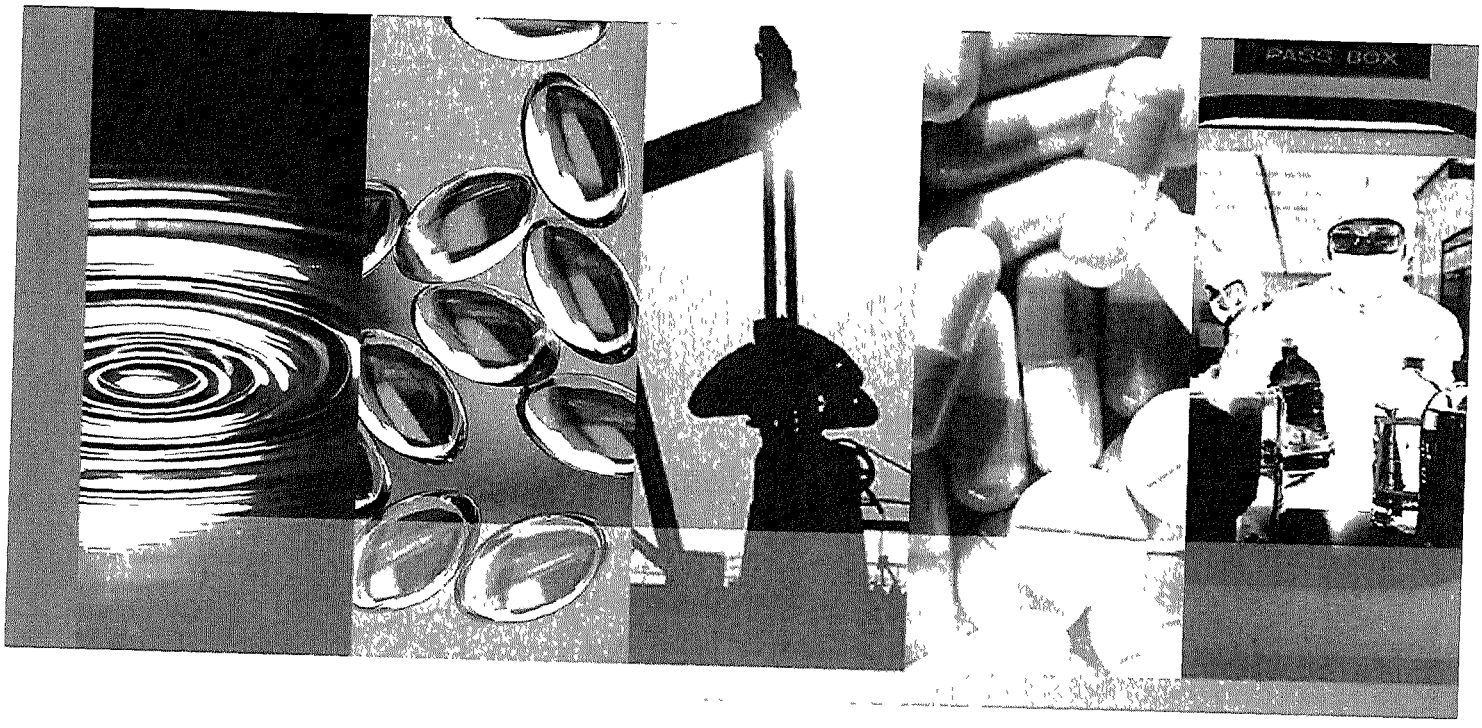

NanoDrop One

User Guide

269-309101 Revision B July 2016

Thermo
SCIENTIFIC

For U S Technical Support, please contact

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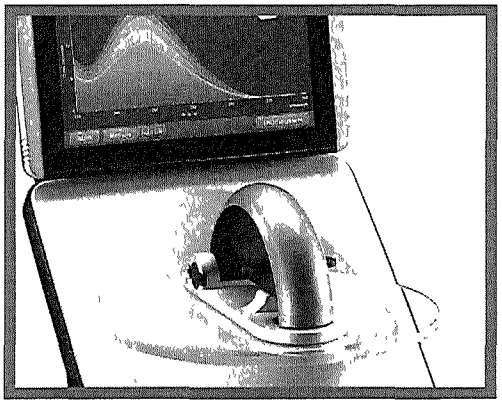
E-mail support madison@thermofisher com

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

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

Contents

About the NanoDrop One Spectrophotometer



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

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

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

Instrument Models and Features

There are two models available for the NanoDrop One spectrophotometer

Optional Accessories

A number of accessories are available for the NanoDrop One instruments

Register Your Instrument

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

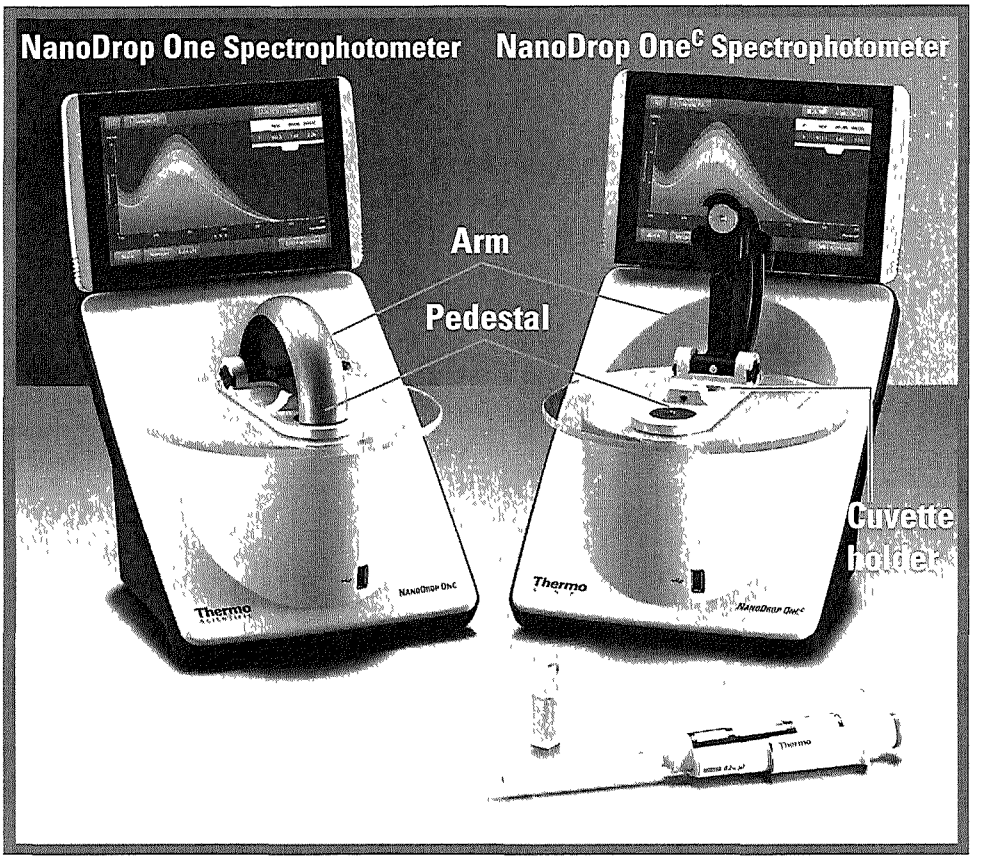
Update Software

Quickly and easily download the latest NanoDrop One software

Instrument Models and Features

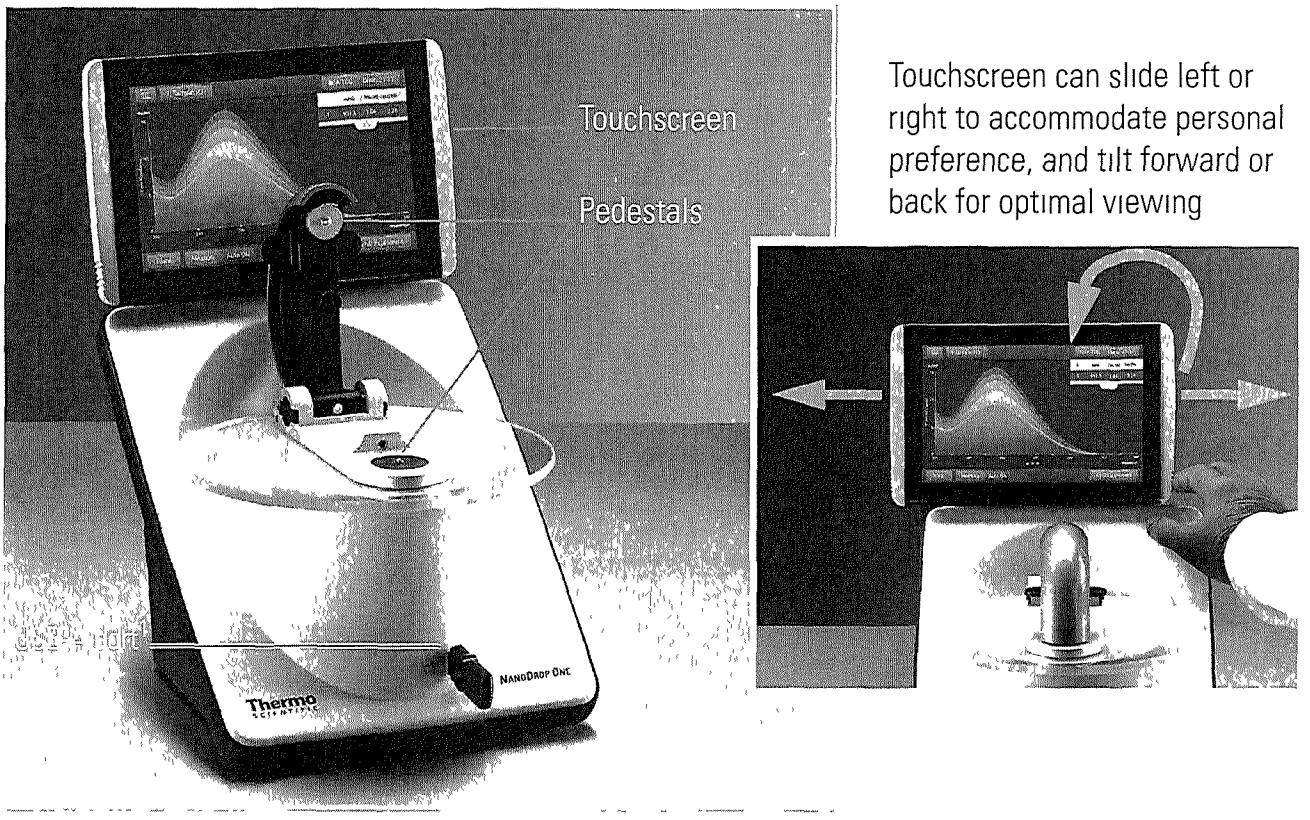
There are two models available for the NanoDrop One spectrophotometer—the NanoDrop One and the NanoDrop One^{C}. Both models include the patented micro-volume sample retention system and general features. The NanoDrop One^{C} model also features a cuvette holder for analyzing dilute samples using standard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

