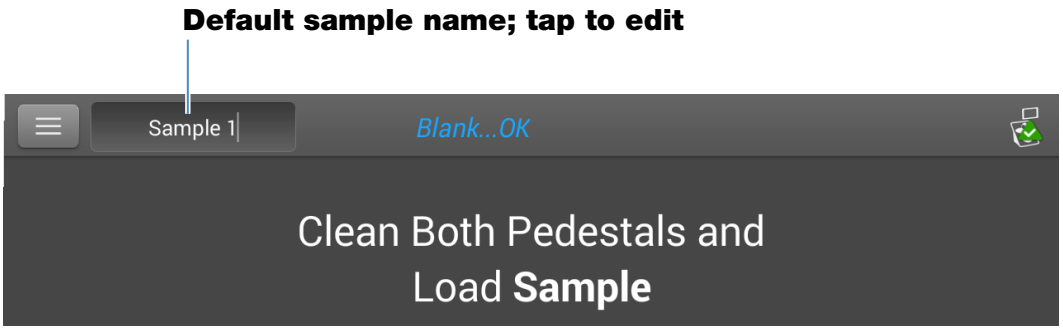
Tap ☰ in any measurement screen to see the available menu options.

Home	Return to NanoDrop One Home screen
[application] Setup	View or change settings for selected application
Settings	View or change instrument settings **Note**: The Dye/Chrom. Editor and Protein Editor tabs appear in Settings only when the Settings tab is opened from the NanoDrop One Home screen or from applications compatible with that functionality.
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.

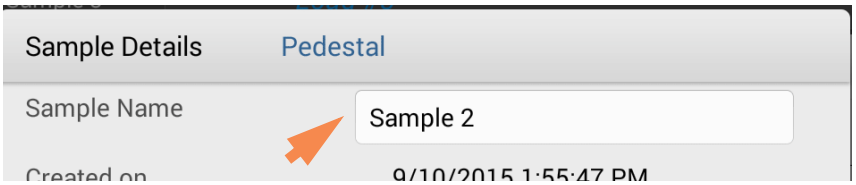
Edit default sample base name

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 History
- 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 to close keyboard
– tap **OK** to close Sample Details box

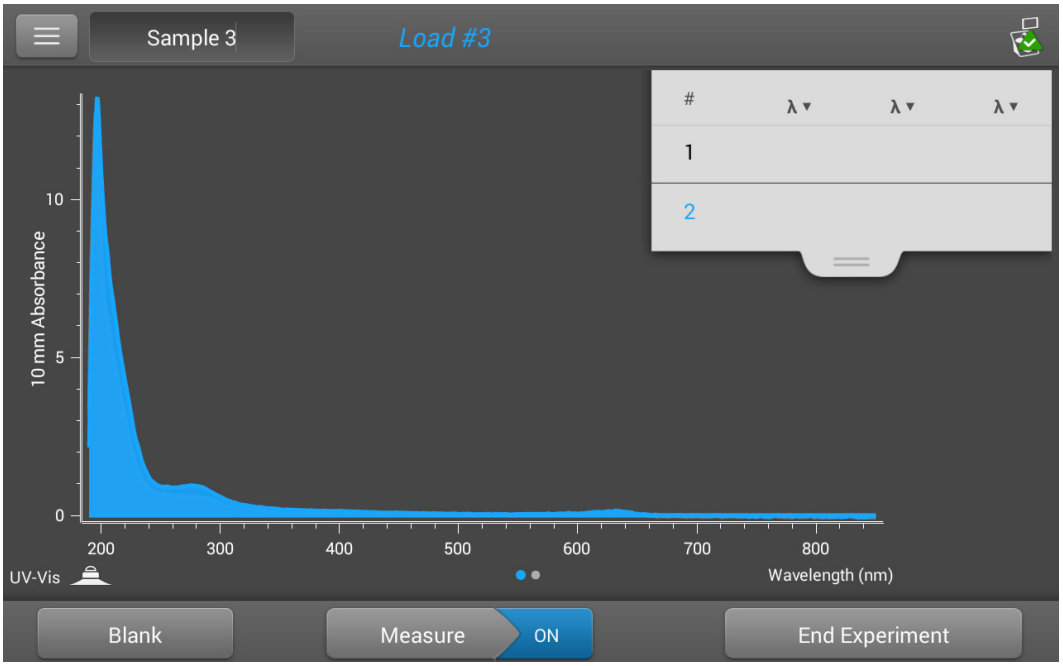
Measurement Results

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

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

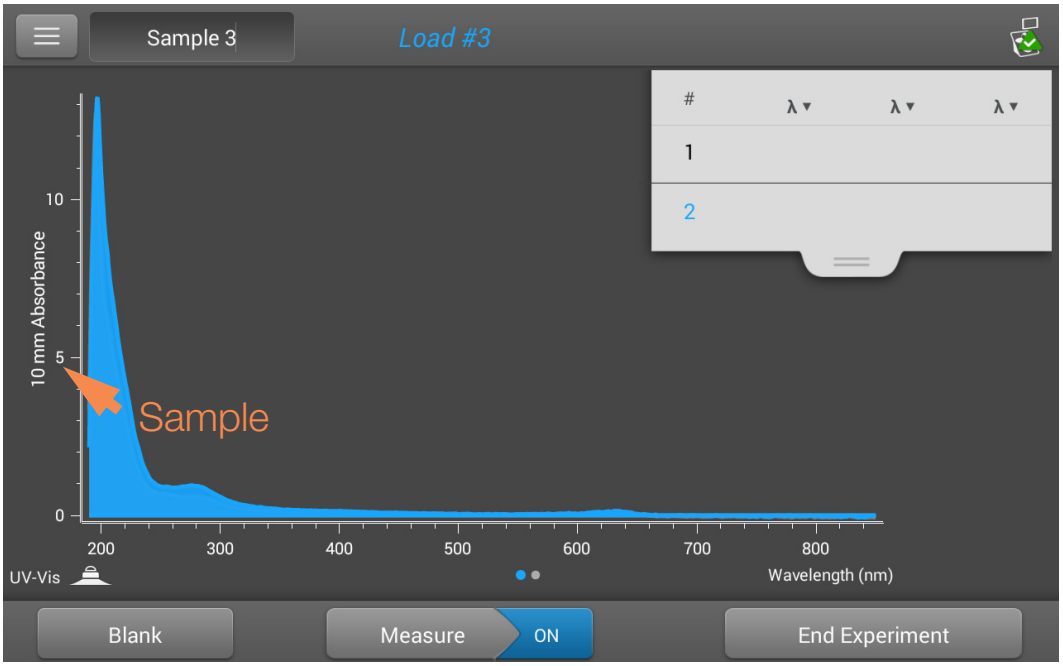
Absorbance Spectrum

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. Here is an example for a UV-Vis method.



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. Here is an example.



Measurement Alerts

The Acclaro Sample Intelligence technology built into the NanoDrop One instruments provides important features to help you assess sample integrity. Tap or click a Sample Intelligence icon in the software to view its associated information.



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 Button

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 Button

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.

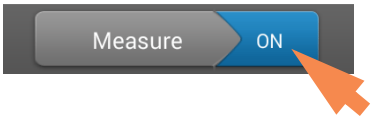
Auto-Measure and Auto-Blank Options

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.

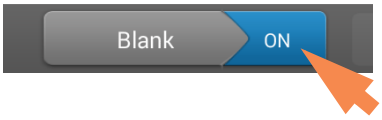
Auto-Measure

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



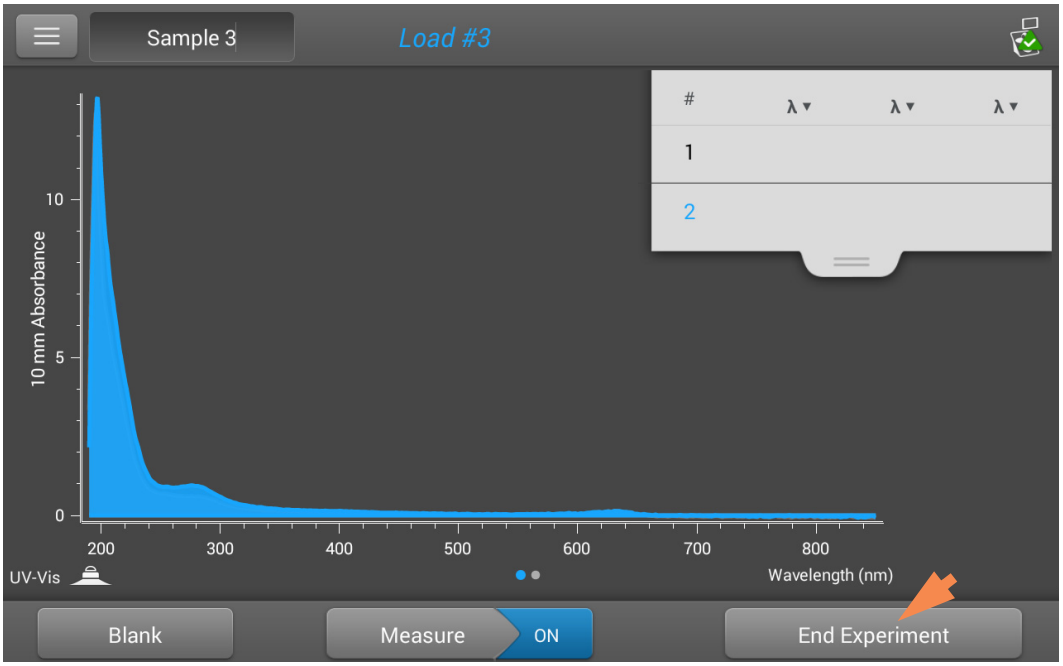
Auto-Blank

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



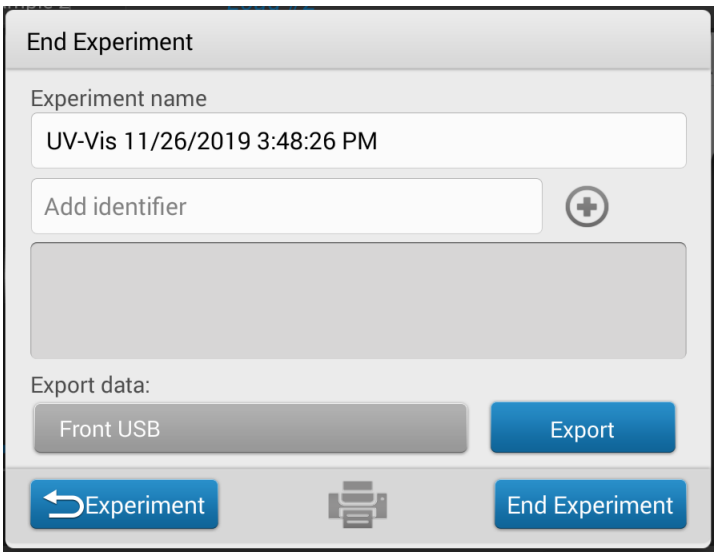
End Experiment Button

Tap **End Experiment** when you are ready to name and save your experiment, add a label to help you locate the experiment later or export the data. Depending on the administrative settings, you may be prompted to sign the experiment upon ending the experiment.



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

After you tap End Experiment, the End Experiment box is displayed:

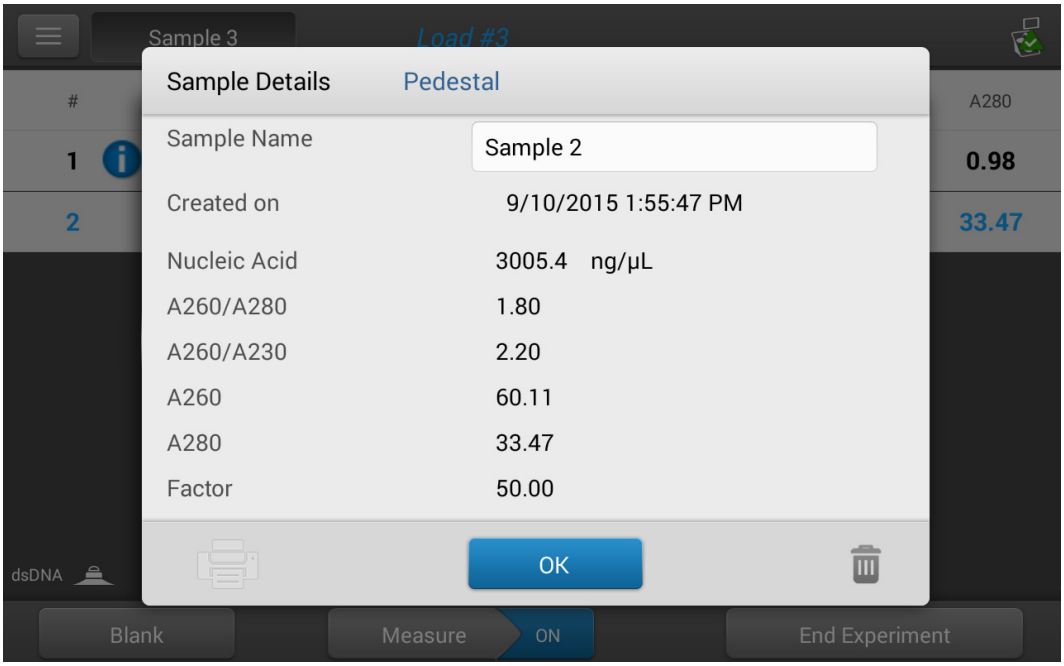


Available options:

Experiment Name	Enter a name for this group of measurements. The measurement results are saved in the selected database location using the entered experiment name.
Add Identifier	Enter a descriptive label to help you find this experiment later or to associate it with another experiment (see Manage identifiers on the instrument for details). Tap the **Add Identifier box** to display a keyboard to enter the label text. Tap the **Add Identifier button**  to add the label; tap the **Done** key to close the keyboard.
Export Data	Select an available location for exporting the measurements in this experiment. Experiments can be exported to a USB device connected to any USB port on the local instrument (front, back-left or back-right) or to a network location.
Export Button 	Allows you to select a file format for exporting the measurements in this experiment and then export the data to a USB device or network. Available export file formats: • comma-separated values spreadsheet (.csv) file • tab-separated values spreadsheet (.tsv) file (spectral data only) • NanoDrop (.sql) file The filename is the entered experiment name (see above). The file is stored in a folder named “NanodropOne” followed by the instrument serial number. (Use System Status to view your instrument serial number.)
Return To Experiment button 	Close the End Experiment box and display the results for the most recent measurement. From there you can add measurements to the current experiment and save it later.
Print button	Print measurement results for current experiment
End Experiment button 	End the experiment and save the measurement results using the entered experiment name. The experiment is saved in the selected database location.

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:



Information about the measured values displayed in Sample Details is provided in this Help system, under the application used to acquire the data.

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.



View History

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 History feature 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 (History) on instrument Home screen

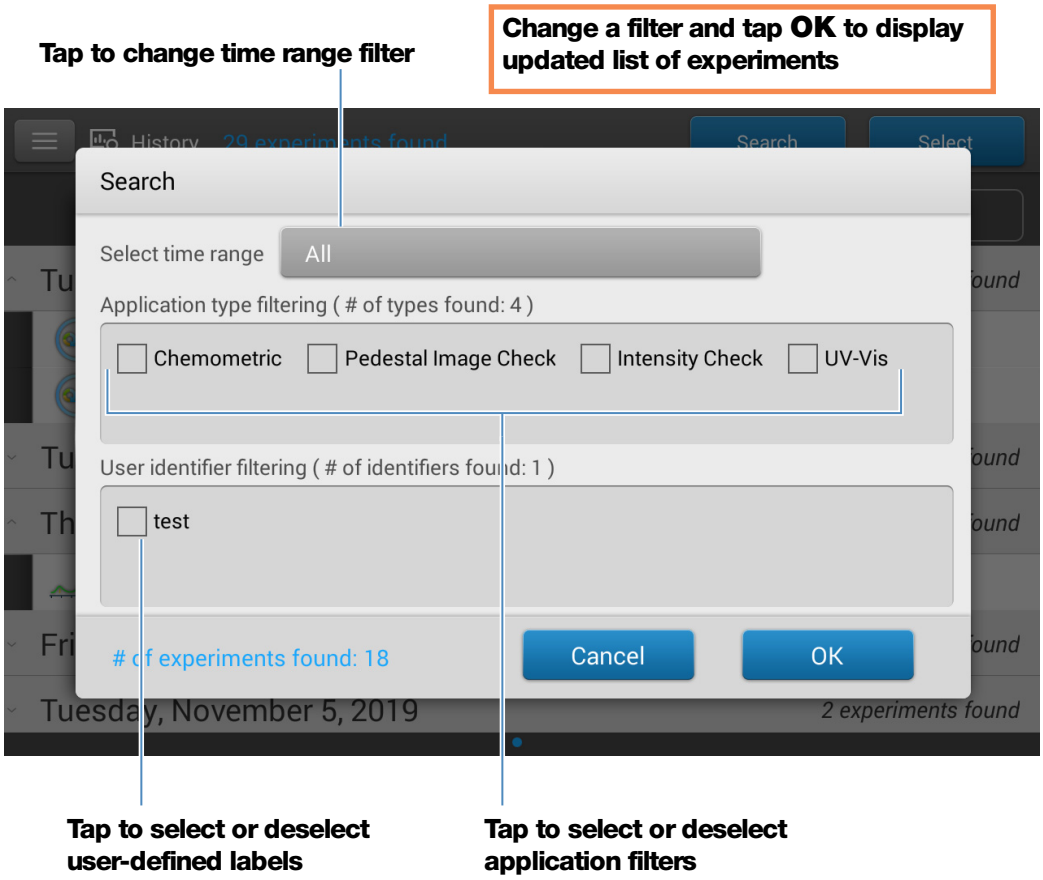
Menu

Tap ☰ in History 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 History 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:

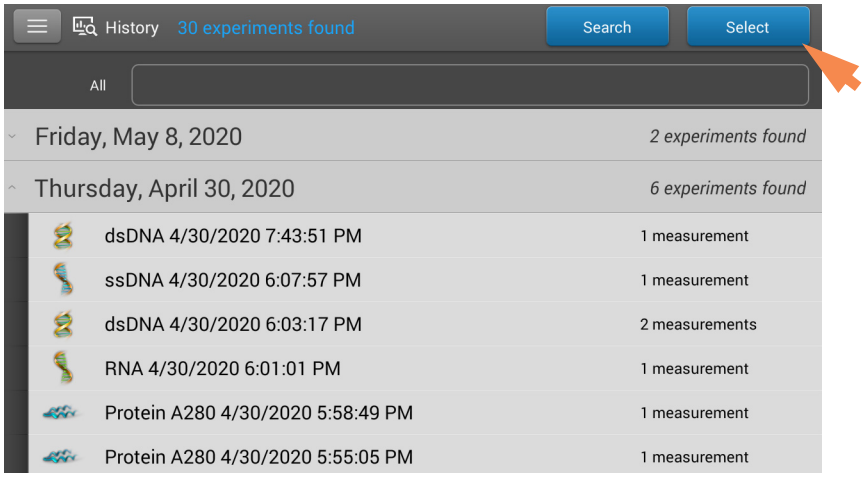


Export Selected Experiments

Use **Select** in History to select experiments to be exported.

Export selected experiments

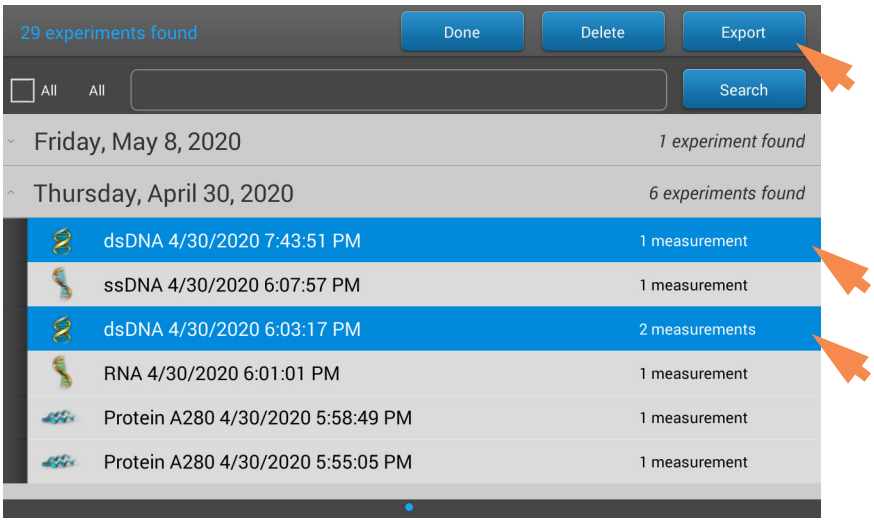
– open History and tap **Select**



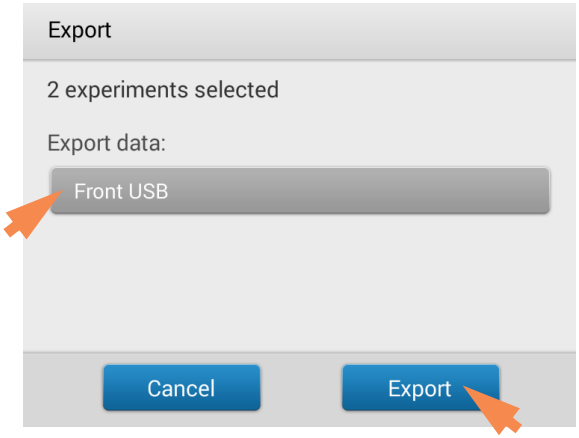
– tap **row** to list experiments acquired on that date, or use Search feature to find experiment

– tap to select one or more experiments to export (tap again to deselect an experiment; to select all experiments in database, select **All)**

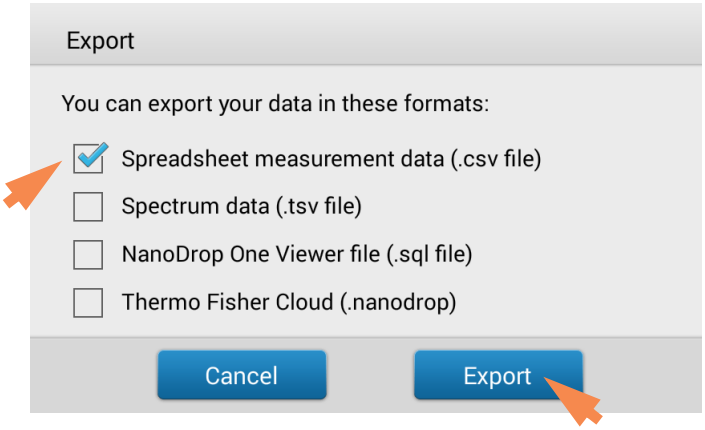
– tap **Export**



– set **Export Data** to an available export location (front, back-left or back-right USB port, or a network location) and select **Export**



– select one or more formats to export to (see "Export Selected Experiments" in General Operations for details) and tap **Export**



– after "Export Success" message, tap **OK**

Delete Selected Experiments

Use **Select** in the History to select experiments to be deleted.

Delete selected experiments

– tap a **row** in History 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**

Note Deleted data cannot be recovered.

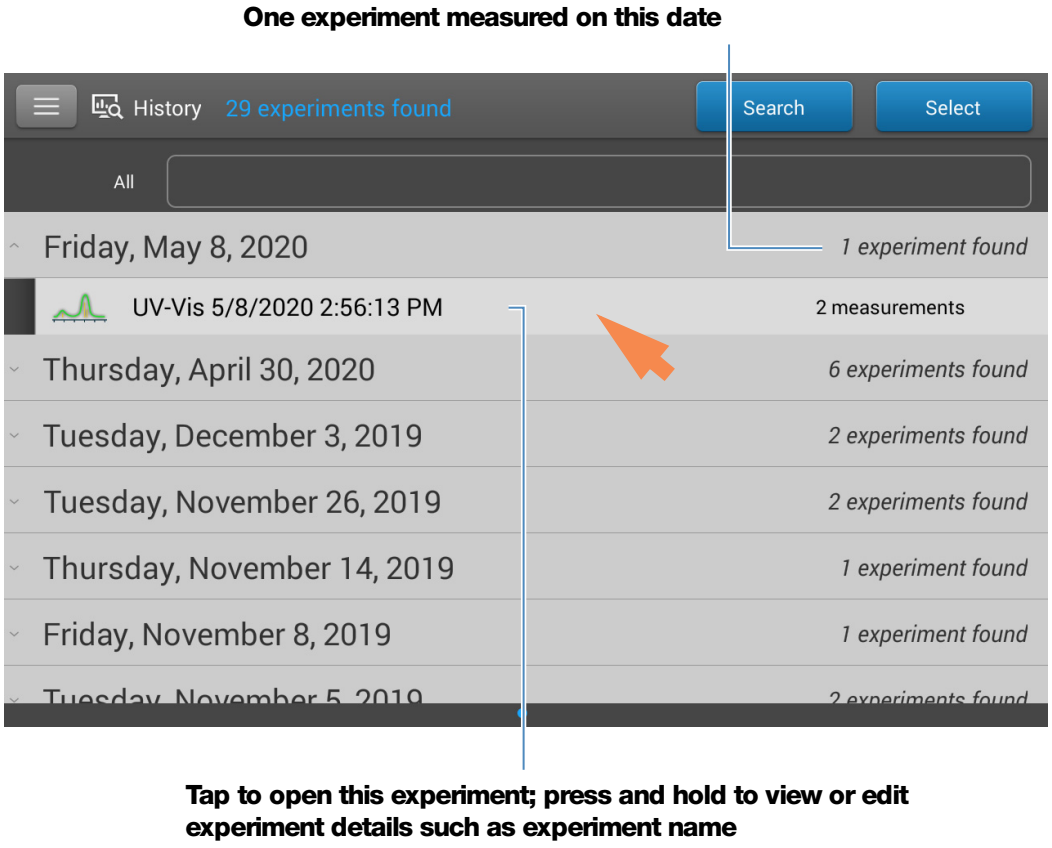
Open Experiment and View Associated Data

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

Open an experiment

- tap a **row** in History 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:

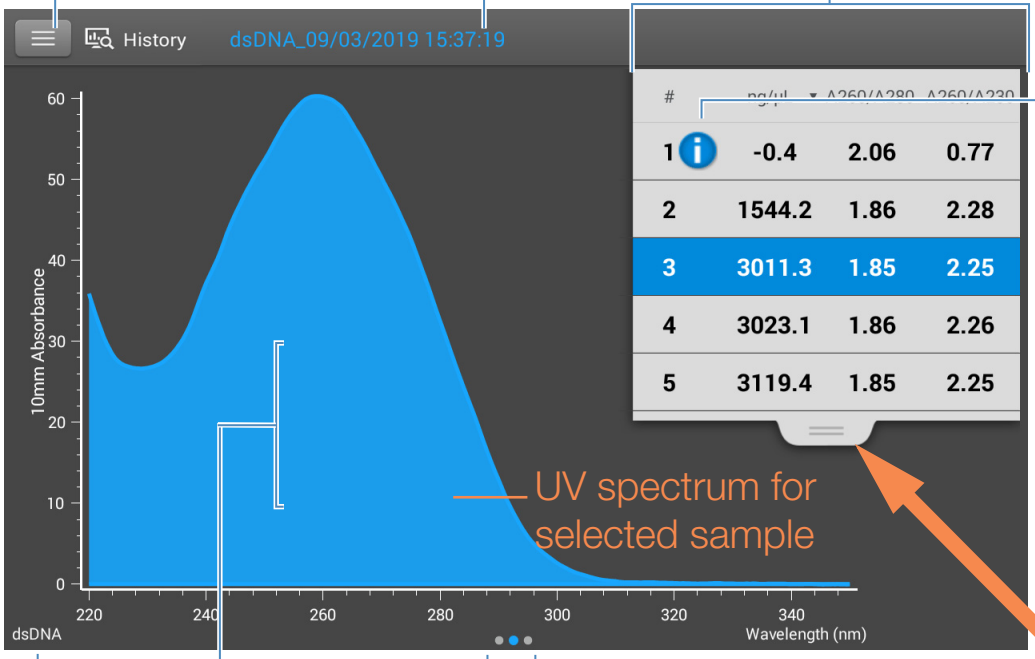


The History provides measurement data as spectral data and data tables, similar to what you see after you complete a measurement.

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.

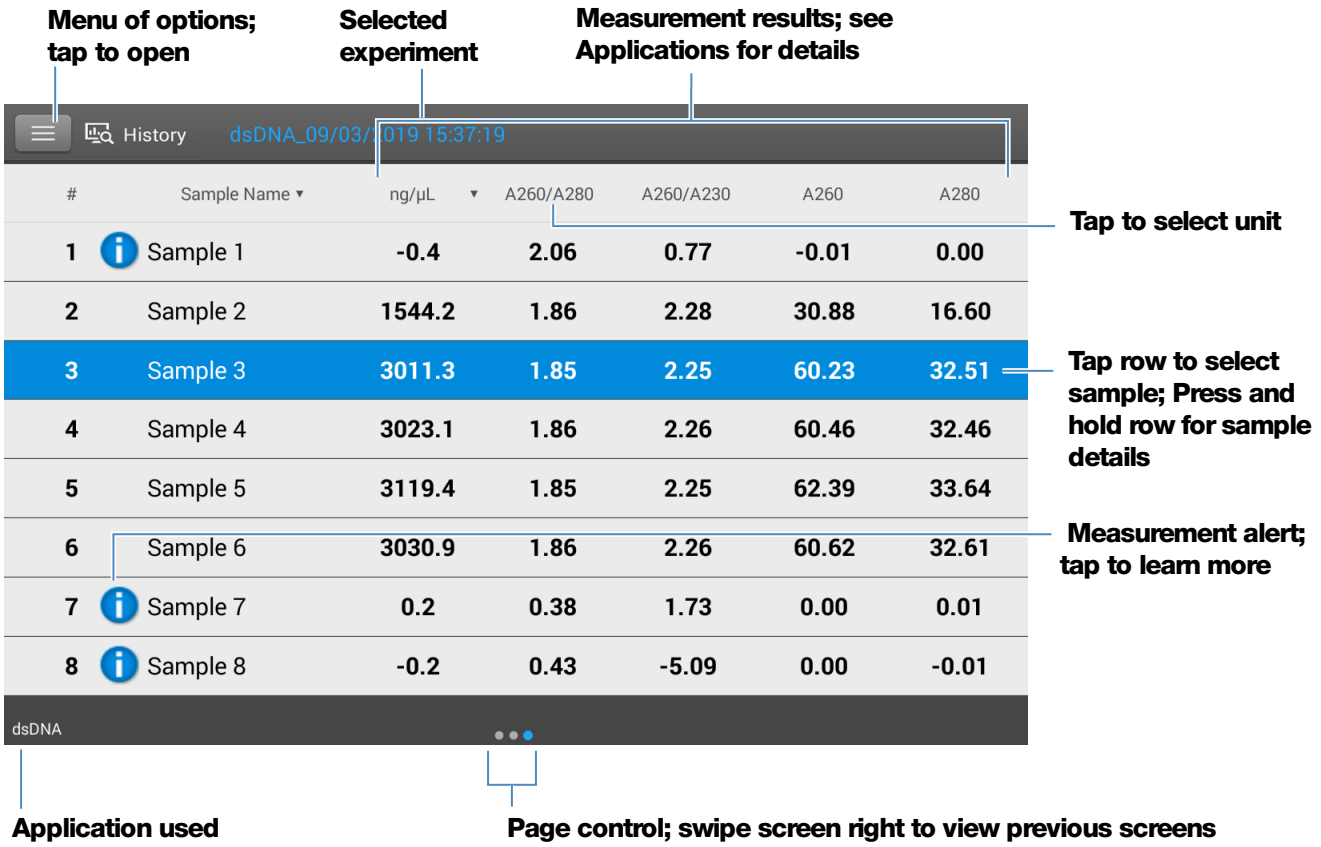
Spectral data—

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 experiments
Print	Print plot or data table for selected measurement results; if no results are selected, prints all results in data table
Settings	View or change instrument settings
Disk Status	View remaining space available for storing measurement data on the instrument

NanoDrop One General Operations

These operations are available from any measurement screen or from the History.

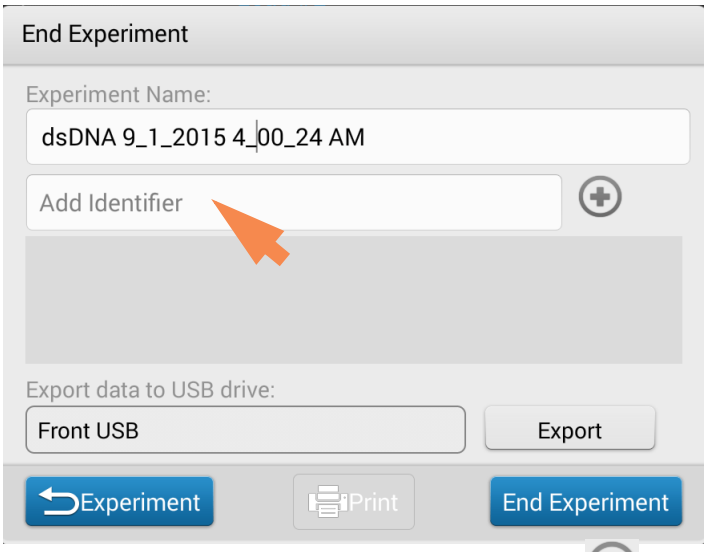
Manage Identifiers (on the instrument)

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 control software installed on a personal computer (see Manage Identifiers on a PC).

Use the History 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 History based on one or more user-defined labels.

Label new experiment when you save it

- after the last sample has been measured, tap End Experiment
- 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 History

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

Find labeled experiments

- from the Home screen, tap  to open History
- 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**

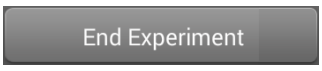
Remove a label

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

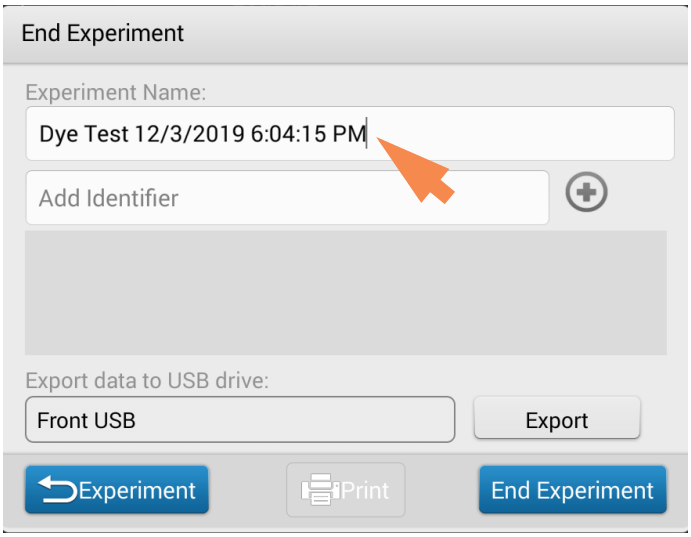
Edit Experiment Name

You can edit the experiment name when you save the experiment or afterwards from the History.