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



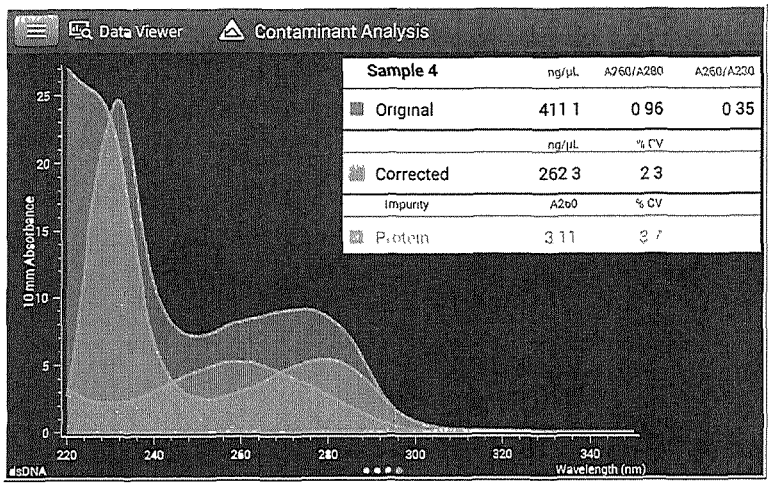
[1]Locate the instrument away from air vents and exhaust fans to minimize evaporation.

Touchscreen



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

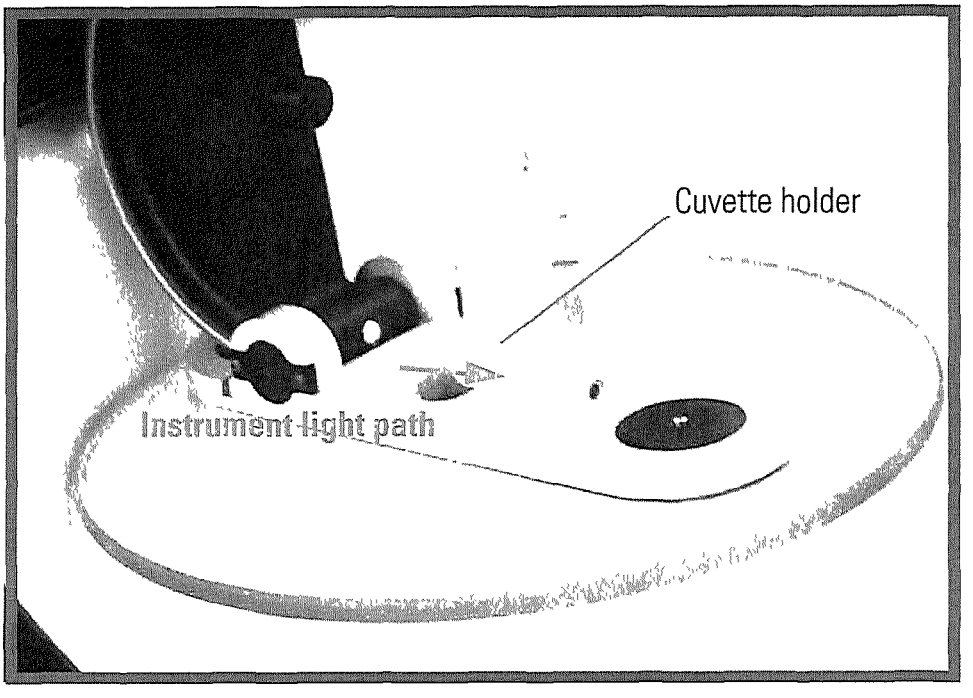
NanoDrop One Software with Acclaro Sample Intelligence Technology



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

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

NanoDrop One^{C} Model Additional Features



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

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

For details, see Measure a Sample using a Cuvette

Optional Accessories

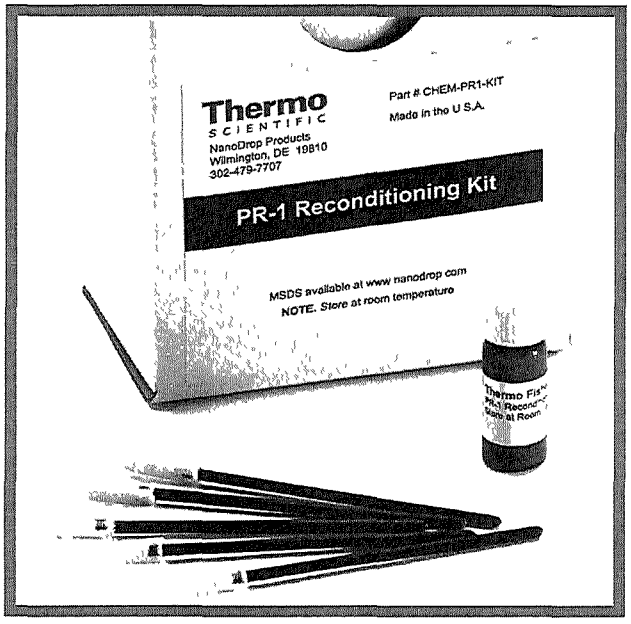
A number of accessories are available for the NanoDrop One instruments To order an accessory, contact your local distributor or visit our website

DYMO™ LabelWriter™ 450 USB Label Printer

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

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

PR-1 Pedestal Reconditioning Kit

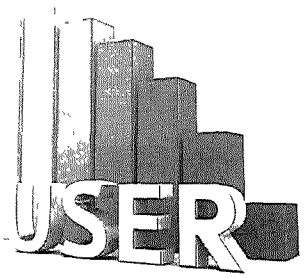


Specially formulated conditioning compound that can be applied to the pedestals to restore them to a hydrophobic state (required to achieve adequate surface tension for accurate sample measurements) The kit includes conditioning compound and applicators For more information, see Reconditioning the Pedestals

PV-1 Performance Verification Solution

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

Register Your Instrument



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

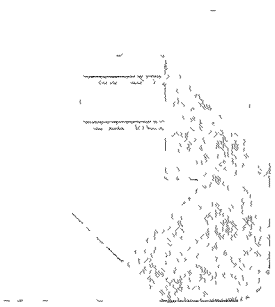
❖ To register your instrument

1 Do one of the following

- From the **NanoDrop One Viewer software** running on a personal computer (PC) that is connected to the Internet, open the Help menu and choose **NanoDrop One Website.**
- From any PC that is connected to the Internet, use any web browser to navigate to our website.

2 On the website, locate NanoDrop One Registration and follow the instructions to register the instrument.

Update Software



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

To install or update NanoDrop One Viewer software

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

To update or upgrade NanoDrop One instrument software

1 Do one of the following

- From the **NanoDrop One Viewer software**, open the Help menu and choose **NanoDrop One Website** to open our website
- From any personal computer that is connected to the Internet, navigate to the NanoDrop website

2 Insert a USB device such as a memory stick into a USB port on the computer

3 On the NanoDrop website, locate the software downloads page, select to update or upgrade NanoDrop One operating software (English version) and follow the instructions to download the installer to the USB device

4 To add a language, including software and Help systems, download the language pack installer(s) to the USB device

5 Insert the USB device into any USB port on the NanoDrop One instrument

6 From the instrument Home screen, tap  (Settings) > **System** > **Update Software**

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

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

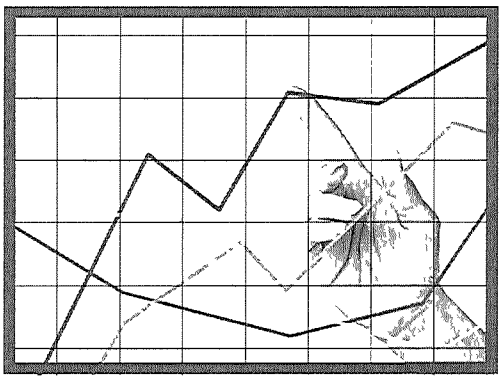
Version 1 2 0 (currently installed version of instrument operating software)
Database version 1 (version of NanoDrop One database on this instrument)