Edit experiment name at end of experiment

- when finished measuring samples, tap 

– enter a name for this group of measurements in the Experiment Name box



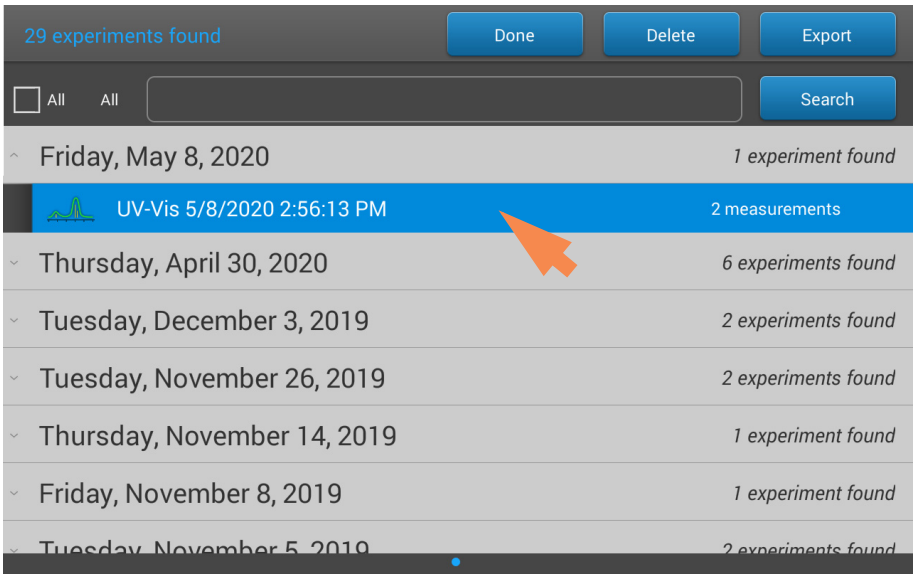
– tap **End Experiment**

Edit experiment name from History

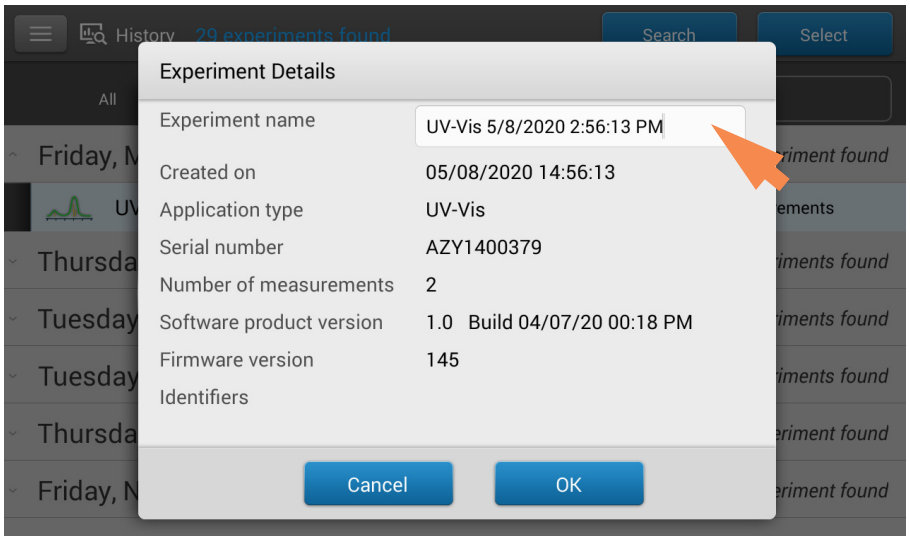
– from Home screen, tap  to open History

– tap **row** to list experiments acquired on that date, or use Search feature to find experiment

– press and hold experiment name to open experiment details box



– tap **Experiment Name** field to display keyboard



– enter new experiment name

– tap **Done** key to close keyboard

– tap **OK** to close Experiment Details box

Export Selected Experiments

You can export measurement data when you save the experiment or afterwards from the History

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 four formats:

- as comma-separated values (.csv) files containing the measurement results and details for each exported experiment
- as tab-separated values (.tsv) files containing x,y coordinates for every spectral data point for each exported experiment
- as NanoDrop (.sql) files containing spectra and measurement results for each exported experiment

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:

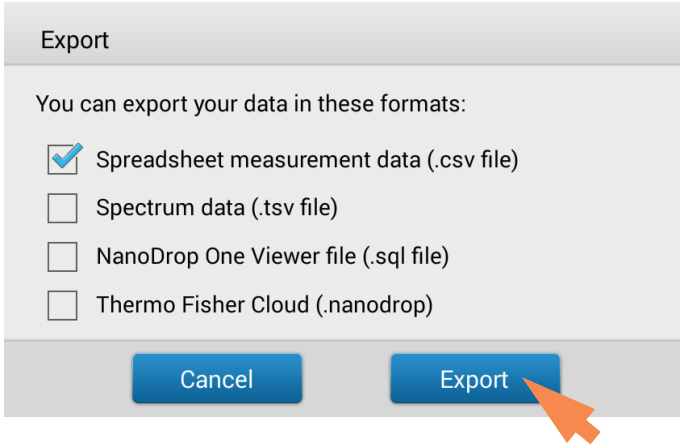
	A	B	C	D	E	F	G	H	I	J
1	Date	Sample Name	Nucleic Acid	A260/A280	A260/A230	A260	A280	Nucleic Acid Factor	Baseline (nm)	
2	4/21/2015 15:37	Sample 1	0.3	0.7	0.56	0.01	0.01	33	340	
3	4/21/2015 15:42	Sample 2	0.37	0.94	0.86	0.01	0.01	33	340	
4	4/21/2015 15:44	Sample 3	0.43	0.98	0.74	0.01	0.01	33	340	
5	4/21/2015 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.

Data can be exported to a USB device connected to any USB port on the local instrument (front, back-left or back-right) or to a network location. If you select multiple experiments for export, each exported experiment has a corresponding file. 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 your instrument serial number.)

Export data at end of experiment

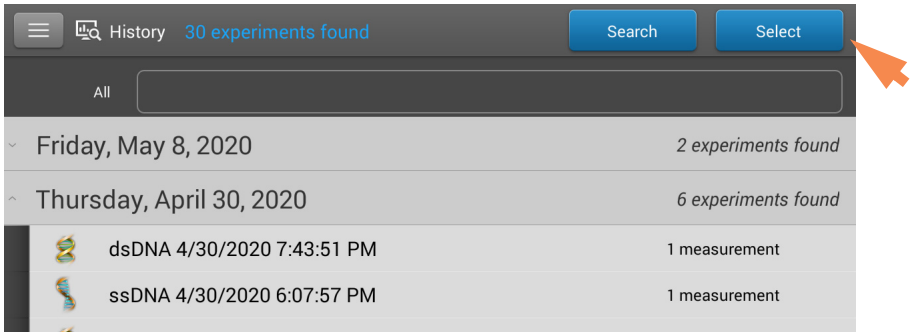
- when finished measuring samples, tap End Experiment
- from End Experiment box, set **Export Data** to an available export location (front, back-left or back-right USB port, or a network location)
- tap Export
- from Export box, select one or more formats to export to (see above for details) and tap **Export**



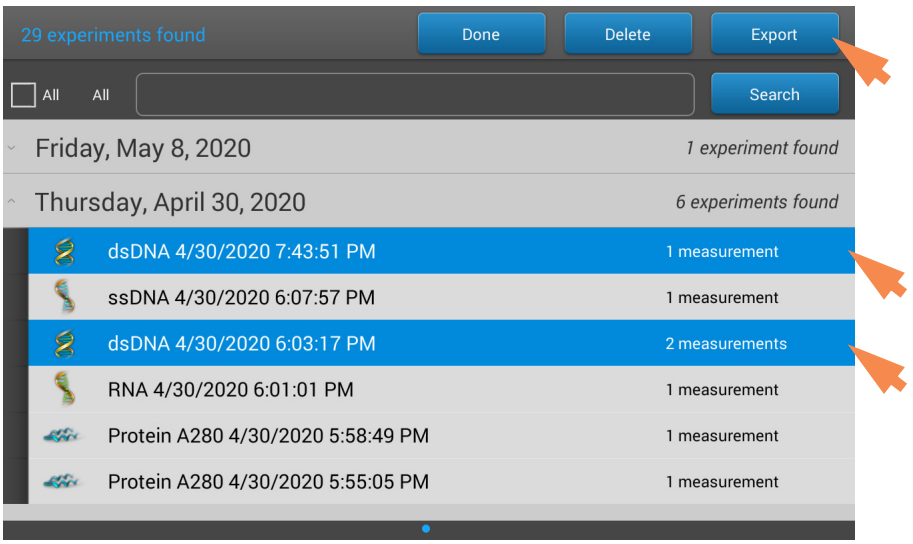
- after “Export Success” message, tap **OK**
- tap **End Experiment**

Export data from History

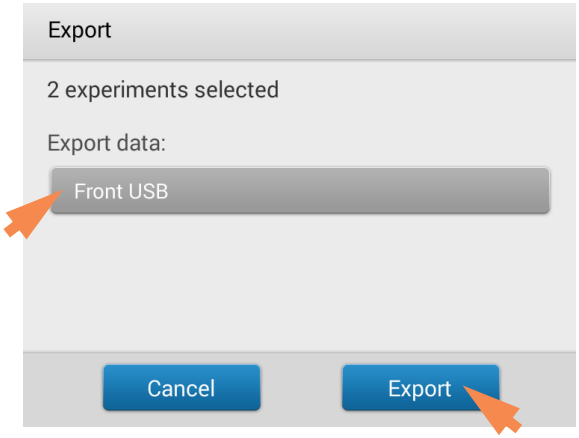
- from Home screen, tap  to open History
- tap **Select**



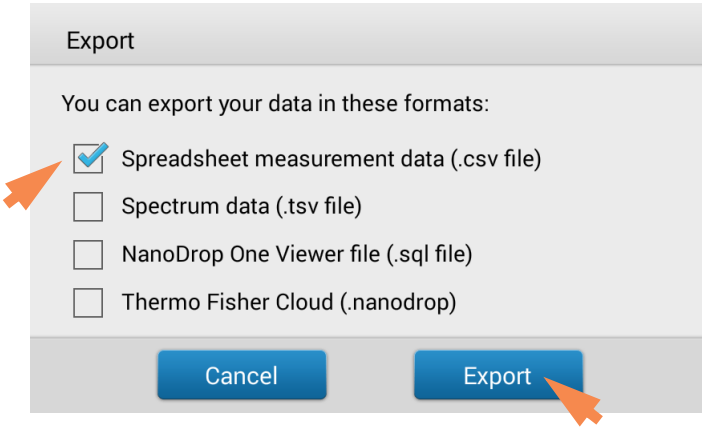
- tap **row** to list experiments acquired on that date, or use Search feature to find experiment
- tap to select one or more experiments to export (tap again to deselect an experiment;
  to select all experiments in database, select **All)**
- tap **Export**



– set **Export Data** to an available export location (front, back-left or back-right USB port, or a network location) and tap **Export**



– select one or more formats to export to (see above for details) and tap **Export**



– after “Export Success” message, tap **OK**

Delete Selected Measurements

You can delete selected sample measurements from any experiment, or all the measurements in the database.

Note 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 History

– from Home screen, tap  to open History

– tap **Select**

- tap **row** in History to list experiments acquired on that date, or use Search feature to find desired experiment
- tap to select one or more experiments to export (tap again to deselect an experiment;
  to select all experiments in database, select **All)**
- tap **Delete**

Print Selected Measurements

Connect a compatible printer to the instrument to quickly print measurement results, including spectral data, standard curves, data tables, sample details and diagnostic results. You can print to a USB printer (label or full service) or to a remote printer through an Ethernet connection or wireless network.

Note

- To select a printer, from the Print Preview window, choose **Printer Options** and select an available printer.
- To add a printer, from the Print Preview window, choose **Printer Options > Manage Printers**.
- A wireless printer or the device it is connected to must be available on the same wireless network as the instrument. The wireless printer must also have its wireless function enabled.
- Full service printer options are not available if you have a label printer connected. Disconnect the label printer to access the full service printer options.

Print data from any measurement screen

- after you have measured a sample, display the measurement results to be printed such as the spectral data, standard curve, data table or sample details (see NanoDrop One Measurement Screens)
- if printing spectral data or the data table, tap to select one or more sample rows to print (tap again to deselect a sample row); if no results are selected in data table, all results will be printed
- tap  and choose  **Print**
- choose **OK** to confirm
- in the Print Preview window, make sure the correct printer is selected and set other print options as desired such as paper size and orientation (“Auto” setting is recommended), margin and alignment to adjust the image in the preview window

  Note The software saves the print settings each time you print.
- choose **Print**

If a label printer is connected to the instrument, the software prints one label for each selected measurement. If a full service printer is connected, the selected measurement screen is printed for each selected measurement.

Print data from History

- from Home screen, tap  to open History
- tap **row** in History to list experiments acquired on that date, or use Search feature to find desired experiment
- tap **experiment name** to open the experiment
- swipe left or right to select the type of data to print (spectral data, standard curve or data table)
- tap to select one or more sample rows to print (tap again to deselect a sample row); if no results are selected in data table, all results will be printed
- tap  and choose  **Print**
- choose **OK** to confirm
- in the Print Preview window, make sure the correct printer is selected and set other print options as desired such as paper size and orientation (“Auto” setting is recommended), margin and alignment to adjust the image in the preview window

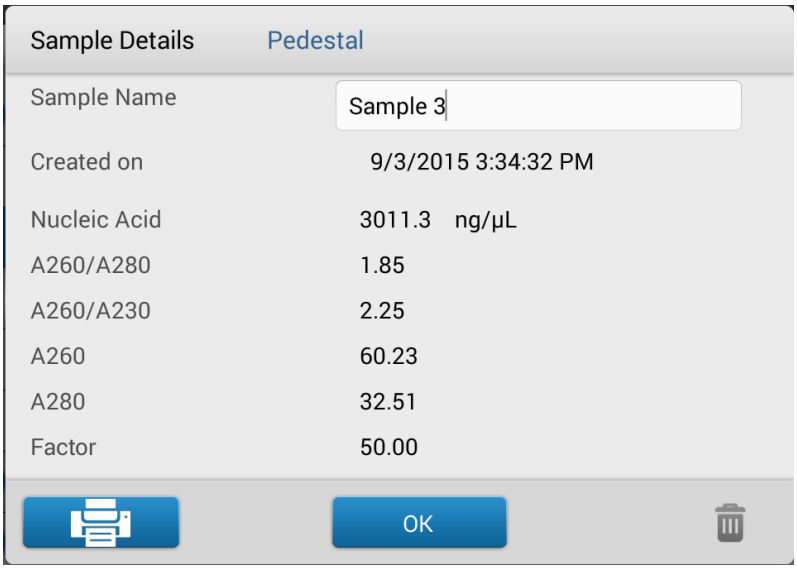
  Note The software saves the print settings each time you print.

- choose **Print**

If a label printer is connected to the instrument, the software prints one label for each selected measurement. If a full service printer is connected, the selected measurement screen is printed for each selected measurement.

Print sample details

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



– tap 

– in the Print Preview window, make sure the correct printer is selected and set other print options as desired such as paper size and orientation (“Auto” setting is recommended), margin and alignment to adjust the image in the preview window

> **Note** The software saves the print settings each time you print.

– choose **Print**

If a label printer is connected to the instrument, the software prints a label for the selected measurement. If a full service printer is connected, the selected sample details screen is printed.

Acclaro Sample Intelligence

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



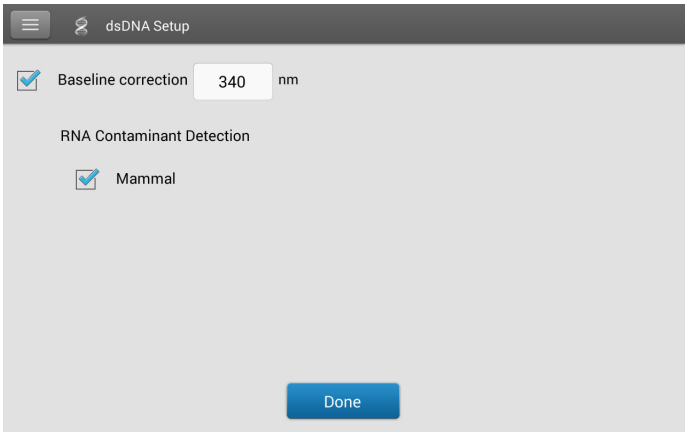
invalid-result alerts (a column sensor monitors for the presence of bubbles or reflective particles that can compromise measurement results)



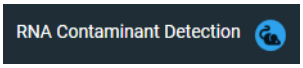
Use these embedded resources to quickly troubleshoot possible problem measurements and make informed decisions on whether to use, re-purify or take other actions with an atypical sample result. The Sample Intelligence feature also serves as a resource for further study and a learning tool for new or novice users.

Activate Detection

In local control, from the application setup screen, select the check box to activate contaminant detection.



When using the PC software, from the measurement screen, select the mouse icon to activate contaminant detection.



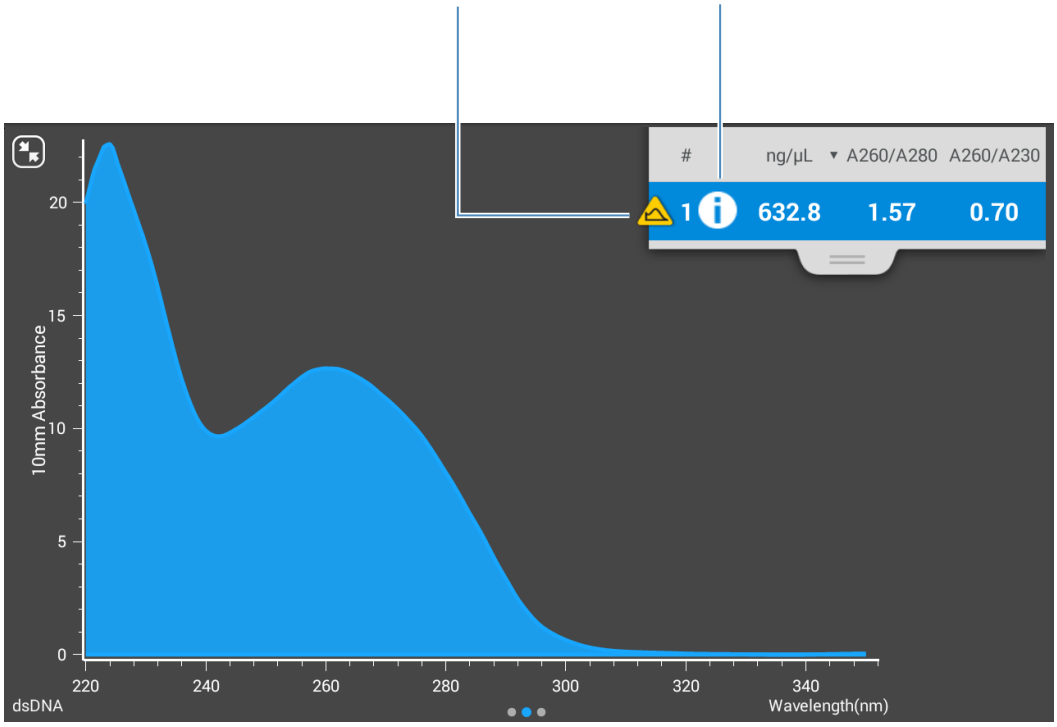
When enabled, RNA Contaminant Detection/DNA Contaminant detection will apply mathematical models to predict the amount of RNA contaminant in dsDNA or dsDNA in RNA. These models are specific to the source of the nucleic acid. The mouse icon is representative of all mammalian sources of nucleic acid. If you are measuring nucleic acid from a source for which we do not have mathematical model, leave the selections unchecked.

View Acclaro Sample Intelligence Information

Measurements that include a contaminant analysis or technical information are flagged automatically (see examples below). Select the icon to review the associated data or information.

Contaminant analysis is available for this measurement

Technical information is available for this measurement



The icons appear next to the measurement results (see above), and in the data table, as well as in History (see below).

#	Sample Name	ng/µL	A260/A280	A260/A230	A260	A280
1	Sample 1	632.8	1.57	0.70	12.66	8.05
2	Sample 2	633.7	1.57	0.70	12.67	8.06
3	Sample 3	518.2	1.56	0.69	10.36	6.63
4	Sample 4	519.3	1.56	0.70	10.39	6.64
5	Sample 5	516.4	1.56	0.69	10.33	6.63
6	Sample 6	876.3	1.80	2.24	17.53	9.71

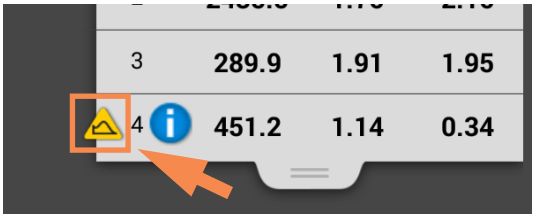
The icons are active in all three places; the information remains with the data indefinitely, even after it has been exported.

Contaminant Analysis

For the dsDNA, RNA and Protein A280 applications, the NanoDrop One software automatically initiates a spectral analysis for several known contaminants during the measurement. Examples of known contaminants include:

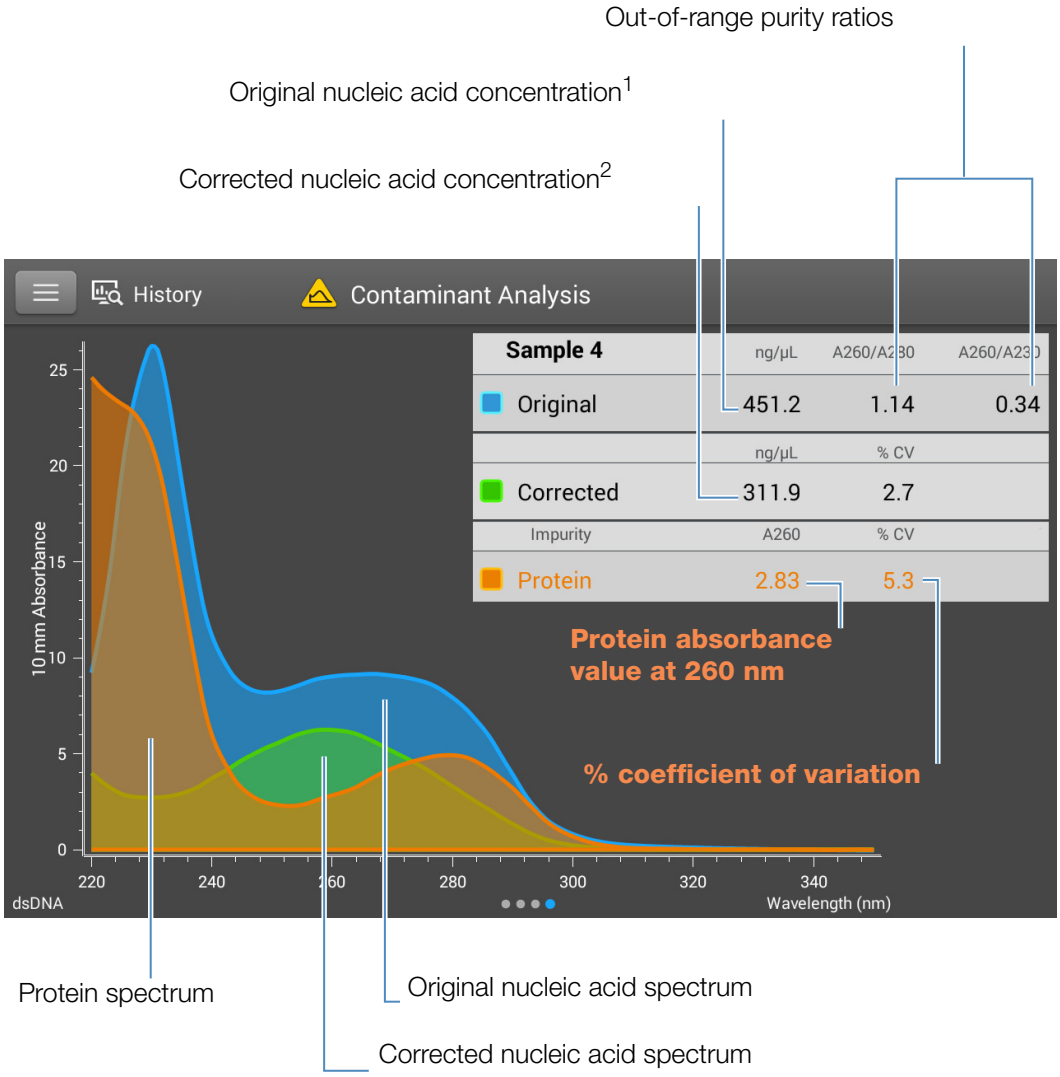
- for dsDNA and RNA measurements:
  - in the analysis region: protein and phenol
  - detects presence of guanidine HCl and guanidinium isothiocyanate
  - detects species-specific dsDNA contamination in the RNA application and detects species-specific RNA contamination is in the dsDNA application
- for protein measurements:
  - in the analysis region: nucleic acids and phenol

If contaminants are identified in a sample, the “Contaminant Analysis” icon appears to the left of the measurement results.



Tap the icon to view the contaminant analysis and associated information.

The following is an example of results from a nucleic acid contaminant analysis that contains enough protein contaminant to affect the measurement results.



[1]Based on total sample absorbance (sample plus contaminant)

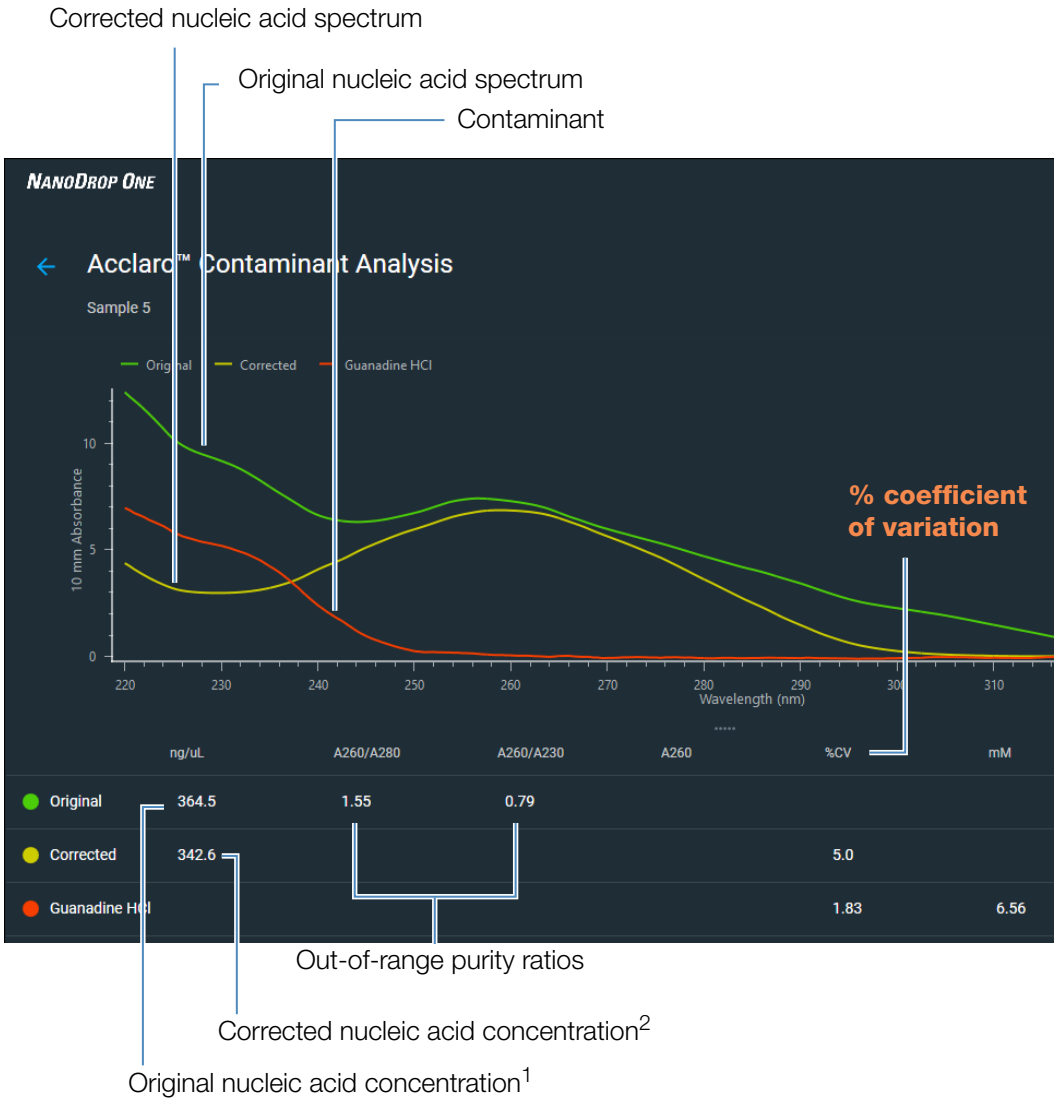
[2]Based on corrected sample absorbance (sample minus contaminant)

Since proteins absorb light near the analysis wavelengths for nucleic acid (230 nm, 260 nm, and 280 nm), the presence of protein in the nucleic acid sample shown above has pushed the A260/A280 and A260/A230 ratios out of range and caused the reported nucleic acid concentration to be higher than the real value. The software identifies the impurity (protein), and reports the following:

- baseline-corrected absorbance due to protein (2.83) at the analysis wavelength (260 nm)

- % coefficient of variation for the measurement result (uncertainty x 100/measurement result = 5.3%; a high %CV indicates the measurement result is close to the instrument detection limit or there is an interfering component)
- original nucleic acid concentration (451.2 ng/µL), which is based on the total baseline-corrected absorbance (sample plus contaminant) at the analysis wavelength
- corrected nucleic acid concentration (311.9 ng/µL), which is based on the corrected absorbance (sample minus contaminant) at the analysis wavelength

Below is an example of results from a nucleic acid contaminant analysis that contains enough guanidine HCl contaminant to affect the measurement results as seen on the PC control software



Theory behind contaminant analysis

UV and UV-visible absorbance measurements are used to quantify nucleic acid and protein samples at 260 nm and 280 nm, respectively. The analysis is based on the fact that the total absorbance of a mixture solution at a given wavelength is the sum of the absorbance values of each component in the mixture.

An ongoing challenge of this method is that a number of materials used in the extraction process can absorb in various regions across the spectrum. When these contaminants are present in a sample, they can interfere with the analysis by artificially inflating the absorbance at the wavelength of interest, which causes the analyte concentration to be overestimated.

Traditionally, purity ratios are used to detect the presence of contaminants that could affect downstream applications. However, purity ratios do not always provide a complete picture of possible contamination. When a purity ratio falls outside the expected range, the spectral profile is often examined qualitatively.

Our Acclaro technology applies a quantitative approach to contaminant analysis. Through sophisticated mathematical algorithms, Acclaro analyzes the spectral data to identify probable contaminants in a sample and removes any contribution due to the contaminant from the sample result. This results in a more accurate concentration value of the analyte of interest and a more quantitative analysis of the level of contamination.

Since the spectrum of a pure compound is unique to that compound, a mixture spectrum of mostly known materials that have few interactions can be mathematically broken down into its component spectra and the components identified. The contaminant analysis algorithm uses a narrow spectral region (220-285 nm) around the analysis wavelength (260 nm for nucleic acids, 280 nm for proteins) to determine any absorbance contribution from possible known contaminants (protein or nucleic acid, and phenol) that absorb in that region. The entire spectrum is analyzed to determine the presence of other possible contaminants such as guanidine HCl and/or guanidinium isothiocyanate, which are common reagents used for nucleic acid purification.

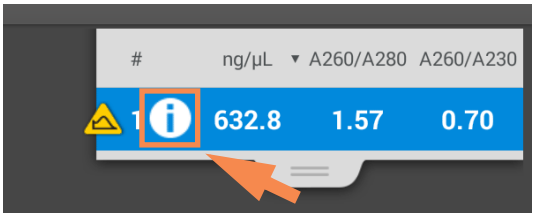
Note Achieving consistent, high quality contaminant analysis results is dependent on the quality of the measured sample spectra, which is dependent on the maintenance status of the instrument. For more information, see Maintenance Schedule.

On-Demand Technical Support

For the dsDNA and Protein A280 applications, the NanoDrop One software monitors all sample measurements for the presence of contaminants or other anomalies that may affect the measurement. Examples of monitored characteristics include:

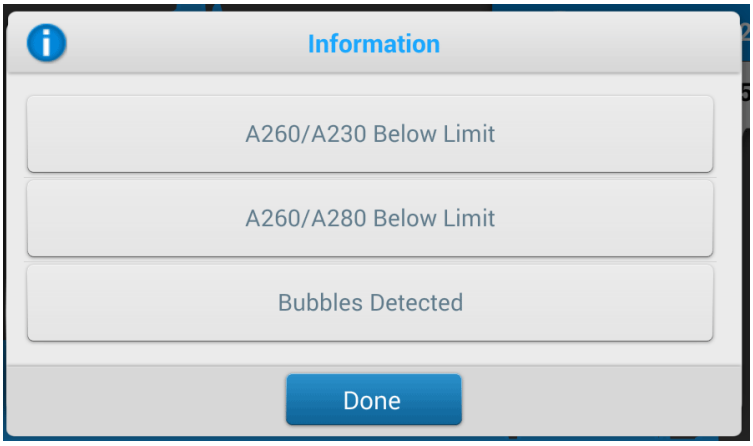
- absorbance ratios, which indicate the presence of compounds that may interfere with sample measurements (also referred to as “purity ratios”). For more information, watch the multimedia training What is a Purity Ratio?.
- bubble check, which looks for bubbles or other reflective materials in a sample or blank. For more information, watch the multimedia training Effects of Bubbles in Samples.

If technical information is available, the “information” icon appears to the left of the measurement results.

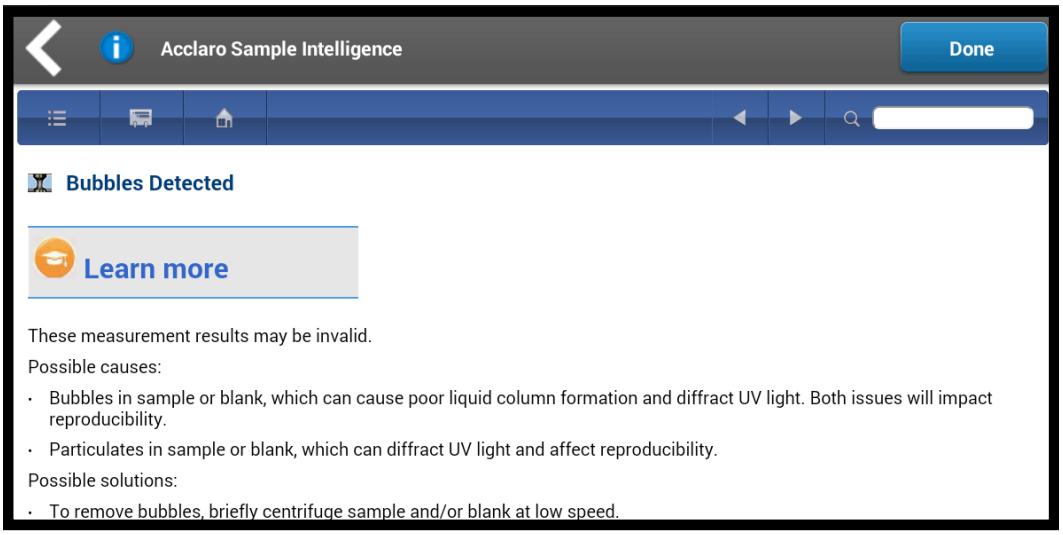


Tap the icon to view the information.

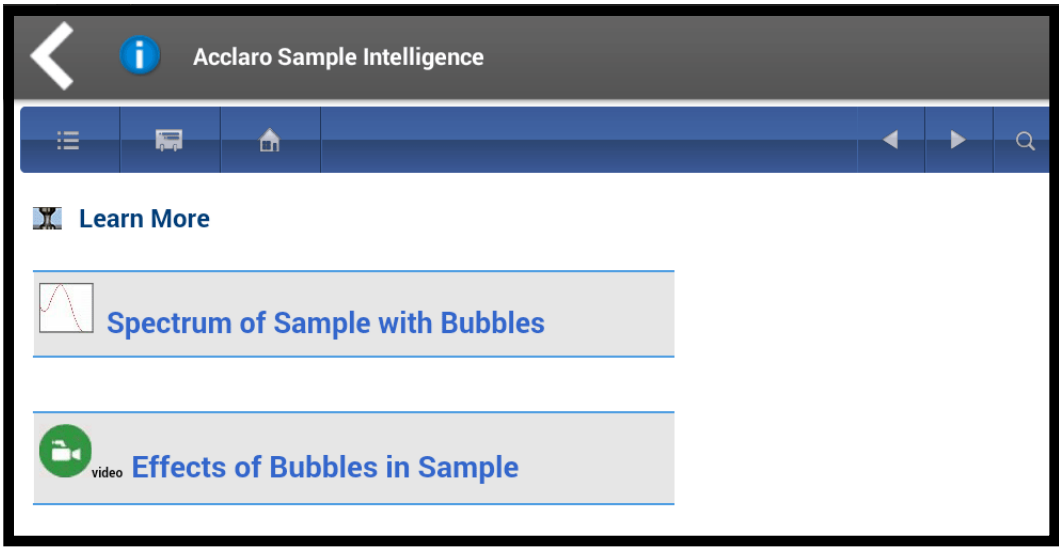
Here are results from a nucleic acid analysis for which two measured purity ratios are below the expected value and the sample contained enough bubbles to possibly affect the measurement results.



Tap an information button for more information. Here is the information provided for the bubble error:



Tap **Learn More** for the next level of information which, for this example, includes a link to a multimedia training video.



Invalid-Results Alerts

The NanoDrop One software uses an embedded image sensor to monitor all measurements for conditions (such as a broken liquid column) that are likely to invalidate the measurement results.

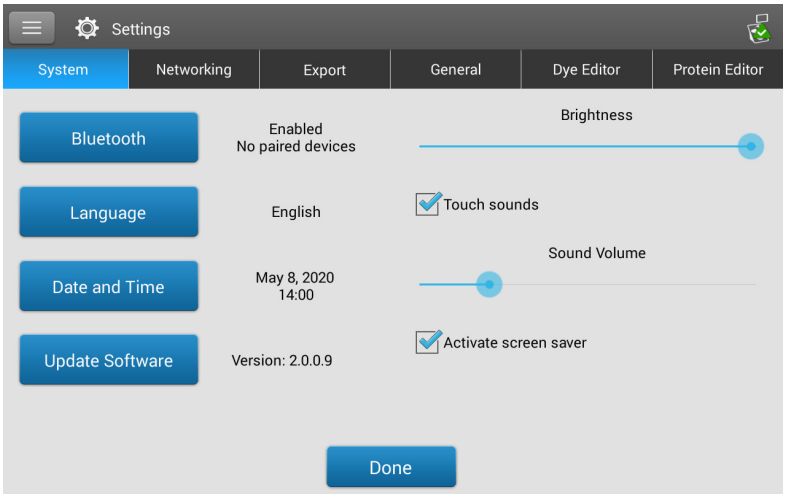
After an invalid-results alert, the Invalid Results icon is displayed and the measurement is stopped. See Troubleshooting for more information.

Instrument Settings

View or change instrument settings

- from Home screen, tap 
- -or-
- from any measurement screen or the History,

  tap  and choose  **Settings**



These instrument settings are available:

System Settings

These options are available:

Bluetooth — Set up Bluetooth connections to wireless input devices for the instrument such as a wireless keyboard, mouse or barcode scanner

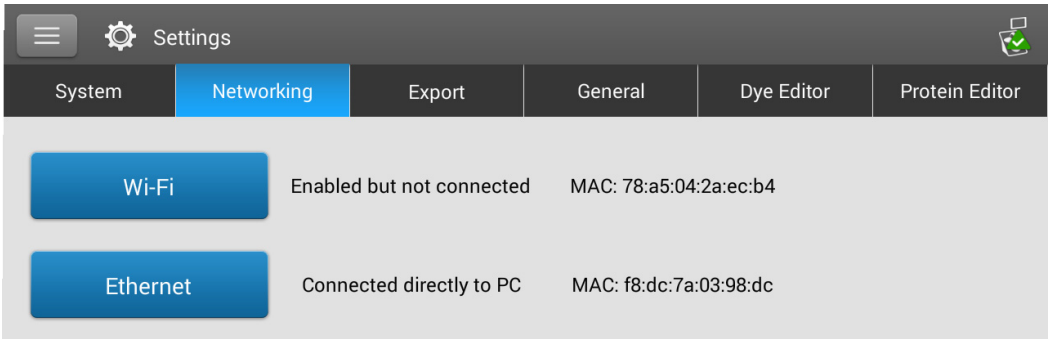
Language — Select language for displaying NanoDrop One software and for any connected input device such as a keyboard, mouse or barcode scanner

Notice: Changing the language requires a software restart.