7 To add a language, including software and Help systems, tap **Update Software** again, select the language and version to install and tap **Update** (No instrument restart is required after a language installer completes)

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

Applications

Detection Limits for All Applications



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

Detection limits for standard applications

Sample Type	Lower Detection Limit	Upper Detection Limit	Typical Reproducibility[a]
dsDNA	2 0 ng/µL (pedestal) 0 20 ng/µL (cuvette)	27,500 ng/µL (pedestal) 75 ng/µL (cuvette)	±2 0 ng/µL for sample concentrations between 2 0 and 100 ng/µL samples, ±2% for samples >100 ng/µL
ssDNA	1 3 ng/µL (pedestal) 0 13 ng/µL (cuvette)	18,150 ng/µL (pedestal) 49.5 ng/µL (cuvette)	±2.0 ng/µL for sample concentrations between 2 0 and 100 ng/µL samples, ±2% for samples >100 ng/µL
RNA	1 6 ng/µL (pedestal) 0 16 ng/µL (cuvette)	22,000 ng/µL (pedestal) 60 ng/µL (cuvette)	±2.0 ng/µL for sample concentrations between 2 0 and 100 ng/µL samples, ±2% for samples >100 ng/µL

Sample Type	Lower Detection Limit	Upper Detection Limit	Typical Reproducibility[a]
DNA Microarray (ssDNA)	1 3 ng/µL (pedestal) 0 13 ng/µL (cuvette)	495 ng/µL (pedestal) 49 5 ng/µL (cuvette)	±2 0 ng/µL for sample concentrations between 2 0 and 100 ng/µL samples, ±2% for samples >100 ng/µL
Purified BSA by Protein A280	0 06 mg/mL (pedestal) 0 006 mg/mL (cuvette)	825 mg/mL (pedestal)	±0 10 mg/mL (for 0 10–10 mg/mL samples), ±2% for samples >10 mg/mL
IgG by Protein A280	0 03 mg/mL (pedestal) 0 003 mg/mL (cuvette)	402 mg/mL (pedestal)	
Purified BSA by Proteins & Labels	0 06 mg/mL (pedestal) 0 006 mg/mL (cuvette)	19 mg/mL (pedestal)	±0 10 mg/mL for 0 10–10 mg/mL samples
Protein BCA	0 2 mg/mL (20 1 reagent/sample volume) 0 01 mg/mL (1 1 reagent/sample volume)	8 0 mg/mL (pedestal) 0 20 mg/mL (cuvette)	2% over entire range 0 01 mg/mL over entire range
Protein Lowry	0 2 mg/mL (pedestal)	4 0 mg/mL (pedestal)	2% over entire range
Protein Bradford	100 µg/mL (50 1 reagent/sample volume) 15 µg/mL (1 1 reagent/sample volume)	8000 µg/mL 100 µg/µL	±25 µg/mL for 100–500 µg/mL samples ±5% for 500–8000 µg/mL samples ±4 µg/mL for 15–50 µg/mL samples ±5% for 50–125 µg/mL samples
Protein Pierce 660	50 µg/mL (15:1 reagent/sample volume) 25 µg/mL (7 5 1 reagent/sample volume)	2000 µg/mL 1000 µg/mL	±3 µg/mL for 50–125 µg/mL samples ±2% for samples > 125 µg/mL ±3 µg/mL for 25–125 µg/mL samples ±2% for samples >125 µg/mL

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

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

- For micro-volume measurements, maximum absorbance at 260 nm (for nucleic acids) or 280 nm (for proteins) should be less than 62 5 A
- For measurements with 10 mm pathlength cuvettes, maximum absorbance at 260 nm (or 280 nm for proteins) should be less than 1 5 A, which is approximately 75 ng/µL dsDNA.

Detection limits for pre-defined dyes

Sample Type	Lower Detection Limit	Upper Detection Limit[a]	Typical Reproducibility[b]
Cy3, Cy3 5, Alexa Fluor 555, Alexa Fluor 660	0.2 pmol/µL (pedestal)	100 pmol/µL (pedestal)	±0 20 pmol/µL for sample concentrations between 0 20 and 4 0 pmol/µL, ±2% for samples >4 0 pmol/µL
Cy5, Cy5 5, Alexa Fluor 647	0 12 pmol/µL (pedestal)	60 pmol/µL (pedestal)	±0 12 pmol/µL for sample concentrations between 0 12 and 2.4 pmol/µL, ±2% for samples >2.4 pmol/µL
Alexa Fluor 488, Alexa Fluor 594	0.4 pmol/µL (pedestal)	215 pmol/µL (pedestal)	±0 40 pmol/µL for sample concentrations between 0 40 and 8.0 pmol/µL, ±2% for samples >8.0 pmol/µL
Alexa Fluor 546	0 3 pmol/µL (pedestal)	145 pmol/µL (pedestal)	±0 30 pmol/µL for sample concentrations between 0 30 and 6 0 pmol/µL, ±2% for samples >6 0 pmol/µL

[a] Values are approximate

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

Measure dsDNA, ssDNA or RNA

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

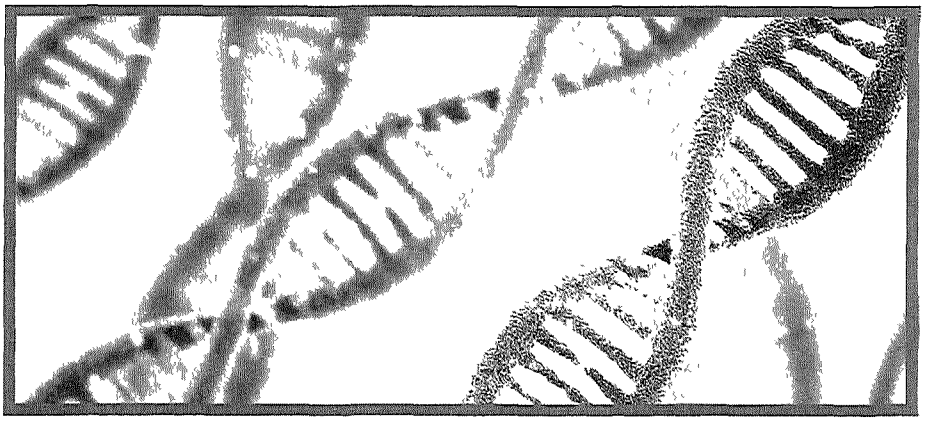
Measure dsDNA, ssDNA or RNA

Reported Results

Settings

Detection Limits

Calculations



Measure dsDNA, ssDNA or RNA

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

To measure dsDNA, ssDNA or RNA samples

NOTICE

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

Before you begin...

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

❖ To measure nucleic acid

1 From the Home screen, select the **Nucleic Acids** tab and tap **dsDNA, ssDNA or RNA**, depending on the samples to be measured

2 Specify a baseline correction if desired

3 Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder

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

4 Tap **Blank** and wait for the measurement to complete

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

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

6 Pipette 1-2 µL sample solution onto the pedestal and lower the arm, or insert the sample cuvette into the cuvette holder

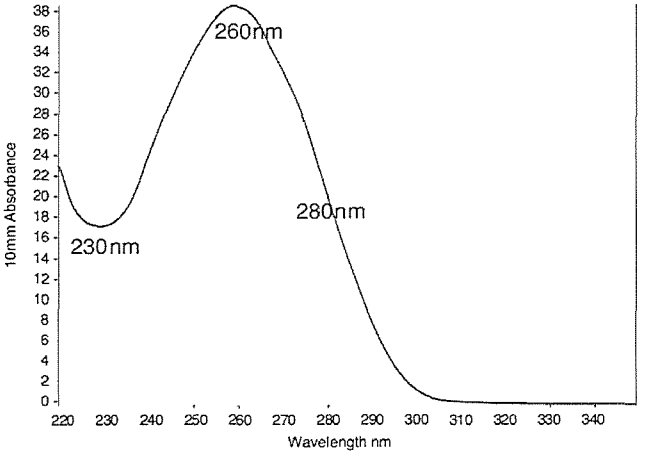
7 Start the sample measurement

- Pedestal If Auto-Measure is On, lower arm, if Auto-Measure is off, lower arm and tap **Measure**
- Cuvette Tap **Measure.**

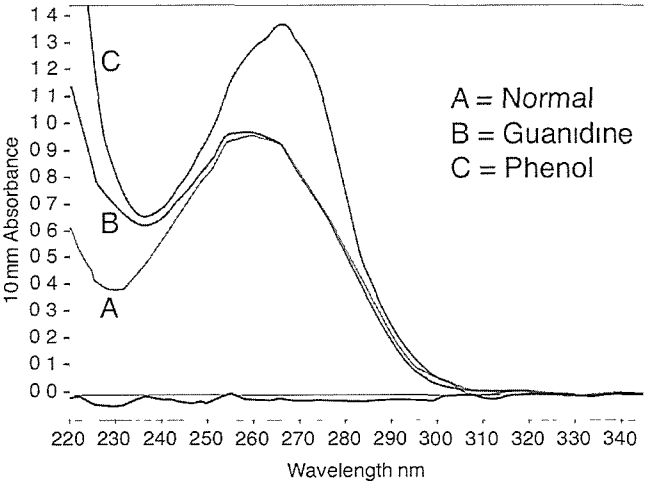
When the sample measurement is completed, the spectrum and reported values are displayed (see the next section).

8 When you are finished measuring samples, tap **End Experiment**

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



Typical nucleic acid spectrum



Comparison of nucleic acid spectra with and without two common contaminants

Best practices for nucleic acid measurements

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

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

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

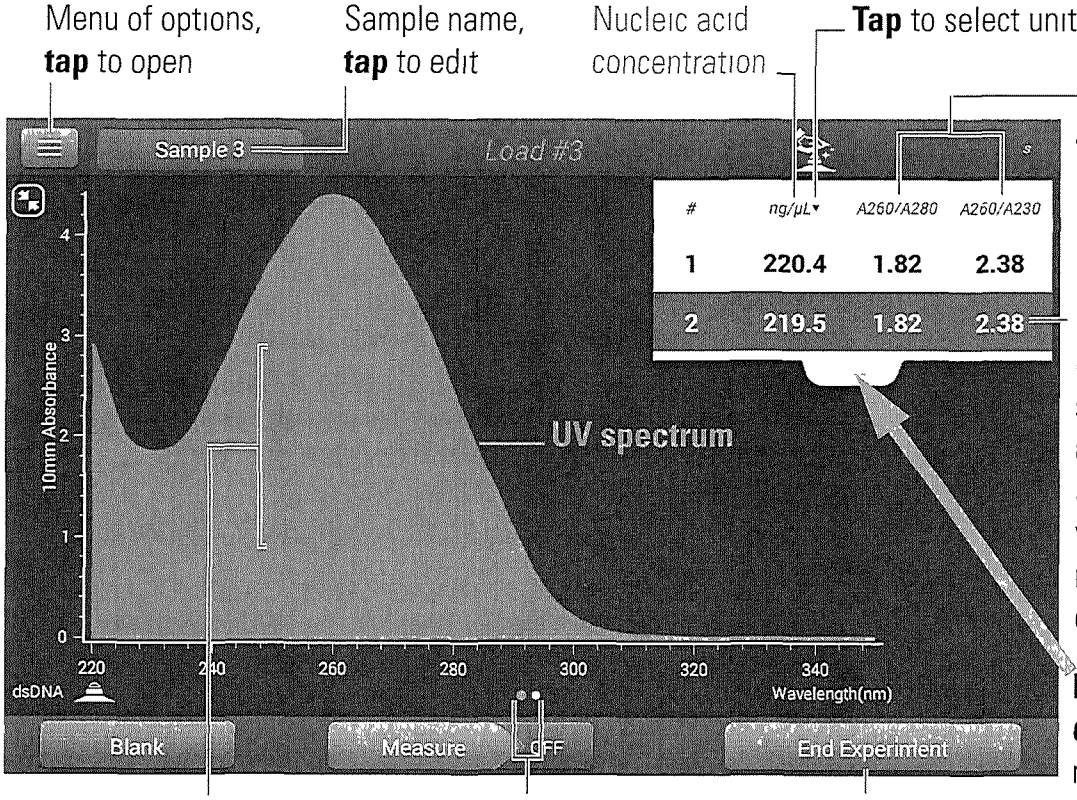
Related Topics

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

Nucleic Acid Reported Results

dsDNA measurement screen

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



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

dsDNA, ssDNA and RNA reported values

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

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

Related Topics

- Basic Instrument Operations
- Nucleic Acid Calculations

Setting for Nucleic Acid Measurements

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

Setting	Available Options	Description
Baseline Correction	On or off Enter baseline correction wavelength in nm or use default value (340 nm)	**Optional user-defined baseline correction** Can be used to correct for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength

Related Topics

- Instrument Settings

Calculations for Nucleic Acid Measurements

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

Beer-Lambert Equation (solved for concentration)

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

where

A = UV absorbance in absorbance units (AU)

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

b = pathlength in cm

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

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

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

Extinction Coefficients vs Factors

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

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

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

Factors Used

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

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

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

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

Measured Values

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

A260 absorbance

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

A230 and A280 absorbance

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

Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1 0 mm and 0.03 mm) based on sample absorbance at the analysis wavelength
- For cuvette measurements, pathlength is determined by the cuvette Pathlength setting in the software (see General Settings)
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent

Reported Values

- **Nucleic acid concentration** Reported in selected unit (i e , ng/µL, µg/uL or µg/mL) Calculations are based on modified Beer's Law equation using corrected nucleic acid absorbance value
- **A260/A280 purity ratio** Ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm An A260/A280 purity ratio of ~1 8 is generally accepted as "pure" for DNA (~2 0 for RNA) Acidic solutions may under represent the reported value by 0.2-0.3, the opposite is true for basic solutions
- **A260/A230 purity ratio** Ratio of corrected absorbance at 260 nm to corrected absorbance at 230 nm An A260/A230 purity ratio between 1 8 and 2 2 is generally accepted as "pure" for DNA and RNA

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

Measure Microarray

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

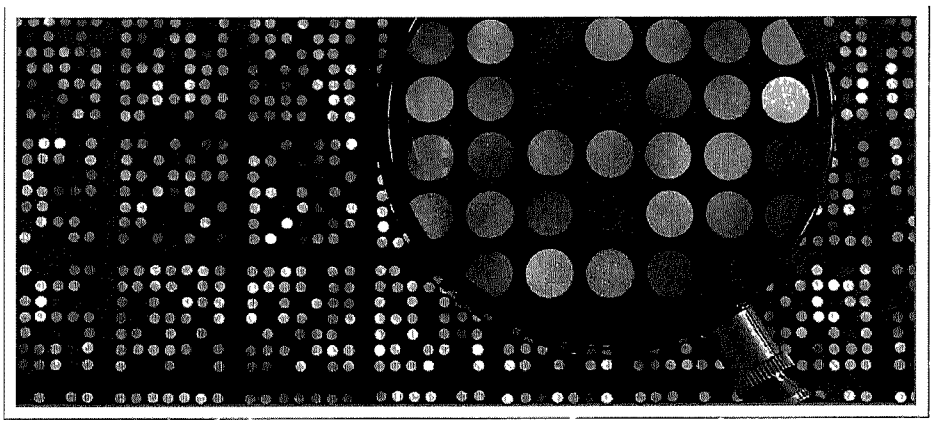
Measure Microarray Samples

Reported Results

Settings

Detection Limits

Calculations



Measure Microarray Samples

Use the Microarray application to quantify nucleic acids that have been labeled with up to two fluorescent dyes The application reports nucleic acid concentration, an A260/A280 ratio and the concentrations and measured absorbance values of the dye(s), allowing detection of dye concentrations as low as 0 2 picomole per microliter

To measure microarray samples

NOTICE

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

Before you begin...

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

❖ To measure a microarray sample

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

2 Specify the sample type and factor and the type of dye(s) used

Tip. Select a dye from the pre-defined list or add a custom dye using the Dye/Chromophore Editor.

3 Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder

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

4 Tap **Blank** and wait for the measurement to complete

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

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

6 Pipette 1-2 µL sample solution onto the pedestal and lower the arm, or insert the sample cuvette into the cuvette holder

7. Start the sample measurement

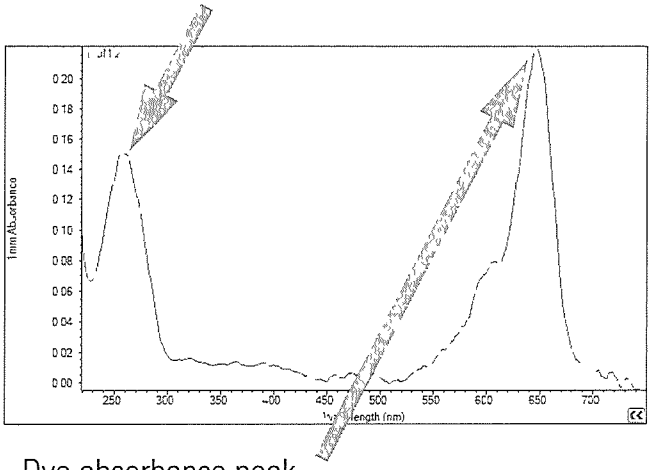
- Pedestal If Auto-Measure is On, lower arm, if Auto-Measure is off, lower arm and tap **Measure**.
- Cuvette Tap **Measure.**

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

8 When you are finished measuring samples, tap **End Experiment**

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

A260 absorbance peak used to calculate nucleic acid concentration



Dye absorbance peak used to calculate dye concentration

Typical microarray spectrum

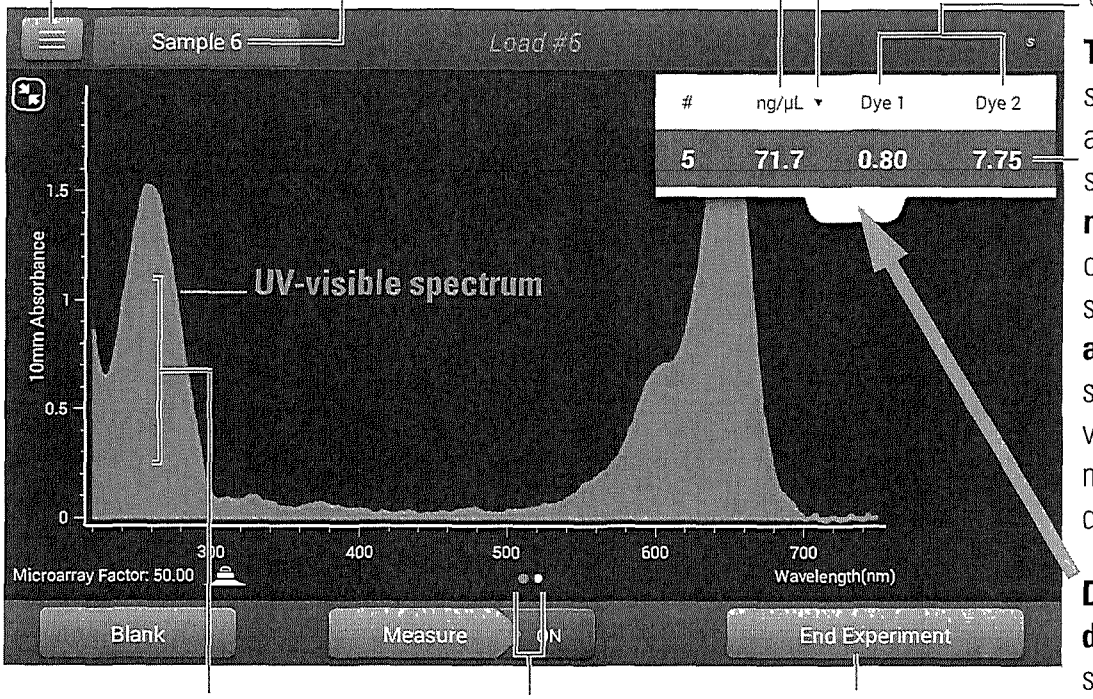
Related Topics

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

Microarray Reported Results

Microarray measurement screen

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



Menu of options, **tap** to open

Sample name, **tap** to edit

Nucleic acid concentration

Tap to select unit

Dye concentration(s)