Date and Time	**Automatic date & time**: synchronize instrument date and time with available network
	Automatic time zone: synchronize instrument time zone with available network
	Set date: manually set instrument date (this option is disabled when Automatic Date & Time is selected)
	Set time: manually set instrument time (this option is disabled when Automatic Date & Time is selected)
	Select time zone: manually select instrument time zone (this option is disabled when Automatic Time Zone is selected)
	Use 24-hour format: use 24-hour time format
	Choose date format: choose an available date format
Update Software	Update NanoDrop One software via USB device connected to instrument; if connected USB device contains multiple eligible update files, you can choose which files to update (see Update Software for details)
	Version: version of NanoDrop One operating software currently installed on this instrument
Brightness	Adjust brightness of instrument touchscreen
Touch Sounds	Provide audible feedback after each interaction with the touch pad
Sound Volume	Adjust volume of instrument touchscreen
Activate Screen Saver	Launch a screen saver application when the instrument has been idle for 30 minutes. To reactivate the instrument, tap the touch pad.

Network Settings

Use this tab to specify a Wi-Fi or Ethernet connection for the instrument.



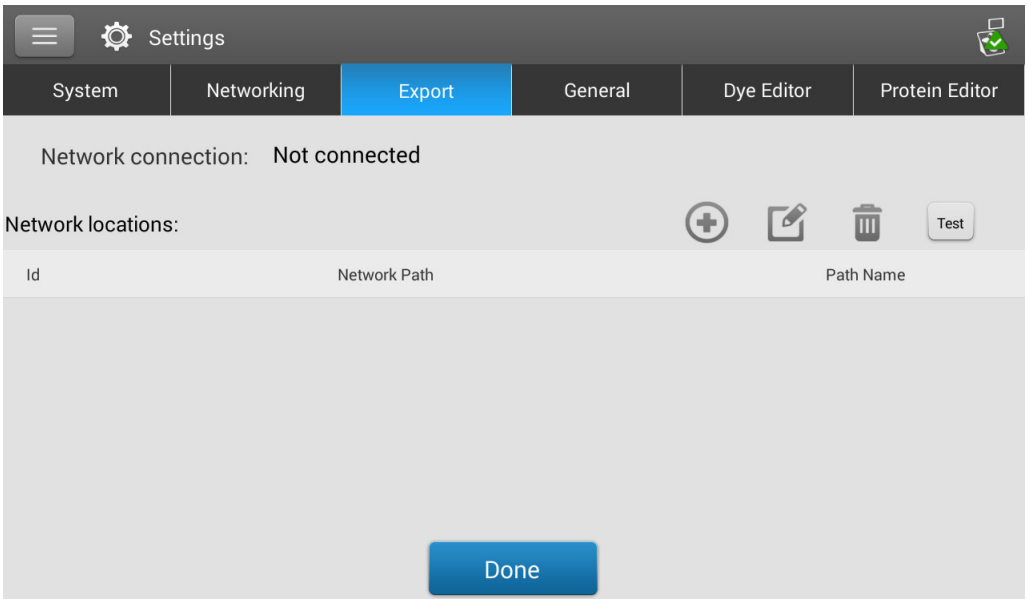
These options are available:

Wi-Fi — Set up wireless local area network (WLAN) connection on the instrument

Ethernet — Set up Ethernet (wired) local area network (LAN) connection between the instrument and a personal computer or network wall jack.

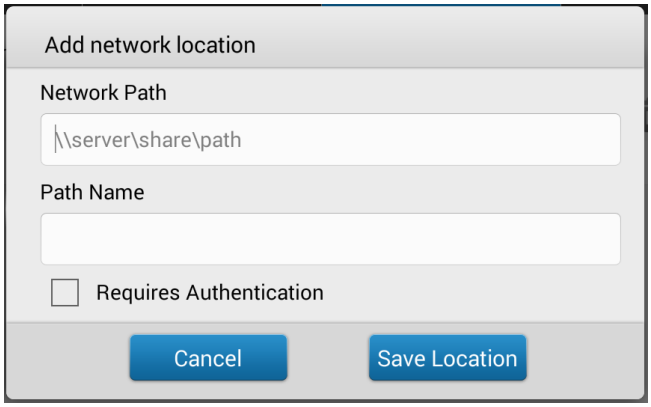
Export Settings

Use this tab to specify one or more network paths for exporting acquired data when the instrument is connected to a network (connection can be wired or wireless). Network paths defined here will appear in the Export Data list box when exporting data, from both the History and the End Experiment box after you complete a measurement.



These options are available:

Add Add a network location:



To add a network location

- Enter a valid network path
- In the **Path Name** box, enter a descriptive name for this network location. The entered name will appear in the Export Data list box when exporting acquired data from the instrument.
- Select **Requires Authentication** if the network path requires a user name and password
- Tap **Save Location**

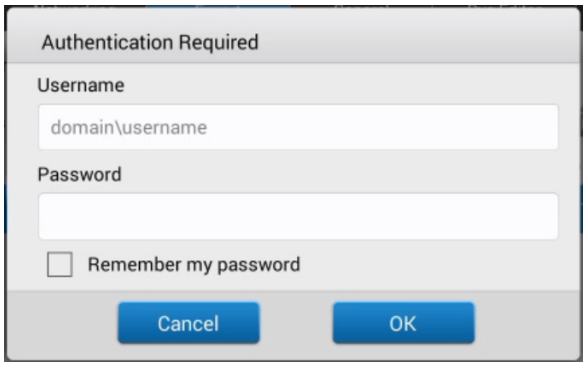
  If the entered network path is valid, its name is displayed in the Network Locations list on the Export Settings tab.

Edit Edit network path, path name or authentication setting for selected network location

Delete Delete selected network location

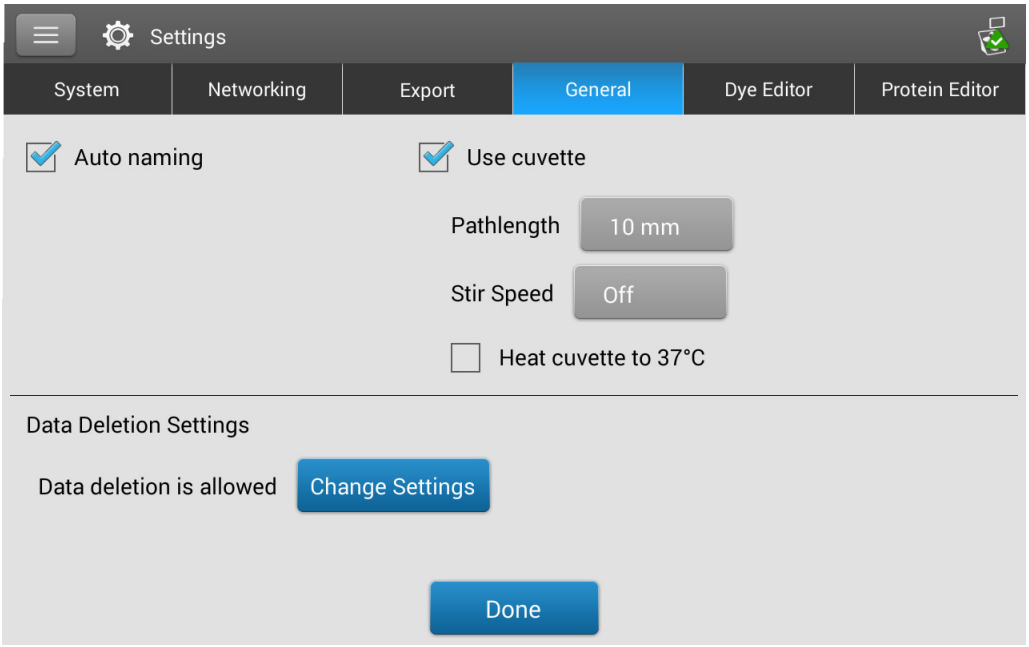
Test

Test connection for selected network location. When asked, enter Username and Password. You have the option to select “Remember my password”. When selected, the Username and Password will be saved as the default account for network export.



General Settings

Use this tab to specify one or more network paths for exporting acquired data when an Ethernet cable is used to connect the instrument to an active network wall jack. Network paths defined here will appear in the Export Data list box when exporting data, from both the History and the End Experiment box after you complete a measurement.



These options are available:

Auto-naming Assign sample names automatically using base name followed by unique number starting with "1." Uses default ("Sample") or user-specified base name. For details, see Sample Name.

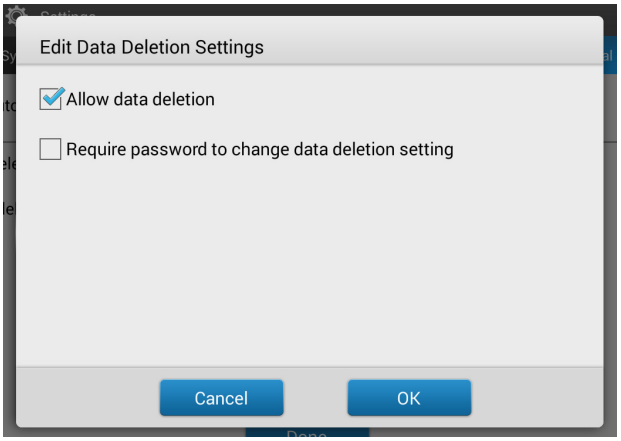
Use cuvette Select cuvette sampling mode. When selected, these additional options are available:

Pathlength: Enter cuvette pathlength (width) before taking blank or sample measurements with cuvettes (see cuvette manufacturer for cuvette specifications)

Stir Speed: If using automatic stirring, drop micro-stir bead into sample cuvette and set Stir Speed (levels 1 through 9 correspond with range from 10 RPM to 850 RPM with controlled ramping from zero)

Heat cuvette to 37 °C: Select this option if sample cuvettes require heating. Cuvette heater increases from room temperature to 37 °C at rate of 5 °C/minute.

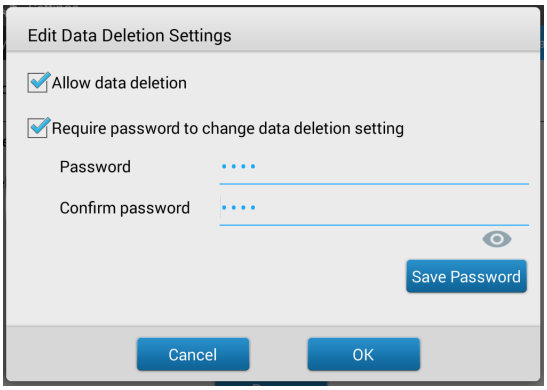
Data Deletion Settings Tap **Change Settings** to edit the data deletion settings. You can enable or disable data deletion and set password requirements. For details, see "Data Deletion Settings."



Data Deletion Settings

Select or de-select the **Allow Data Deletion** checkbox to allow or disallow deletion of instrument data and both custom and chemometric methods.

You can select **Require password to change data deletion** and set your password to secure deletion settings.

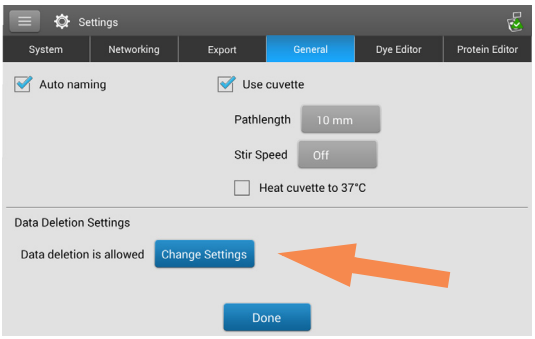


Enter your desired password and select Save Password.

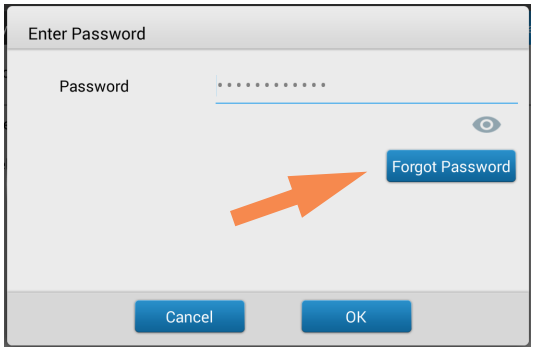
You have the option to save a password reset key to a USB drive. Saving a password reset key to a USB drive allows you to reset the password in the event you do not remember or have access to your password.

Reset Instrument password

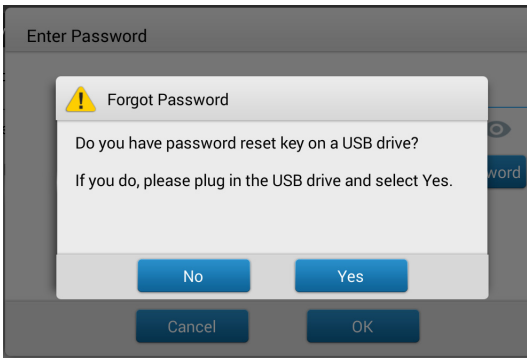
1. From General Settings, select **Change Settings**



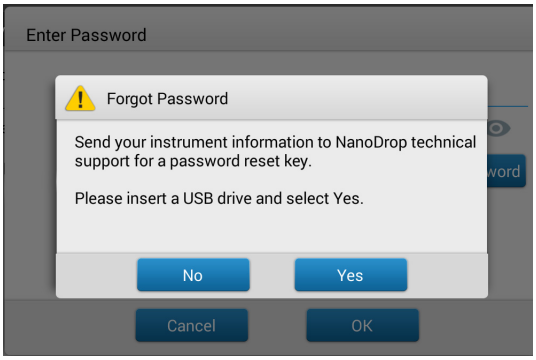
2. Select **Forgot Password**



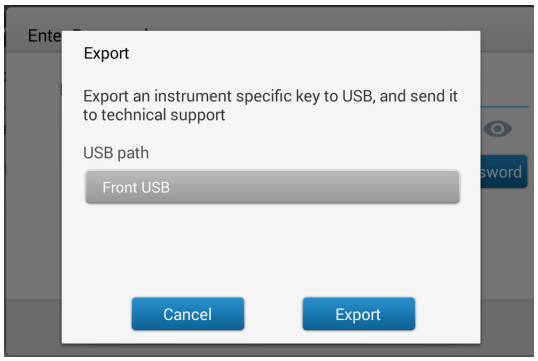
3. Implement password key
   - If you have a USB with a password key, insert the USB drive into the instrument and select Yes. The password is reset and you will be now be able to enter a new password.
   - If you do not have a password key, select **No**. Continue to step 4



4. With a USB drive inserted into the instrument, select **Yes**.



5. Select **Export**. This will generate a file for you to send to NanoDrop support so they can provide you with a password reset key.



PC Control Software

Control your NanoDrop One from a PC through a direct Ethernet connection with the PC Control software. If your PC does not have an Ethernet port, you can use an Ethernet-to-USB adapter. You can store or view data acquired with a NanoDrop One instrument on the PC, as well as change instrument settings, and create or edit custom methods.

PC Control Home Screen overview

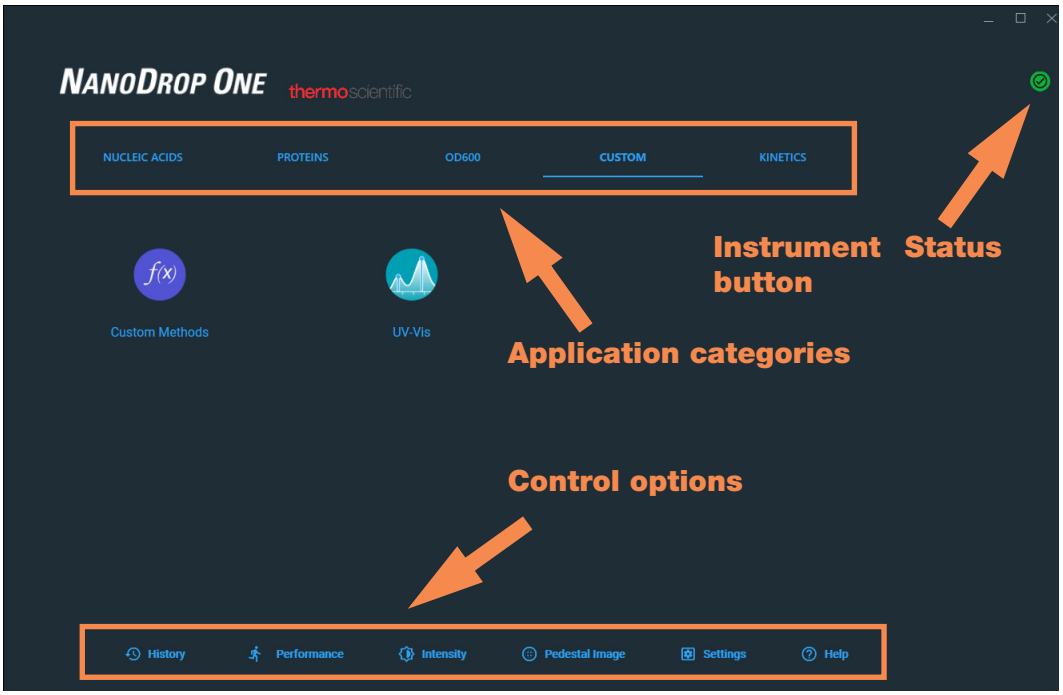


Figure 1. PC Control Home Screen

Select your application from the categorized icons just as you would with the NanoDrop One instrument Home Screen.