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

- A baseline correction is performed at 750 nm (absorbance value at 750 nm is subtracted from absorbance values at all wavelengths in sample spectrum)
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10 0 mm pathlength equivalent

Microarray reported values

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

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

Related Topics

- Basic Instrument Operations
- Microarray Calculations

Settings for Microarray Measurements

Microarray settings

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

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

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

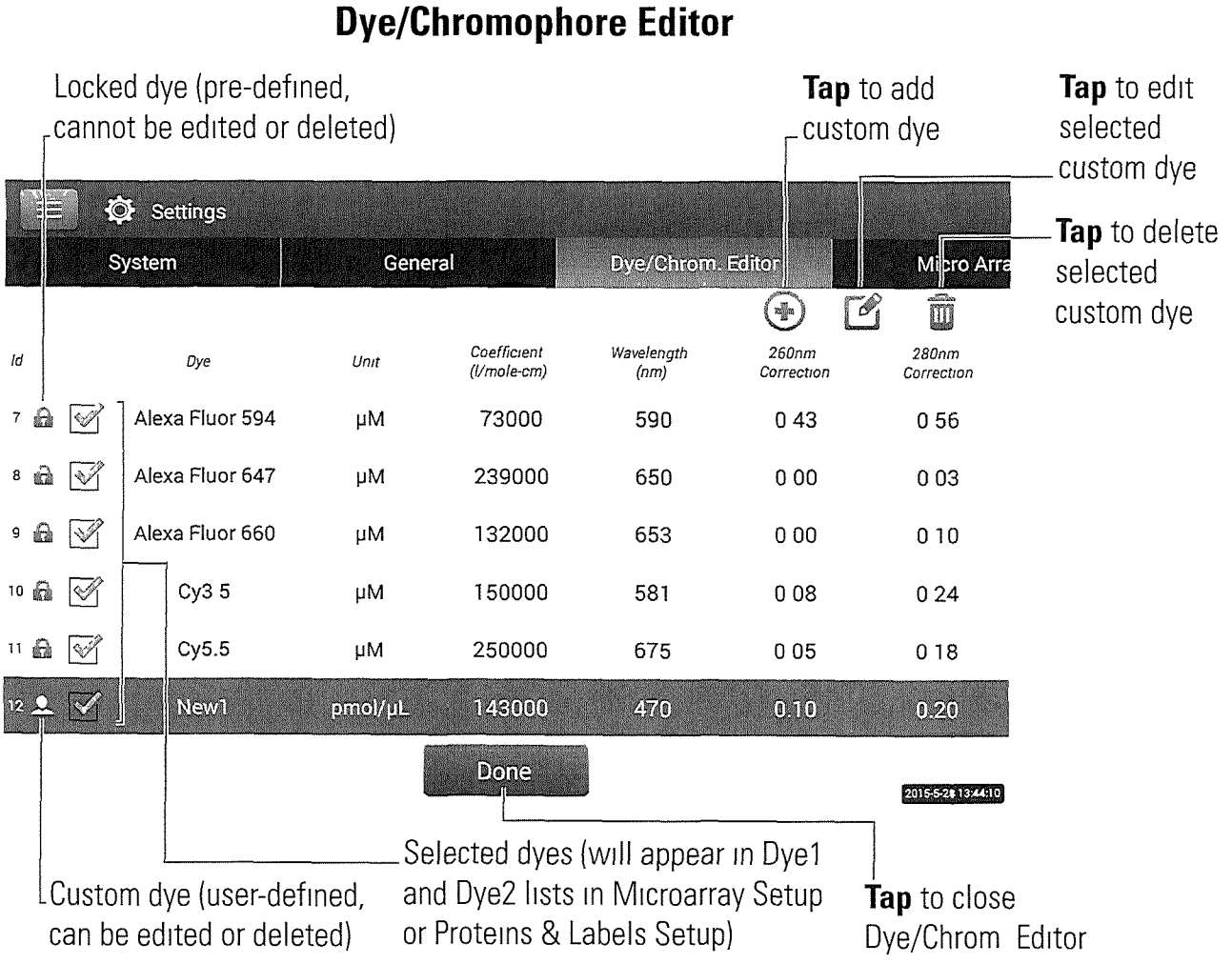
[b] The Analysis Correction affects the calculation for nucleic acid concentration only

Dye/chromophore editor

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

To access the Dye/Chromophore Editor

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

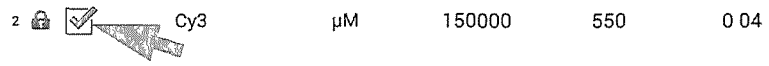


These operations are available from the Dye/Chromophore Editor

Add or remove a dye

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

- select or deselect corresponding checkbox



Add custom dye

- tap ⊕ to show New Dye box
- enter unique **Name** for new dye (tap field to display keyboard, tap **Done** key to close keyboard)
- *select default* **Unit** *that will be used to display dye concentration*
- enter dye's **Extinction Coefficient** (or molar absorptivity constant) in L/mole-cm (typically provided by dye manufacturer)
- specify **Wavelength** in nm (between 450 nm and 700 nm) that will be used to measure dye's absorbance
- specify dye's correction values at 260 nm and 280 nm
- tap **Add Dye**

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

- use instrument to measure pure dye and note absorbance at 260 nm, 280 nm and at analysis wavelength for dye (see above)
- calculate ratio of $A_{260}/A_{\text{dye wavelength}}$ and enter that value for 260 nm Correction
- calculate ratio of $A_{280}/A_{\text{dye wavelength}}$ and enter that value for 280 nm Correction

---

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

Edit custom dye

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

- tap to select custom dye
- tap ✎

- edit any entries or settings
- tap **Save Dye**

Delete custom dye

Tip Dyes pre-defined in the software cannot be deleted

- tap to select custom dye
- tap 

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

Related Topics

- Instrument Settings

Calculations for Microarray Measurements

As with the other nucleic acid applications, the Microarray application uses a modification of the Beer-Lambert equation to calculate sample concentration where the extinction coefficient and pathlength are combined and referred to as a "factor" The Microarray application offers six options (shown at right) for selecting an appropriate factor for each measured sample, to be used in conjunction with Beer's Law to calculate sample concentration

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

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

Available Options for Factors

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

Note See Sample Type for more information

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

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

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

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

Measured Values

A260 absorbance

Note: The absorbance value at 750 nm is subtracted from all wavelengths in the spectrum. As a result, the absorbance at 750 nm is zero in the displayed spectra. Also, for micro-volume absorbance measurements and measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent.

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

A280 absorbance

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

Dye absorbance

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

Dye correction

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

Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1.0 mm and 0 03 mm) based on sample absorbance at the analysis wavelength
- For cuvette measurements, pathlength is determined by the cuvette Pathlength setting in the software (see General Settings)
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent
-

Reported Values

- **Nucleic acid concentration** Reported in selected unit (i.e , ng/µL, µg/uL or µg/mL) Calculations are based on modified Beer's Law equation using corrected nucleic acid absorbance value
- **A260/A280 purity ratio** Ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm An A260/A280 purity ratio of ~1 8 is generally accepted as "pure" for DNA (~2 0 for RNA) Acidic solutions may under represent the reported value by 0 2-0 3, the opposite is true for basic solutions
- **Dye1/Dye2 concentration** Reported in pmol/µL Calculations are based on Beer's Law equation using (sloping) baseline-corrected dye absorbance value(s)

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

Related Topics

- Calculations for Nucleic Acid Measurements

Measure using a Custom Factor

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

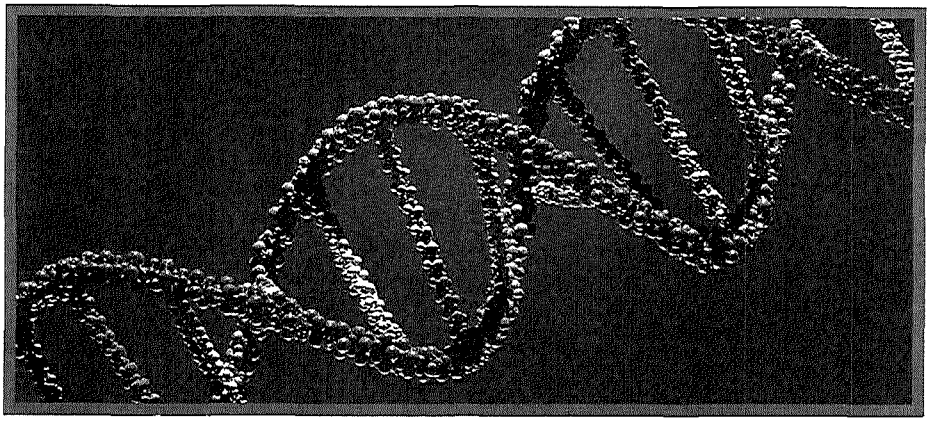
Measure using Custom Factor

Reported Results

Settings

Detection Limits

Calculations



Measure Nucleic Acid using a Custom Factor

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

To measure nucleic acid samples using a custom factor

NOTICE

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

Before you begin...