Control options

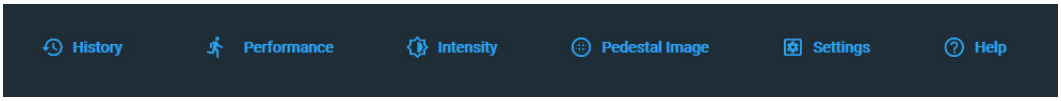
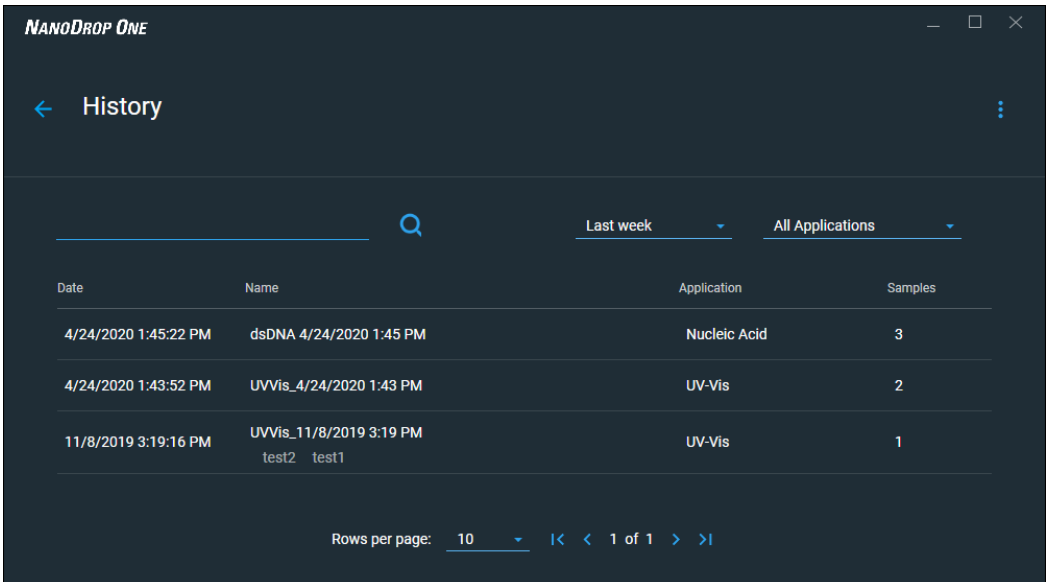


Figure 2. Control options

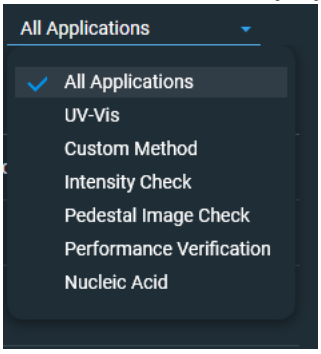
History:	View data stored locally. Filter by date or application.
Performance:	Performance verification process using PV-1 solution. See “Performance Verification” on page 281
Intensity:	Run an intensity check for the cuvette or pedestal. See “Intensity Check” on page 279.
Pedestal Image:	Run a pedestal image check. See “Pedestal Image Check” on page 286.
Settings:	Set security server location and path if desired. See “User Account Control” on page 16
Help:	View help

History

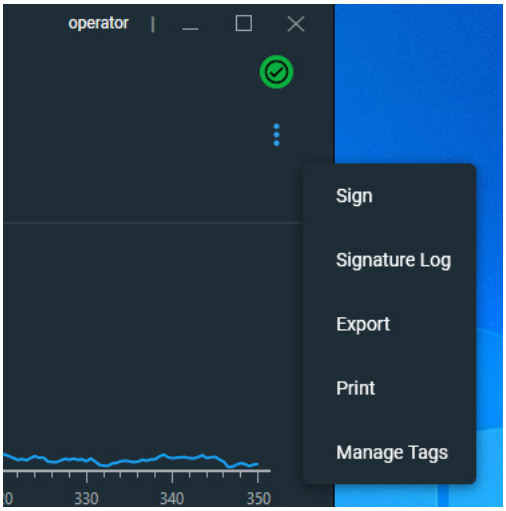
The History option functions similarly to the instrument History. You can view all experiments performed from the local PC.



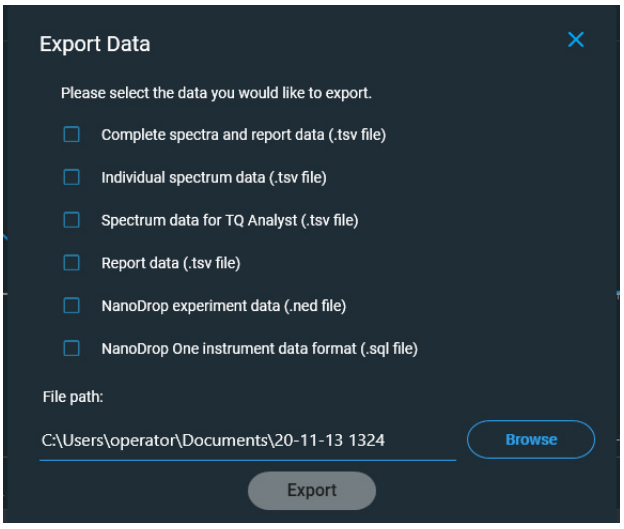
You can search history by name, filter by date, and filter by application.



When viewing an experiment in History, you can **Sign**, view the **Signature Log**, **Export**, **Print**, or **Manage Tags** from the drop-down menu.

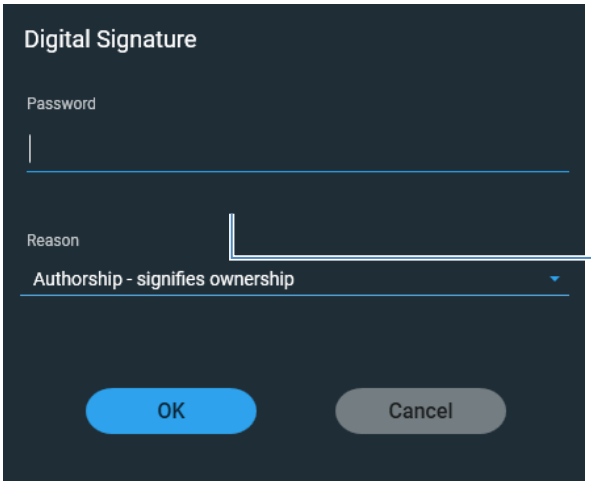


When exporting data from History or at the end of an experiment, click the check box in the dialog box to select the data you want to export. You can also browse to change the file path if desired.



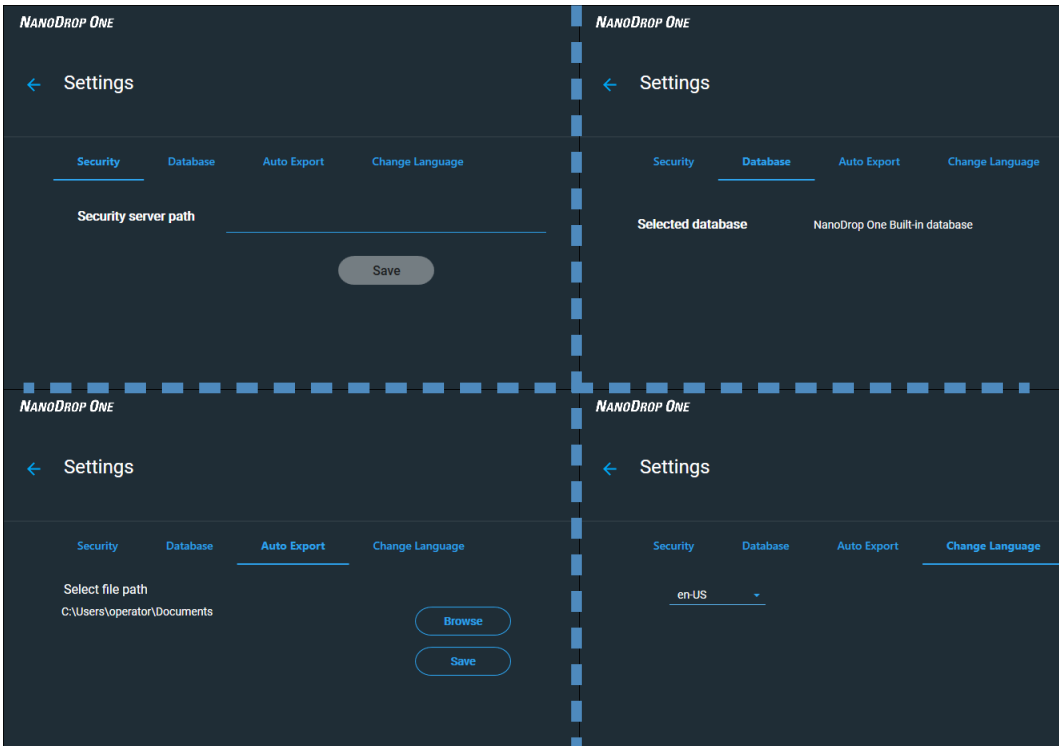
If you make any changes to an experiment from History, you may be asked to sign for the changes upon exiting.

– Enter your password to sign the changes if prompted.



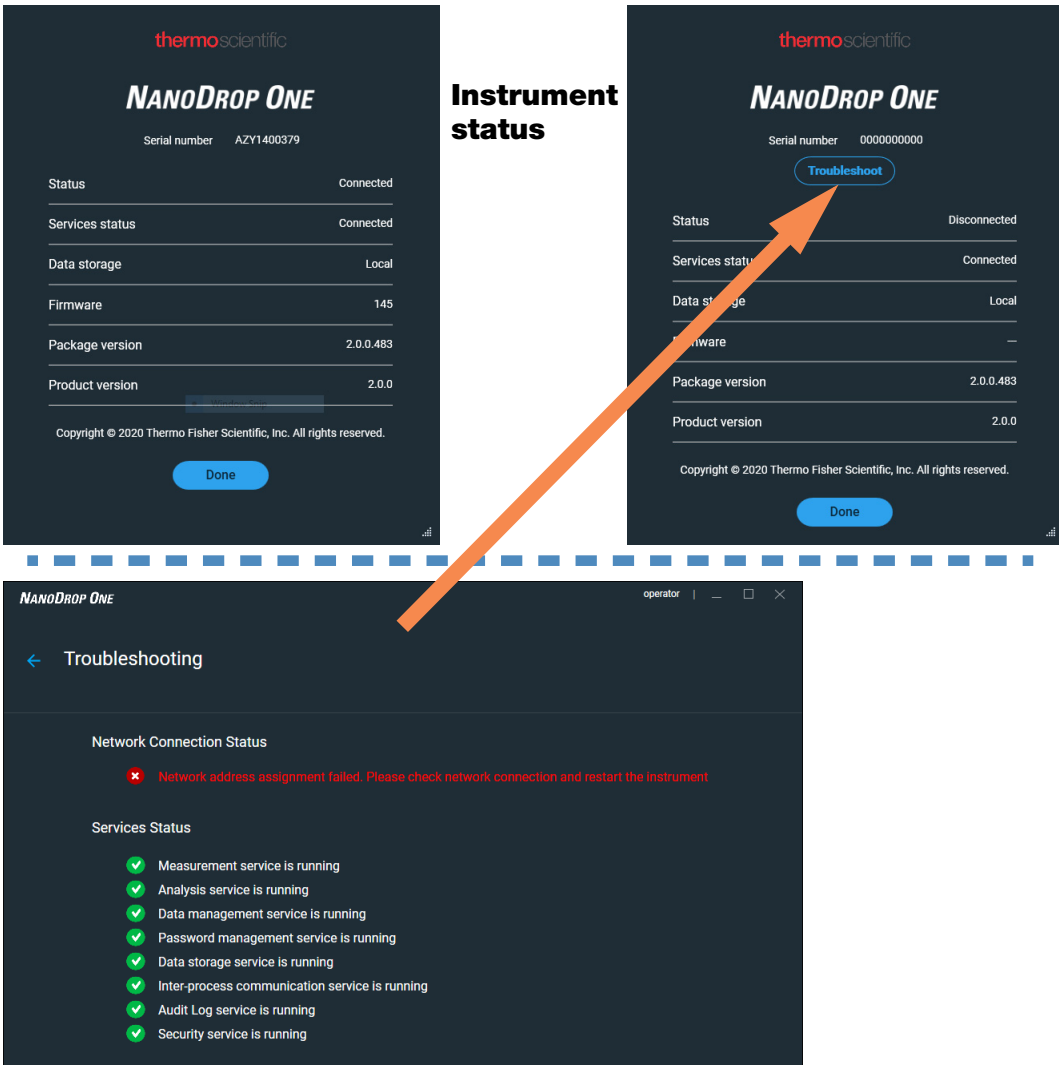
Settings

Use Settings to modify security settings, view the selected database, select a file path for exporting data, and change the interface language.



Instrument Status

Select the Instrument Status button to view the status and instrument configuration. If the Instrument status button is red, select the button and then click **troubleshoot** to learn more about the problem.



Measurement Screen Display Options

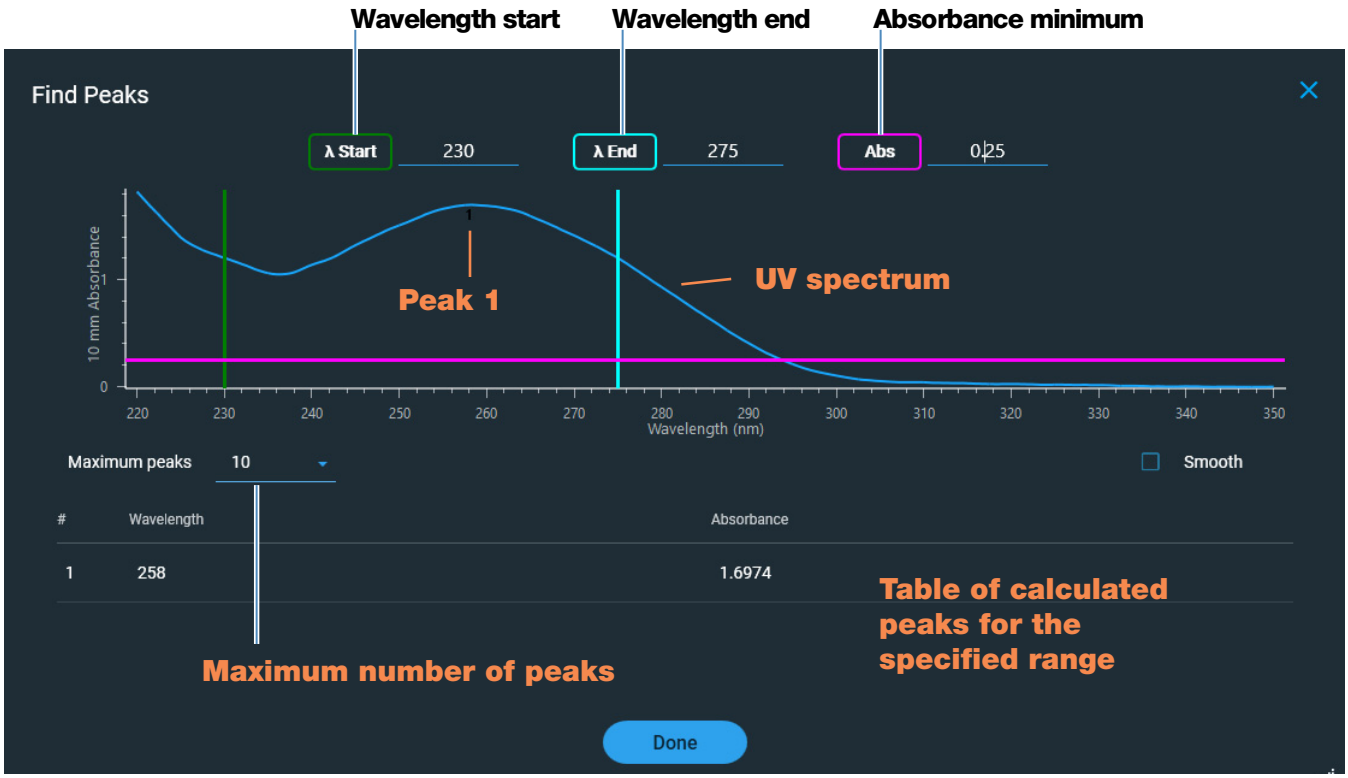
When performing measurements using the PC Control software, right-click the graph on the measurement screen to bring up the following display options:

Overlay Mode	**Display multiple samples in overlay.**
✓ Show CrossHairs	**Hover spectrum to see plot data**
✓ Show Legend	**Display graph legend**
Find Peaks	**Calculate peaks for specified range**
Autoscale	**Scale axes to fit spectra measurement**
Format X-axis	**Select to manually enter x-axis range**
Format Y-axis	**Select to manually enter y-axis range**

Display options- Right click graph to view

Find Peaks

Select **Find Peaks** to view calculated peaks for specified range. You can enter the range by dragging the color-coded limit lines, or enter values into the fields at the top of the spectrum. Found peaks for the defined range are listed in the table below the spectrum.



10

Maintenance

- Maintenance Schedule 270
- Cleaning the Touchscreen 271
- Maintaining the Pedestals 271
- Decontaminating the Instrument 276
- Maintaining the Cuvette Sampling System 278
- Instrument Diagnostics 278

Maintenance Schedule

Daily Maintenance

- Clean pedestals with deionized water



Periodic Maintenance

- Clean touchscreen
- Clean pedestals with 0.5M HCl
- Recondition pedestals

Every 6 Months

- Recondition pedestals
- Run Intensity Check
- Run Performance Verification
- Run Pedestal Image Check

If you are experiencing an issue with your system, refer to the troubleshooting information. If the issue persists, contact us. If you are outside the U.S.A. and Canada, please contact your local distributor.

If your instrument requires maintenance or repair, contact us or your local distributor.

Cleaning the Touchscreen

Note To avoid causing permanent damage to the touchscreen, do not:

- clean the touchscreen with abrasive material such as paper towel
- apply excessive pressure
- spray liquid directly onto the touchscreen
- apply lubricant to the touchscreen slide mechanism

To clean the touchscreen

Gently wipe the touchscreen with a soft, lint-free cloth such as microfiber.

If necessary, use a cleaner intended for glass LCD displays and follow the manufacturer's recommendations.



Maintaining the Pedestals

The pedestals require periodic maintenance to maintain measurement integrity. Time lines and procedures for cleaning and reconditioning the pedestals are provided below.

Cleaning the Pedestals

To avoid carryover and cross contamination, clean the pedestals before the first blank or sample measurement and at the end of each measurement. Additional cleaning (see below) or reconditioning may be required for periodic maintenance.

Note

- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.
- 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.
- Do not attempt to remove the diaphragm around the lower pedestal as it is permanently affixed to the instrument.
- Do not allow HCl, alcohol, bleach, acetone or any other solvent to remain on the diaphragm for more than one minute or it may loosen the seals. If the diaphragm becomes loose, contact us.
-

Note Solutions containing detergent or isopropyl alcohol may uncondition the pedestals. If these are required for sample analyses, follow immediately with 3–5 µL DI H_2O.

Supplies needed

- lint-free laboratory wipes
- deionized water (DI H_2O)
- for thorough cleaning: PR-1 kit or 0.5M HCl



To clean the pedestals between measurements

Lift the instrument arm and clean the upper and lower pedestal with a new laboratory wipe.

To clean the pedestals between users

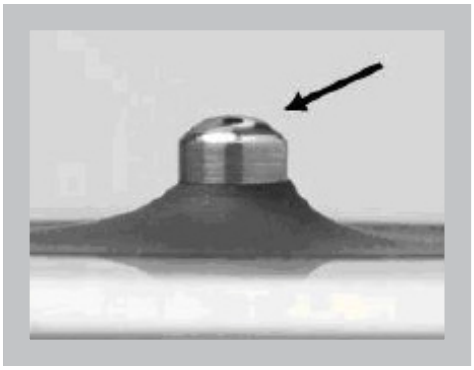
1. Lift the arm and clean both pedestals with a new laboratory wipe.
2. Pipette 3–5 µL DI H_2O onto the lower pedestal.
3. Lower the arm and wait 2–3 minutes.
4. Lift the arm and clean both pedestals with a new wipe.

Tip: When thorough cleaning is required (for example, to remove dried sample left on the pedestals), substitute 0.5M HCl for the DI H2O in the procedure above and follow with 3-5 µL DI H2O. You can also recondition the pedestals using PR-1 compound.

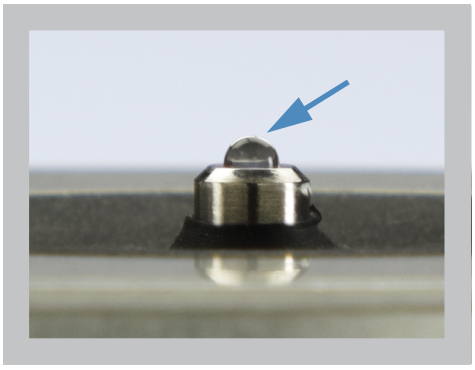
Reconditioning the Pedestals

The pedestal surfaces may lose their “conditioned” properties over time, especially after measurements with isopropyl alcohol or solutions that contain surfactants or detergents such as the Bradford reagent. An unconditioned pedestal causes droplets on the lower pedestal to “flatten out,” preventing proper formation of the liquid column when the arm is lowered. The resulting spectrum may look “rough” or “jagged.”

If samples flatten out on the pedestal (rather than “beading up” or forming a rounded droplet) or the liquid column breaks during a measurement, recondition the pedestals.



Unconditioned pedestal
(droplet flattens out)



Properly conditioned pedestal
(droplet beads up)

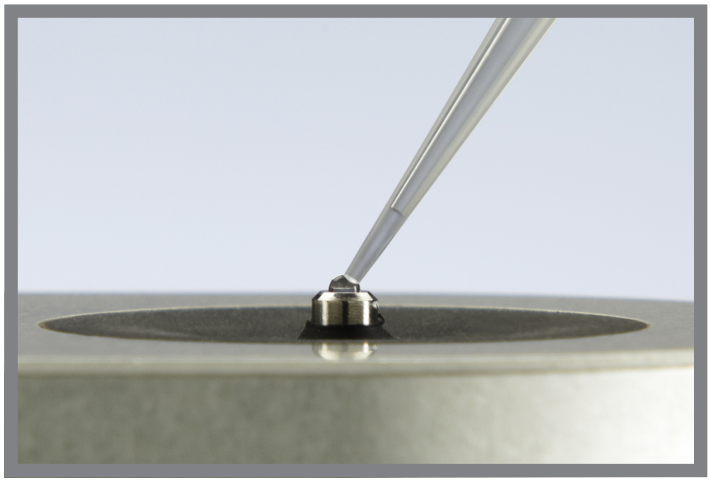
Supplies needed

- lint-free laboratory wipes
- PR-1 pedestal reconditioning kit (available from us or a local distributor)
- calibrated precision pipettor (0-2 µL)
- canned air

To recondition the pedestals







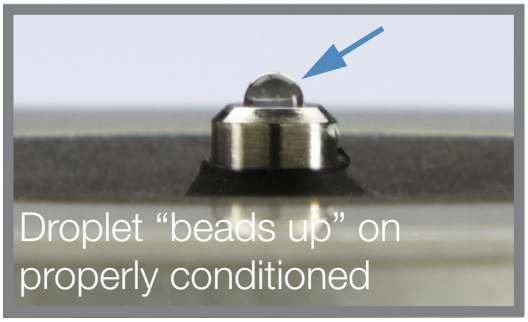
1. Open the container of PR-1 compound and use the provided applicator to remove a pin-head sized amount of the compound.
2. Apply a thin, even layer of reconditioning compound to the surface of the upper and lower pedestal.

   Wait 30 seconds for the PR-1 compound to dry.
3. Fold a clean laboratory wipe into quarters and use it to vigorously buff the surface of each pedestal.

   Notice: Support the instrument arm with one hand while you buff the upper pedestal to avoid damaging the arm.

   Tip: Black residue on the wipe is normal.
4. Repeat step 3 with a new folded wipe until all residue is removed and the pedestals buff clean.
5. Use canned air to remove any paper residue from the pedestals.
6. Pipette 1 µL DI H_2O onto the lower pedestal.

   The DI H_2O should “bead up” or form a rounded droplet.



Tip The PR-1 pedestal reconditioning compound is the easiest way to recondition the pedestals. If you don't have a PR-1 kit, follow these steps:

1. Lift the instrument arm and pipette 3 µL 0.5M HCl onto the lower pedestal.
2. Lower the arm and wait 2–3 minutes.
3. Lift the arm and clean both pedestals with a new laboratory wipe.
4. Pipette 3 µL DI H_2O onto the lower pedestal.
5. Lower the arm and wait 2–3 minutes.
6. Lift the arm and clean both pedestals with a new wipe.

   NOTICE: Support the instrument arm with one hand while you buff the upper pedestal to avoid damaging the arm.
7. Fold a clean laboratory wipe into quarters and use it to vigorously buff the surface of each pedestal at least 50 times.
8. Use canned air to remove any paper residue from the pedestals.

Decontaminating the Instrument

Decontaminate the instrument after measurements with samples that contain hazardous materials and before returning the instrument to us for maintenance or repair.

Note If your instrument requires maintenance or repair, contact us or your local distributor.

Note

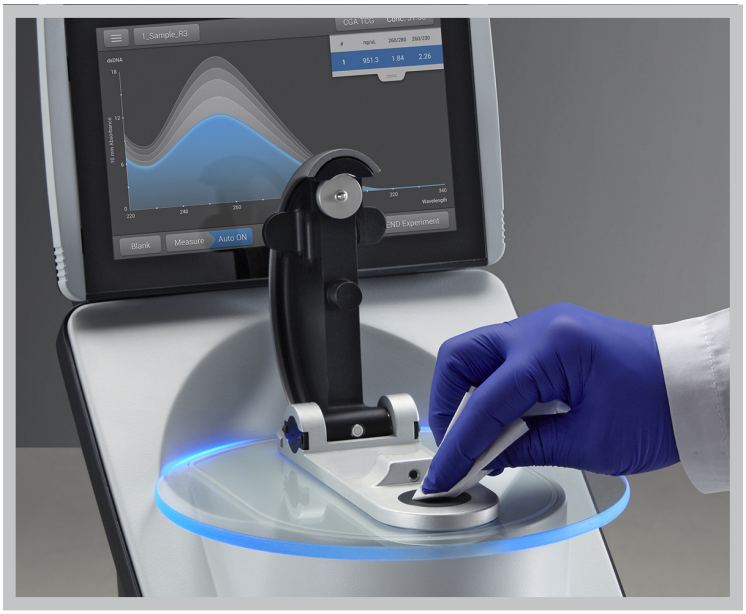
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.
- To prevent damage from spills, keep containers of liquids away from the instrument.
- Do not use a squirt or spray bottle on or near the instrument as liquids will flow into the instrument and may cause permanent damage.
- Do not attempt to remove the diaphragm around the lower pedestal as it is permanently affixed to the instrument.
- Do not allow HCl, alcohol, bleach, acetone or any other solvent to remain on the diaphragm for more than one minute or it may loosen the seals. If the diaphragm becomes loose, contact us.
-

Supplies needed

- lint-free laboratory wipes
- deionized water (DI H_2O)
- 0.5% sodium hypochlorite solution (1:10 dilution of commercial bleach, freshly prepared)
- pipettor

To decontaminate the pedestals

1. Lift the instrument arm and clean the upper and lower pedestal with a new laboratory wipe.
2. Pipette 2–3 µL diluted bleach solution (see Supplies needed) onto the lower pedestal.
3. Lower the arm and wait 2–3 minutes.
4. Lift the arm and clean both pedestals with a new wipe.
5. Pipette 3–5 µL DI H_2O onto the lower pedestal.
6. Lower the arm and wait 2–3 minutes.
7. Lift the arm and clean both pedestals with a new wipe.



To decontaminate the instrument surfaces

1. Dampen a clean, soft cloth or laboratory wipe with the diluted bleach solution (see Supplies needed) and use it to gently wipe the outside surfaces of the instrument.
2. Use a clean cloth or wipe dampened with DI H_2O to remove the bleach solution.



Maintaining the Cuvette Sampling System

The cuvette sampling system is included only with the NanoDrop One^{C} model instrument. For information about compatible cuvettes, see Measuring a Sample using a Cuvette.

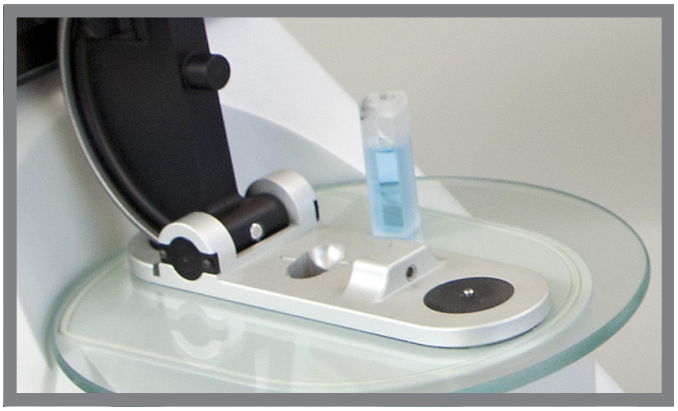
Note Clean and dry cuvettes after each measurement. Use cuvettes that are free of scratches and avoid fingerprints which may affect results.

Note 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 maintain the cuvette sampling system

- Keep the instrument arm closed when the instrument is not in use.
- Use canned air to remove any dust from the cuvette holder.
- Clean up any spills inside the cuvette holder with a new laboratory wipe.

To clean and maintain cuvettes, follow the recommendations of the cuvette manufacturer.



Instrument Diagnostics

Every 6 months, run the following performance and quality checks to verify instrument operation.

Intensity Check 279

Performance Verification 281

Pedestal Image Check 286

Diagnostics can be performed using the NanoDrop One instrument or the PC Control software. **Intensity Check**, **Performance Verification**, and **Pedestal Image Check** are all accessible from the PC Control software Home screen:

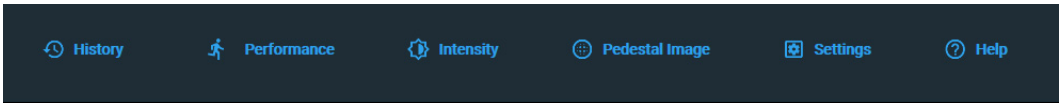


Figure 3. Control options

History:	View data stored locally. Filter by date or application.
Performance:	Performance verification process using PV-1 solution
Intensity:	Run an intensity check for the cuvette or pedestal
Pedestal Image:	Run a pedestal image check
Settings:	Set security server location and path if desired
Help:	View help

Intensity Check

Run Intensity Check every 6 months to verify operation of the instrument's internal components. The test measures the intensity of light from the xenon source through the instrument to verify that throughput, wavelength accuracy, and bias are within specifications. The test is automatically performed using the pedestal and the cuvette optical paths.

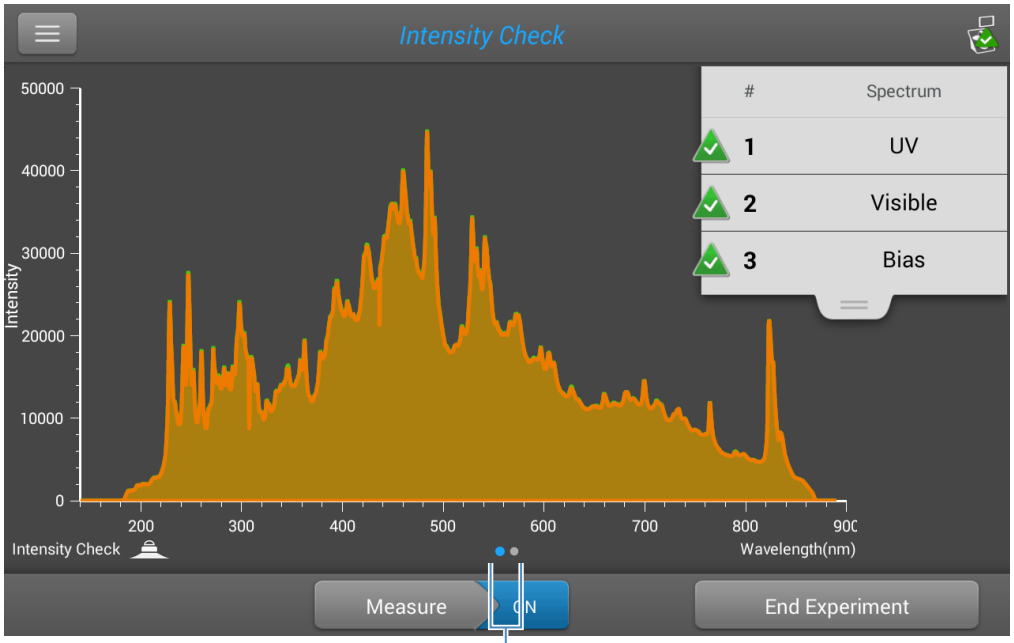
Supplies needed

- lint-free laboratory wipes

To run intensity check

1. Lift the instrument arm and clean the upper and lower pedestal with a new laboratory wipe.
2. Remove any cuvette from the cuvette holder.
3. Lower the arm.
4. From the instrument home screen, tap  (Diagnostics) and then tap **Intensity Check**. If you are using the PC Control software, from the Home screen, select **Intensity**.
5. On the instrument, Tap **Measure** and wait for the measurements to complete.

Here is an example of a typical intensity check result screen.

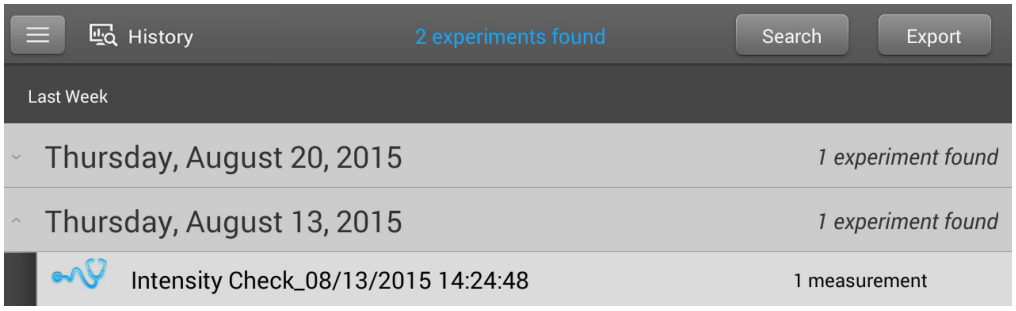


Swipe screen left to view detailed results

If you are using the PC Control software, select **Run**.

6. To rerun the intensity check, tap **Measure**.
7. When finished, tap **End Experiment**.

After the test is completed, the results are available from the History (see example below). See Manage identifiers on the instrument for details.



To interpret intensity check results

If one of these indicators:

- UV
- Visible
- Bias

has an adjacent yellow triangle instead of the green check marks shown above, clean the pedestals with deionized water and then repeat the Intensity Check.

If a yellow triangle appears next to the Bias indicator, make sure the room is within the temperature specifications for the instrument.

If the Intensity Check fails again, contact us.

Performance Verification

Run Performance Verification every 6 months to confirm pathlength accuracy is within specifications.

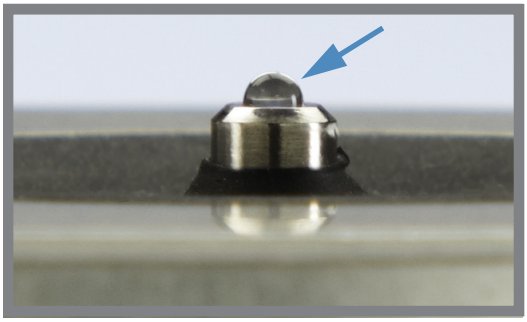
Supplies needed

- lint-free laboratory wipes
- deionized water (DI H_2O)
- calibrated precision pipettor (0–2 µL)
- PV-1 performance verification solution (liquid photometric standard available only from us or a local distributor)
- laboratory gloves

Note The PV-1 solution comes in a single-use ampoule. Before you open the ampoule, shake it vigorously and then allow the liquid to collect in the bottom portion of the ampoule. After the ampoule is opened, its contents must be used within one hour. Pipette directly from the ampoule; do not transfer the solution.

Before you begin

First make sure the pedestals are properly conditioned. To test pedestal conditioning, clean the pedestals with a new laboratory wipe, then pipette 1 µL DI H_2O onto the lower pedestal. The droplet should "bead up" as shown below. If it does not, recondition both pedestals.

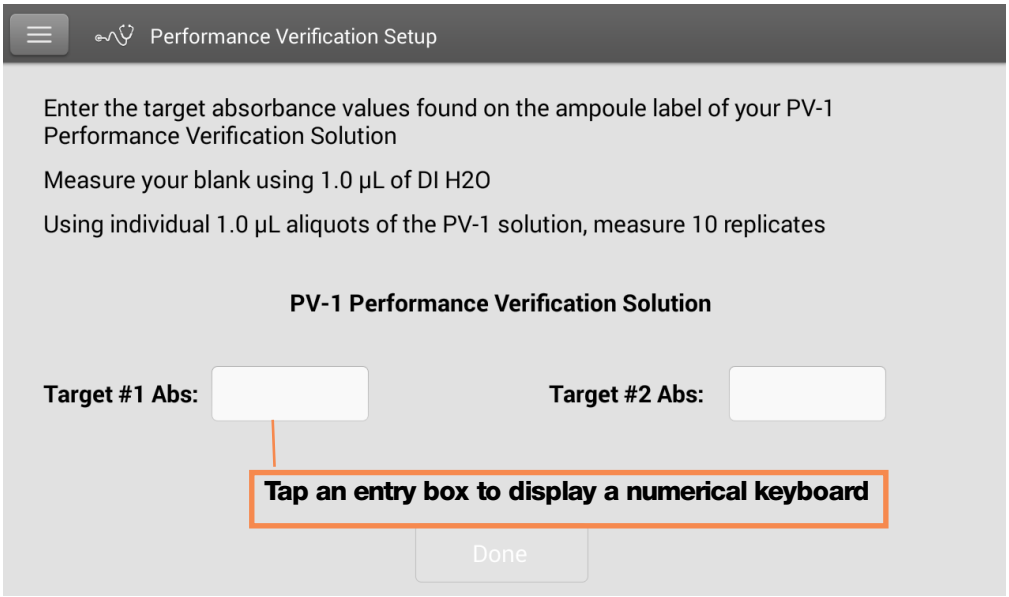


Droplet "beads up" on properly conditioned pedestal

To run performance verification

1. From the instrument home screen, tap  (Diagnostics) and then tap **Performance Verification**. If you are using the PC Control software, from the Home screen, select **Performance**.