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

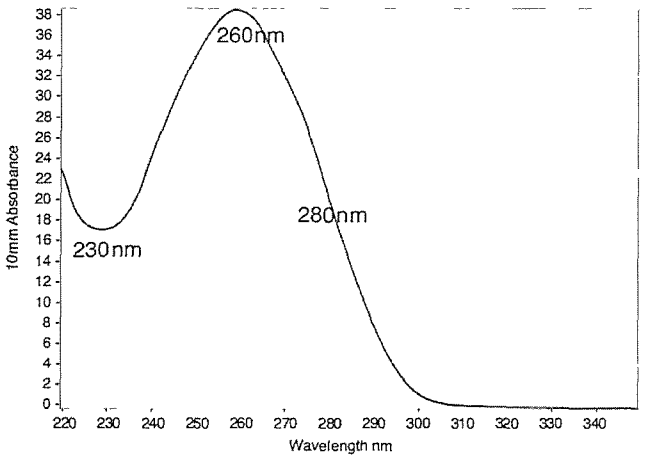
❖ To measure using a custom factor

1. From the Home screen, select the **Nucleic Acids** tab and tap **Custom Factor.**
2. Enter the factor to be used for the calculations and specify a baseline correction if desired.
3. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder.

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

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

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



Typical nucleic acid spectrum

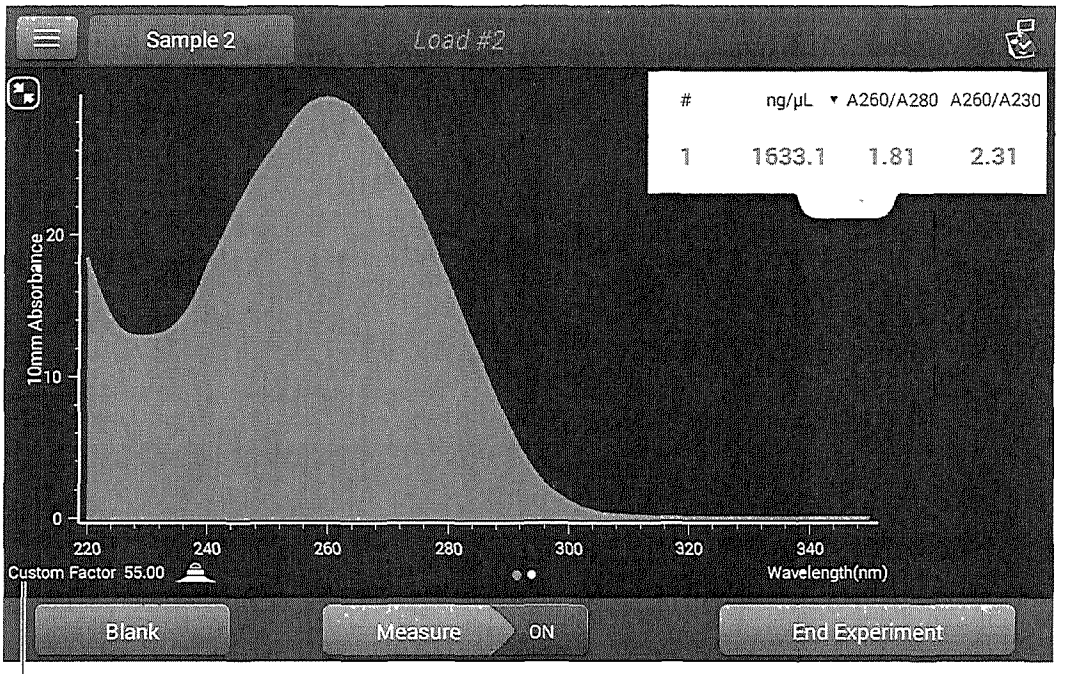
Related Topics

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

Custom Factor Reported Results

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

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



Custom factor used to calculate nucleic acid concentration

Related Topics

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

Settings for Nucleic Acid Measurements using a Custom Factor

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

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

Related Topics

- Instrument Settings

Detection Limits for Nucleic Acid Measurements using a Custom Factor

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

To calculate upper detection limits for nucleic acid samples

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

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

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

(550 AU * 55 ng-cm/µL) = 30,250 ng/µL

Note For measurements with 10 mm pathlength cuvettes, the upper absorbance limit is 1 5 AU, which is approximately 75 ng/µL for dsDNA

Related Topics

- Detection Limits for All Applications

Measure Oligo DNA or Oligo RNA

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

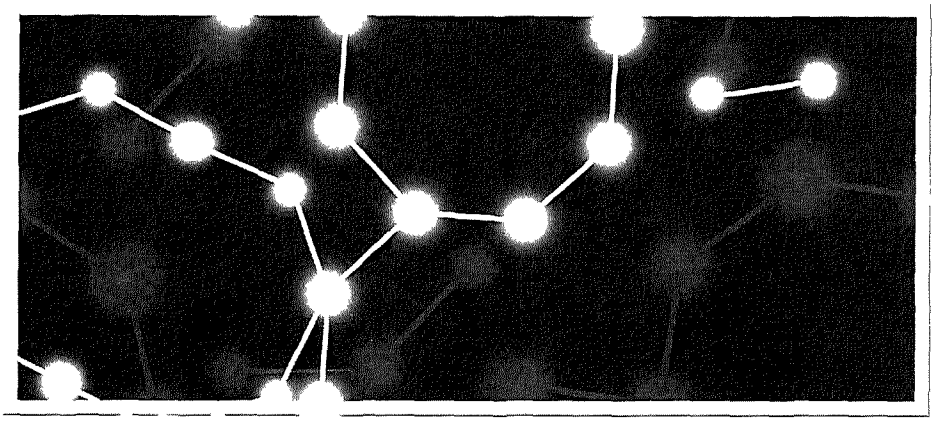
Measure Oligo DNA or RNA

Reported Results

Settings

Detection Limits

Calculations



Measure Oligo DNA or Oligo RNA

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

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

To measure Oligo DNA or Oligo RNA samples

NOTICE

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

Before you begin...

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

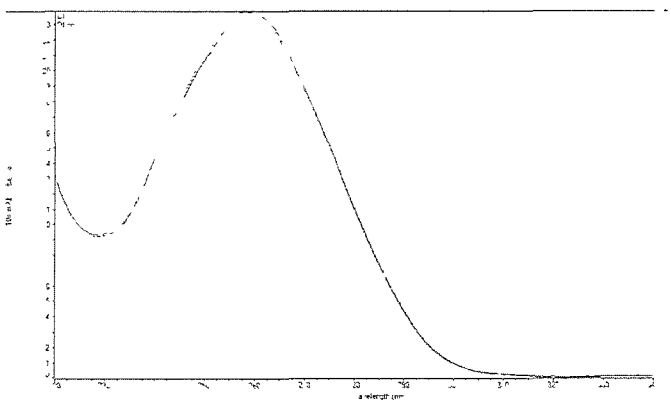
❖ To measure an oligonucleotide sample

1. From the Home screen, select the **Nucleic Acids** tab and tap either **Oligo DNA** or **Oligo RNA**, as needed
2. Specify the Oligo base sequence and a baseline correction if desired
3. Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder

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

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

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



Example Oligo DNA spectrum

Related Topics

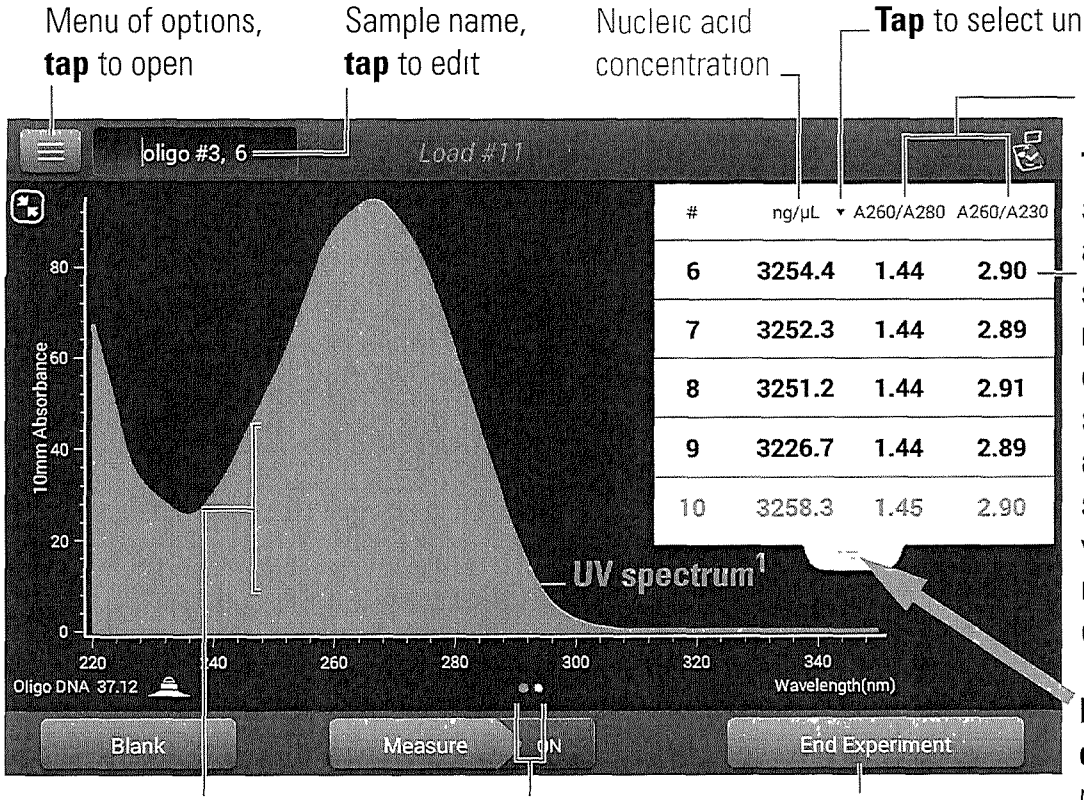
- Best Practices for Nucleic Acid Measurements

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

Oligo Reported Results

Oligo DNA measurement screen

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



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

Note Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10 0 mm pathlength equivalent.

Oligo DNA and Oligo RNA reported values

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

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

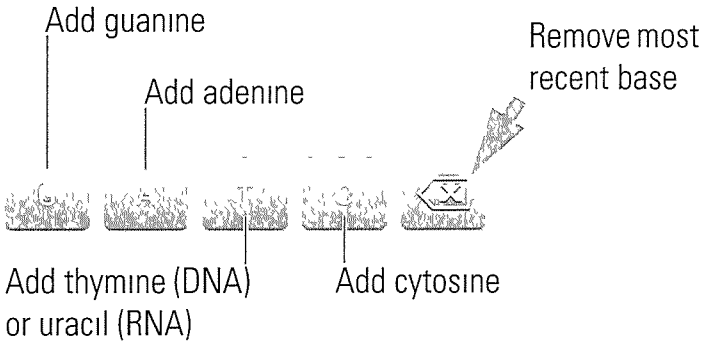
Note The five nucleotides that comprise DNA and RNA exhibit widely varying A260/A280 ratios. See Oligo Purity Ratios for more information

Related Topics

- Basic Instrument Operations
- Oligo Calculations

Settings for Oligo DNA and Oligo RNA Measurements

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

Setting	Available Options	Description
Oligo Base Sequence	for DNA Use the G, A, T and C keys to specify the DNA base sequence for RNA Use the G, A, U and C keys to specify the RNA base sequence	Specify your DNA or RNA base sequence by tapping the corresponding keys 
		Each time a base is added to the sequence, the software calculates the following • **Factor** Constant used to calculate oligonucleotide concentration in modified Beer's Law equation Based on extinction coefficient and pathlength $f = 1/(\varepsilon_{260} * b)$ 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

Related Topics

- Detection Limits for All Applications

Calculations for Oligo DNA and Oligo RNA Measurements

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

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

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

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

Extinction Coefficients for Oligonucleotides

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

$$\varepsilon_{260} = \sum_{1}^{N-1} \varepsilon_1 - \sum_{2}^{N-1} \varepsilon_2 + \sum_{1}^{N} \varepsilon_3$$

where
ε = molar extinction coefficient in L/mole-cm
$\varepsilon_1 = \varepsilon_{\text{nearest neighbor}}$
$\varepsilon_2 = \varepsilon_{\text{individual bases}}$
$\varepsilon_3 = \varepsilon_{\text{modifications, such as fluorescent dyes}}$

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

Measure Protein A280

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

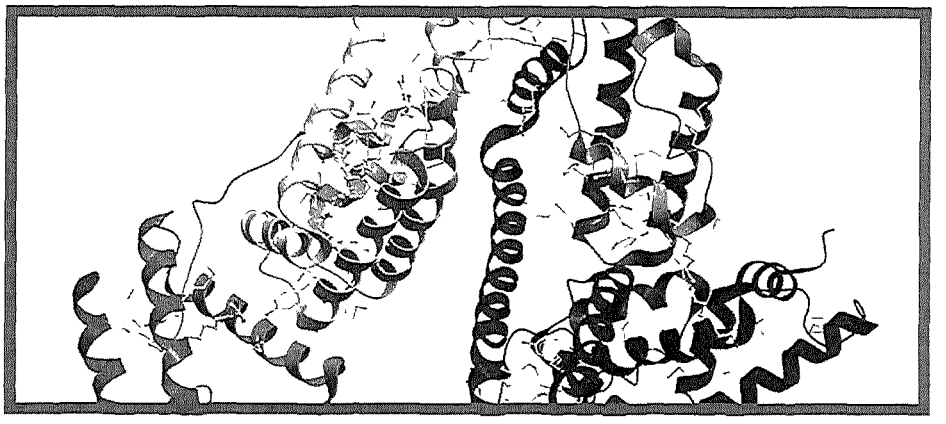
Measure A280 Proteins

Reported Results

Settings

Detection Limits

Calculations



Measure Protein Concentration at A280

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

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

To measure Protein A280 samples

NOTICE

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

Before you begin...

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

❖ To measure a Protein A280 sample

1 From the Home screen, select the **Proteins** tab and tap **Protein A280**

2 Specify a sample type and baseline correction if desired

3 Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder

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

4 Tap **Blank** and wait for the measurement to complete

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

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

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

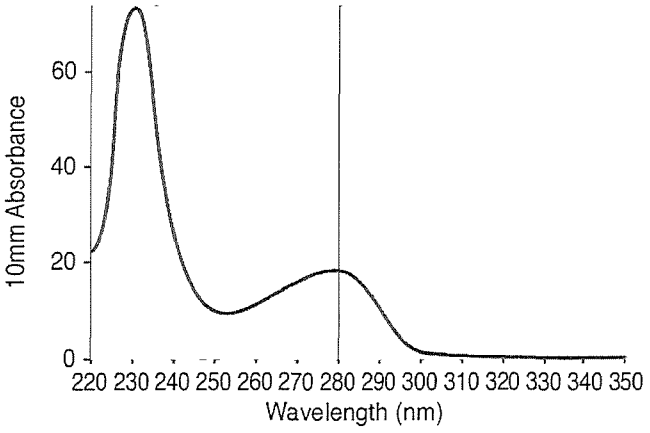
7 Start the sample measurement

- Pedestal: If Auto-Measure is On, lower arm, if Auto-Measure is off, lower arm and tap **Measure**
- Cuvette: Tap **Measure**

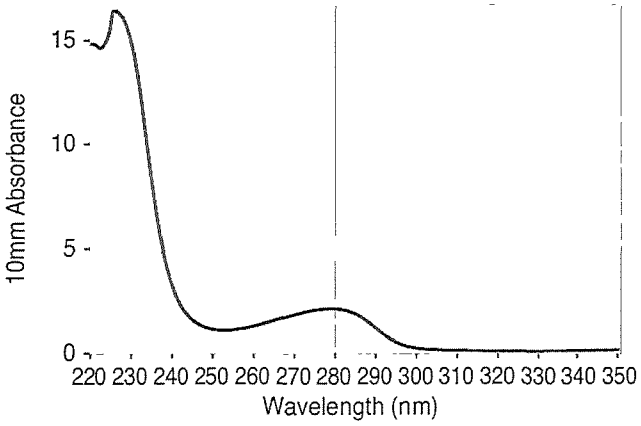
When the sample measurement is completed, the spectrum and reported values are displayed (see the next section)