   A message asks for target absorbance values.



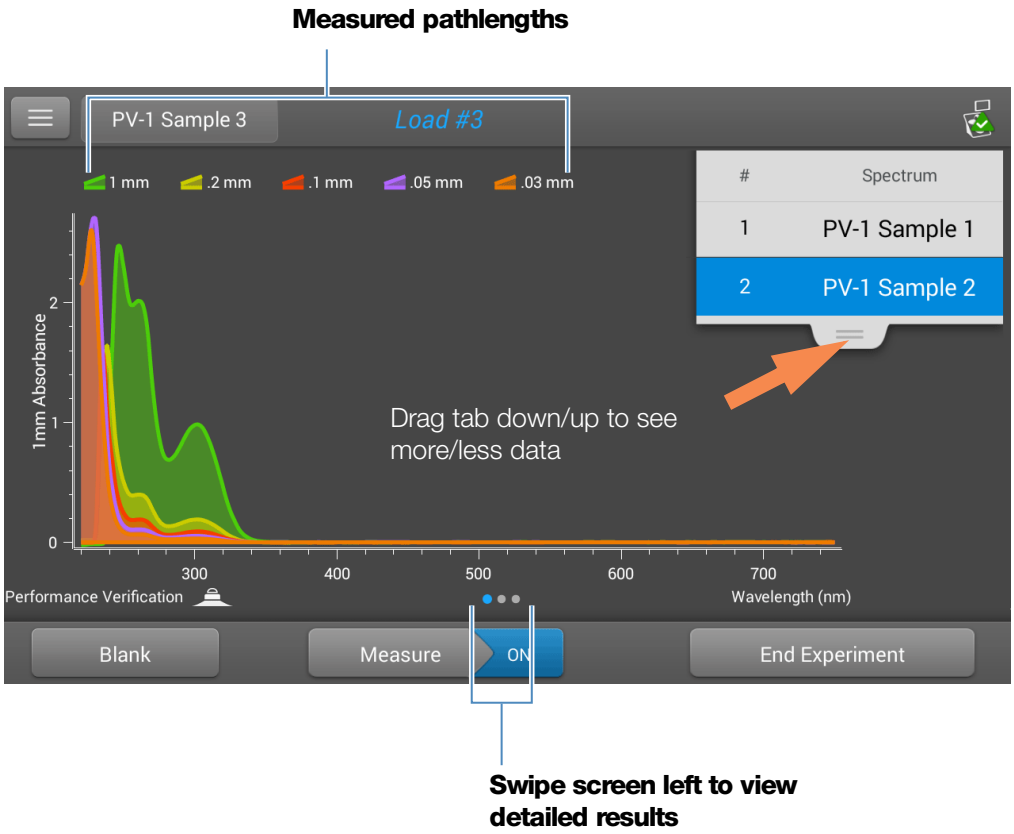
2. Enter each lot-specific target absorbance value from the label on the PV-1 ampoule in its associated entry box and then tap **Done**.
3. Lift the instrument arm and clean the upper and lower pedestal with a new laboratory wipe.

4. Pipette 1 µL DI H_2O onto the lower pedestal, lower the arm and tap **Blank**.
5. Lift the arm and clean both pedestals with a new wipe.

> **Note** Vigorously shake the ampoule of PV-1 solution, allow the liquid to collect in the bottom portion of the ampoule and then follow standard practices to open it.

6. Pipette 1 µL PV-1 solution onto the lower pedestal and start the sample measurement:
   - If Auto-Measure is On, lower arm
   - If Auto-Measure is off, lower arm and tap **Measure** or from the PC Control software, select **Run**.

   After the measurement, the software displays the results. Here is an example of the performance verification result screen.

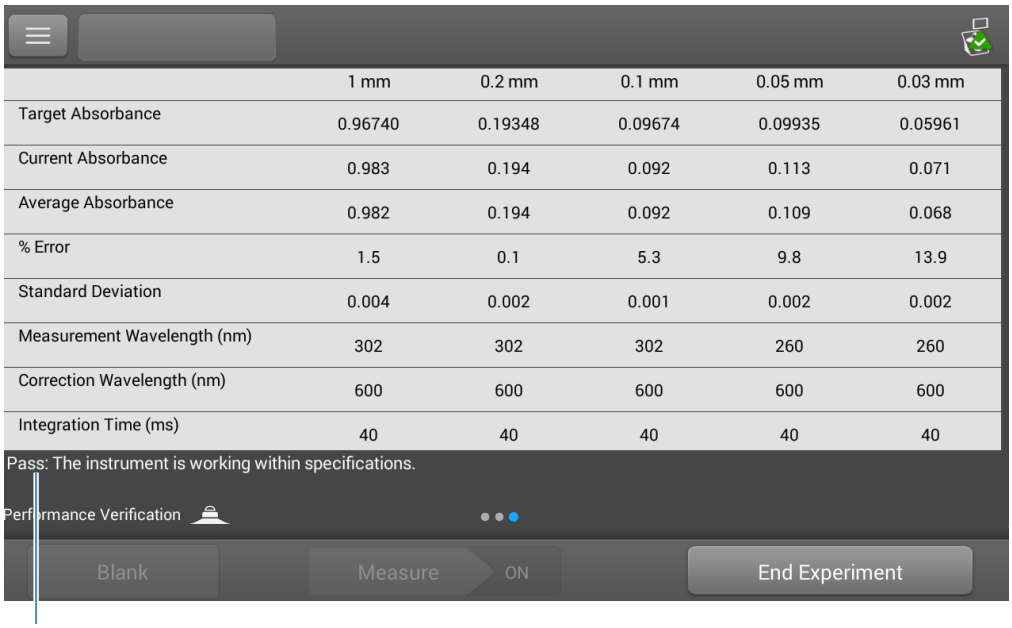


7. Repeat step 6 to measure the PV-1 solution nine more times using a new 1 µL aliquot for each measurement and cleaning both pedestals after each measurement.

After each measurement, a new sample result is added to the display. Swipe the screen left to see a summary of the 10 sample results.

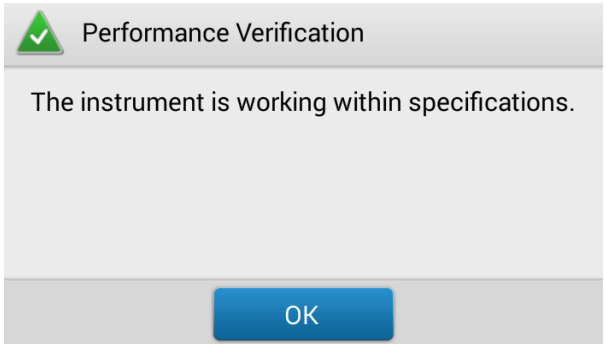


Swipe left again to see additional measurement details, along with the overall test result.



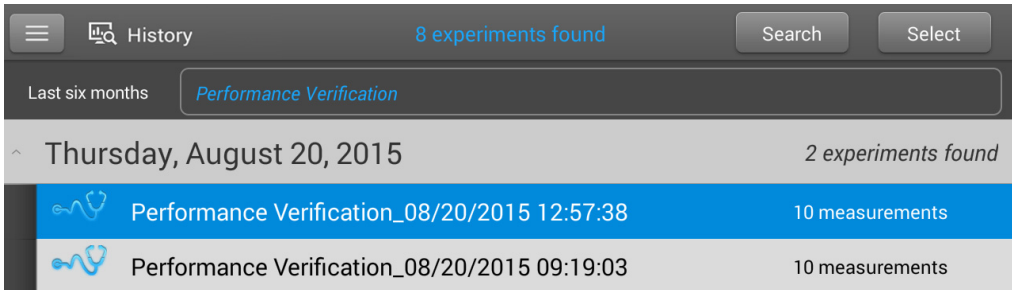
Performance test result

After the tenth measurement, a message indicates whether the instrument passed or failed performance verification:



8. If the instrument failed, immediately repeat step 6 using ten 2 µL aliquots of the PV-1 solution.
9. When finished, tap **End Experiment** and clean the pedestals with 3–5 µL DI H_2O.

   After the test is completed, the results are available from the History (see example below). See Manage identifiers on the instrument for details.



To interpret performance verification results

If your instrument failed performance verification and you repeated ten measurements using 2 uL aliquots, contact us.

Pedestal Image Check

Run the Pedestal Image Check periodically to verify the instrument's column sensor which monitors for possible errors such as an empty column or bubbles in a sample. The Pedestal Image Check can be used for routine quality control purposes. It also provides important diagnostic information if a detection system component fails.

Supplies needed

- lint-free laboratory wipes

To run pedestal image check

1. Lift the instrument arm and clean the upper and lower pedestal with a new laboratory wipe.
2. Lower the arm.
3. From the instrument home screen, tap  (Diagnostics) and then tap **Pedestal Image Check**. If you are using the PC Control software, from the Home screen, select **Pedestal Image**.
4. Tap **Measure** or select **Run.**

   The instrument runs a series of tests to check pedestal position and image quality. After the measurements are completed, the results are displayed. A green check mark indicates the instrument passed the Pedestal Image Check.
5. When finished, tap **End Experiment**.

To interpret pedestal image check results

If the Pedestal Image Check displays a yellow triangle instead of the green check mark, follow the on-screen instructions to fix any possible problems. Then rerun the Pedestal Image Check. If the instrument fails again, contact us.

11

Safety and Operating Precautions

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Note Be sure that all persons operating this system read the safety manual first.

Operating Precautions



CAUTION Do not remove the instrument cover. Removing the cover exposes the operator to sharp edges and delicate fiber optic cables. The instrument warranty is void if the cover has been removed.

NanoDrop One spectrophotometers are designed to operate indoors in an environment that meets our specifications. For details, see the site preparation guide for your instrument.

Follow these precautions to avoid damaging your NanoDrop spectrophotometer during use:

- Use a grounded power cord appropriate for your electrical service. If the supplied power cord is incompatible or if it becomes damaged, contact us.
- Do not remove the instrument cover.
- The plate below the arm assembly is made of heat tempered glass. The LCD display uses heat treated, chemical tempered glass. Both are rugged and difficult to break. However, should either the plate or display become cracked or broken, contact us for replacement.
- Use solvents that are compatible with the instrument (see Hazardous Materials)
- Do not use hydrofluoric acid (HF) on the pedestals. Fluoride ions will permanently damage the quartz fiber optic cables.
- 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.
- Do not attempt to remove the diaphragm around the lower pedestal as it is permanently affixed to the instrument.
- Do not allow HCl, alcohol, bleach, acetone or any other solvent to remain on the diaphragm for more than one minute or it may loosen the seals. If the diaphragm becomes loose, contact us.

Safety Information

Before operating a NanoDrop One instrument, please read the safety information and follow its recommendations for the system.

Safety and Special Notices

In many cases, safety information is displayed on the instrument itself. The symbol indicates that there is additional safety information in the documentation and failure to heed the safety precautions could result in injury.



WARNING Indicates a hazardous situation which, if not avoided, could result in death or serious injury.



CAUTION Indicates a hazardous situation which, if not avoided, could result in minor or moderate injury.

Note Follow instructions with this label to avoid damaging the system hardware or losing data.

Note Contains helpful supplementary information.

The following table lists some of the safety symbols and their indications that may appear in the user documentation.

Symbols	Indication
	This is a mandatory action symbol. It is used to indicate that an action shall be taken to avoid a hazard.
	This is a prohibition symbol. The graphic in this symbol is used to alert the user to actions that shall not be taken or shall be stopped.
	This is the general warning sign. Failure to heed the safety precautions could result in personal injury.
	Avoid shock hazard. If you see either of these symbols, there is a risk of electrical shock in the vicinity. Only qualified persons shall perform the related procedures.
	Avoid fire hazard. Do not test flammable or explosive samples. Read and follow the associated instructions carefully.
	Avoid eye injury. If you see these symbols, there is a risk of exposure to ultraviolet light, which can harm your eyes if safety glasses are not worn.
	Avoid Biohazard. This icon informs of a biological hazard in the area. Read and follow the associated instructions carefully.
	Avoid chemical burns. This symbol alerts you to possible skin irritation. Wear gloves when handling toxic, carcinogenic, mutagenic, or corrosive or irritant chemicals. Use approved containers and proper procedures to dispose of waste.

Symbol	Description
	Alternating current
	Earth terminal or ground
	Direct current
	Protective conductor terminal
	Frame or chassis terminal
	Fuse
	Power on
	Power off

When the System Arrives



WARNING Avoid personal injury. If this equipment is used in a manner not specified in the accompanying documentation, the protection provided by the equipment may be impaired.



CAUTION Avoid personal injury. Perform *only* those procedures described in the documentation. If there are other problems, contact us. Any other service must be performed by trained personnel.



CAUTION Avoid shock hazard. Do not remove the cover of the instrument. All service to the instrument must be performed by trained personnel.

When the instrument arrives, check the exterior of the shipping box for signs of damage. If damage is apparent, contact us or your local distributor for instructions.

- Move the shipping box to the installation location at least 24 hours before installation.

  Note

  - Inside the shipping box, the instrument is sealed in a plastic bag to keep the unit dry.
  - Allow 24 hours for the instrument to reach room temperature before opening the bag. If the bag is opened before the instrument reaches room temperature, moisture could condense on the optical components and cause permanent damage.

- Keep the instrument upright at all times.

The warranty will not cover:

- Damage due to improper moving techniques.
- Damage due to removing the sealed plastic bag before the instrument has come to room temperature.

Note It is important to have all system utilities installed before the instrument arrives. Utility installations must comply with all local building and safety codes.

Lifting or Moving the Instrument

To avoid risk of injury, use proper lifting techniques when lifting or moving the instrument or other system components.

Electrical Requirements and Safety

Power supplied to the system must be from dedicated, uninterrupted sources. Power must be free of voltage dropouts, transient spikes, frequency shifts, and other line disturbances that impair reliable performance.

If you suspect power quality problems at your site, or if your system will be installed in a heavy industrial environment, we recommend a power quality audit before installation. Contact us or your local electrical authority for more information.

CAUTION Avoid shock hazard.



- Only a qualified person using the appropriate measuring device shall check the line voltage, current and frequency.
- Only our trained and certified service representatives shall attempt to service a component that carries this symbol.
- If a protective cover on a system component appears damaged, turn off the system and secure it against any unintended operation. Always examine the protective cover for transport stresses after shipping.
- Even after this instrument has been disconnected from all voltage sources, capacitors may remain charged for up to 30 seconds and can cause an electrical shock.
- Do not allow liquid to run over or into any surface where it may gain entry into the instrument.
- Do not attempt to remove the cover of the instrument.

Grounding



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.

Power Cords

Be sure to use an appropriate grounded power cord for your electrical service. If the power cord received is not appropriate for the electrical system in your location, or if the power cord becomes damaged, contact us.

Power Line Conditioning Accessories

A UPS reduces the probability of a system shutdown if power is lost elsewhere in the building. Power line conditioners (which ensure that your service is free from sags, surges or other line disturbances) also are available in the U.S.A. from us for 120 volt operation. Line conditioners for 220 volt operation can be purchased locally. Contact technical support for information about power conditioners and UPS.

Electrical Service Specifications

The following table lists the specifications for electrical service. Contact our service representative in your area if you have questions about the requirements.

Requirements	Specifications
Input current	5.0 A (max.)
Input voltage	100-240 VAC
Line frequency	50-60 Hz
Line disturbances	Sags, surges or other line disturbances must not exceed 10% of input voltage (even for a half cycle).
Noise	< 2 V (common mode) < 20 V (normal mode)

Power Consumption

Generally, 50% more power should be available than the entire system (including accessories) typically uses. Maximum power consumption and heat dissipation specifications for the spectrometer and accessories are shown below. The values are approximate.

Item	Power Consumption	Max. Heat Dissipation
instrument	60 W	205 Btu/hr

Fire Safety and Burn Hazards

Note Do not position the instrument so that it is difficult to operate the power switch or access the power supply and power cord.

To avoid a burn injury and the risk of fire or explosion:

- Use caution when testing flammable or explosive samples (see the “Hazardous Materials” section)
- Never block any of the vents on the instrument or its power supply
- Only use exact replacement power supplies from us

Optical Safety

This instrument was designed with a protective housing to prevent user exposure to ultraviolet light.



WARNING Avoid personal injury. Never look at the lamp while illuminated.

Hazardous Materials

Many standard spectroscopy methods are based on the use of solvents. Others involve corrosive samples or pressurized samples in a gaseous state.

Volatile Solvents and Flammable Samples



CAUTION Avoid personal injury. Do not leave solvents or flammable samples near the instrument. Be sure that the workspace is properly ventilated.

Compatible Solvents

Most solvents typically used in life science laboratories are compatible with the fiber optic pedestals of all NanoDrop spectrophotometers. However, the high vapor pressure properties of some solvents may not be conducive to small volume measurements when using the pedestal for measurements on any of the NanoDrop instruments. If you are measuring samples with high vapor pressures, use an instrument with provision for measuring samples in cuvettes.

The following solvents are compatible for use on the pedestals of all NanoDrop instruments.

Note Spillage of these solvents on surfaces other than the pedestals may damage the instrument.

- methanol
- ethanol
- n-propanol
- isopropanol
- butanol
- acetone
- ether
- chloroform
- carbon tetrachloride
- DMSO
- DMF
- acetonitrile
- THF
- toluene
- hexane
- benzene
- sodium hydroxide
- sodium hypochlorite (bleach)
- dilute HCl
- dilute HNO_3
- dilute acetic acid

It is recommended that all corrosive solvents be wiped from the pedestal immediately upon completion of a measurement. It is also recommended that the user end a series of measurements with a diH_2O sample to ensure that solvents are not inadvertently left on the pedestal.

The diaphragm around the pedestal of the NanoDrop is permanently affixed to the instrument. Do not attempt to remove the diaphragm or break the seal. Avoid prolonged exposure of the diaphragm to HCl, alcohol, bleach, acetone or other solvents as the adhesive securing the seal may be affected. If the seal comes loose please contact us.

Note All forms of Hydrofluoric Acid (HF) are incompatible as the fluoride ion will etch the fiber optic cable.

Biohazard or Radioactive Materials and Infectious Agents

Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Wear appropriate protective equipment. Individuals should be trained according to applicable regulatory and organization requirements before working with potentially infectious materials. Follow your organization's Biosafety Program protocols for working with and/or handling potentially infectious materials.



WARNING Reduce the risk associated with potentially infectious samples:

- Do not spill samples on any of the instrument components.
- If spill occurs, disinfect the external surfaces immediately following your laboratory protocols.

Instruments, accessories, components or other associated materials should not be disposed of and may not be returned to us or other accessory manufacturers if they are contaminated with biohazard or radioactive materials, infectious agents, or any other materials and/or conditions that could constitute a health or injury hazard to employees. Contact us if you have questions about decontamination requirements.