8 When you are finished measuring samples, tap **End Experiment**

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



High concentration BSA sample



Low concentration BSA sample

Best practices for protein measurements

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

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

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

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

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

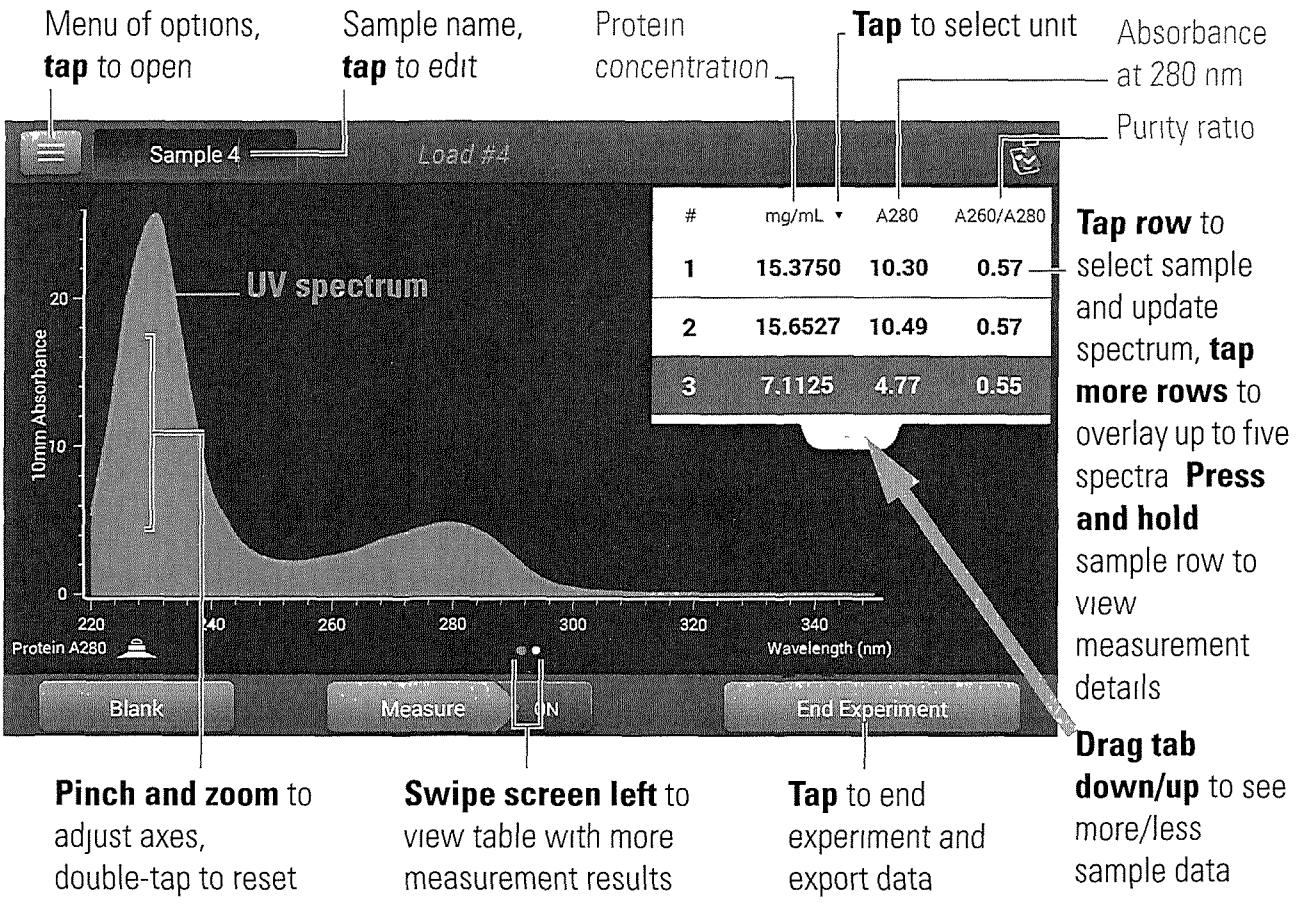
Related Topics

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

Protein A280 Reported Results

Protein A280 measurement screen

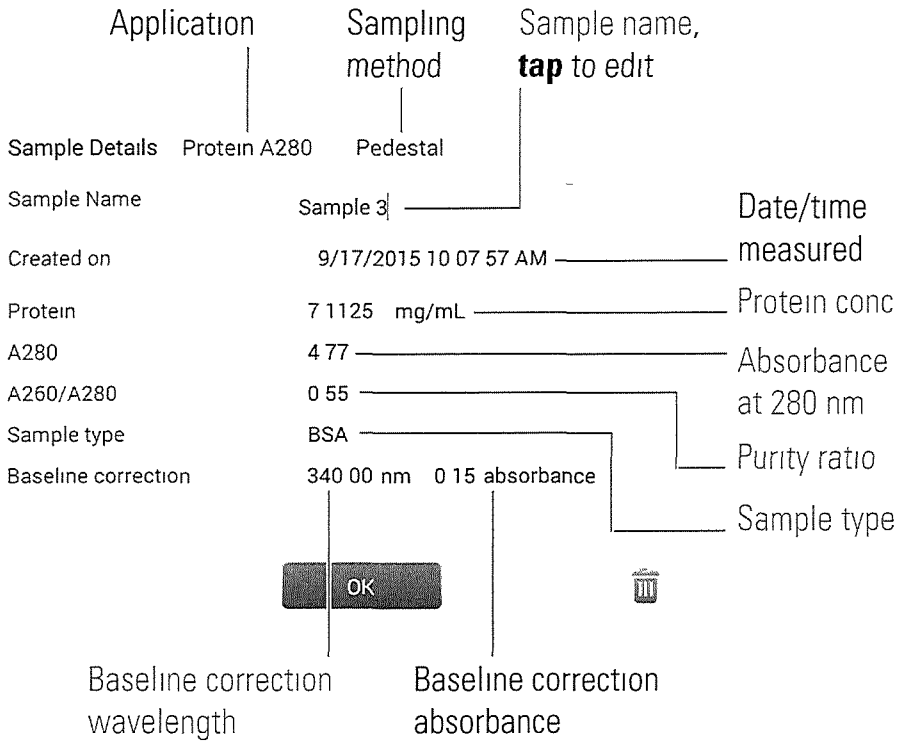
For each measured sample, this application shows the absorbance spectrum and a summary of the results Here is an example



Note Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10 0 mm pathlength equivalent

Protein A280 reported values

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



Related Topics

- Basic Instrument Operations
- Protein A280 Calculations

Settings for Protein A280 Measurements

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

Protein A280 settings

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

Each sample type applies a unique extinction coefficient to the protein calculations. If the extinction coefficient of the sample is known, choose the **ε** + MW (molar) or **ε**1% (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., **ε**1% = 10.
	BSA	6.67	Calculates BSA (Bovine Serum Albumin) protein concentration using mass extinction coefficient (**ε**) of 6.67 L/gm-cm at 280 nm for 1% (i.e., 10 mg/mL) BSA solution. Assuming MW is 66,400 daltons (Da), molar extinction coefficient at 280 nm for BSA is approximately 43,824 $M^{-1}cm^{-1}$
	IgG	13.7	Suitable for most mammalian antibodies (i.e., immunoglobulin G or IgG). Calculates protein concentration using mass extinction coefficient (**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 $M^{-1}cm^{-1}$

Setting	Available Options	Mass Ext. Coefficient (L/gm-cm)	Description
	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.
Baseline Correction	On or off Enter baseline correction wavelength in nm or use default value (340 nm)	N/A	Corrects for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum As a result, absorbance of sample spectrum is zero at specified baseline correction wavelength **Tip** If the sample has a modification that absorbs light at 340 nm, select a different correction wavelength or turn off Baseline Correction

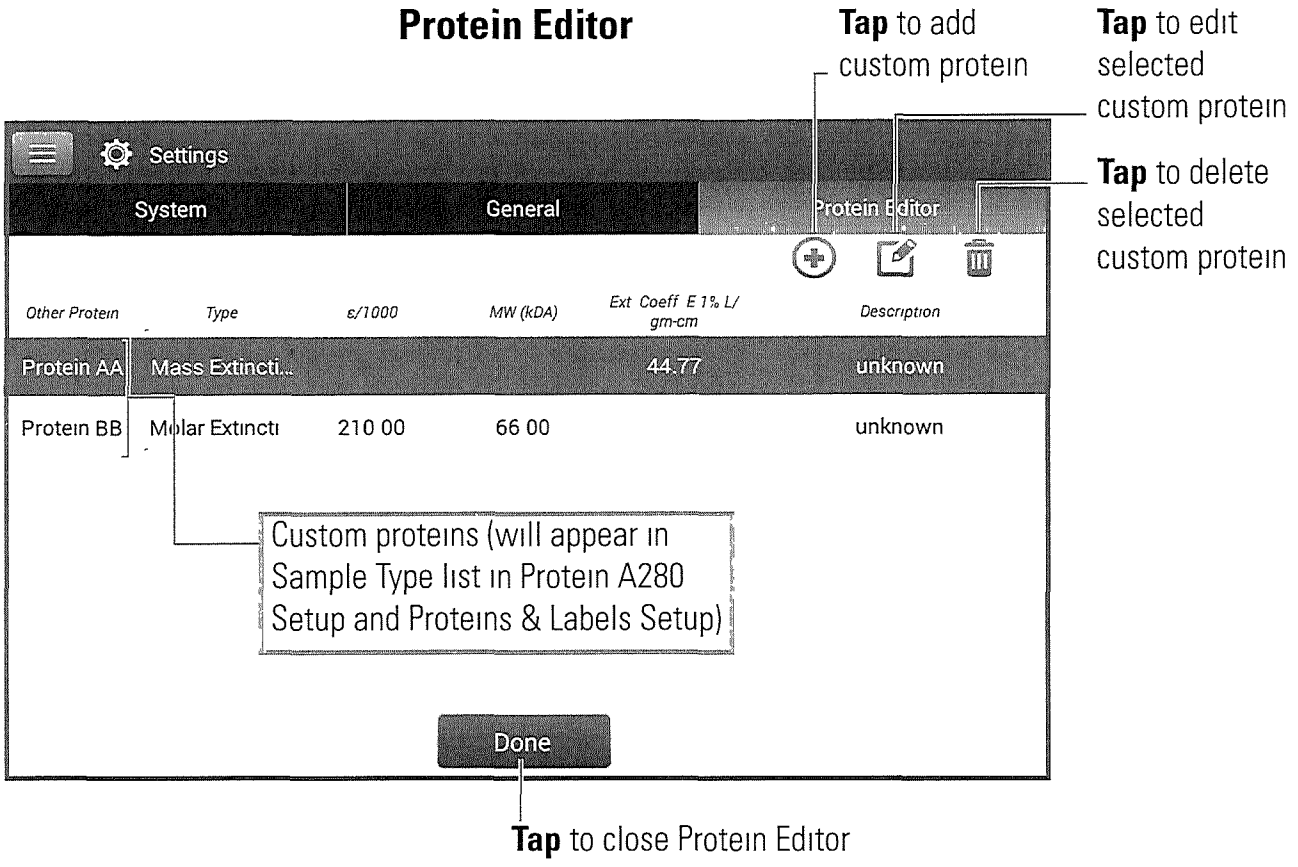
[a] To add or edit a custom protein, use Protein Editor

Protein editor

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

To access the Protein Editor

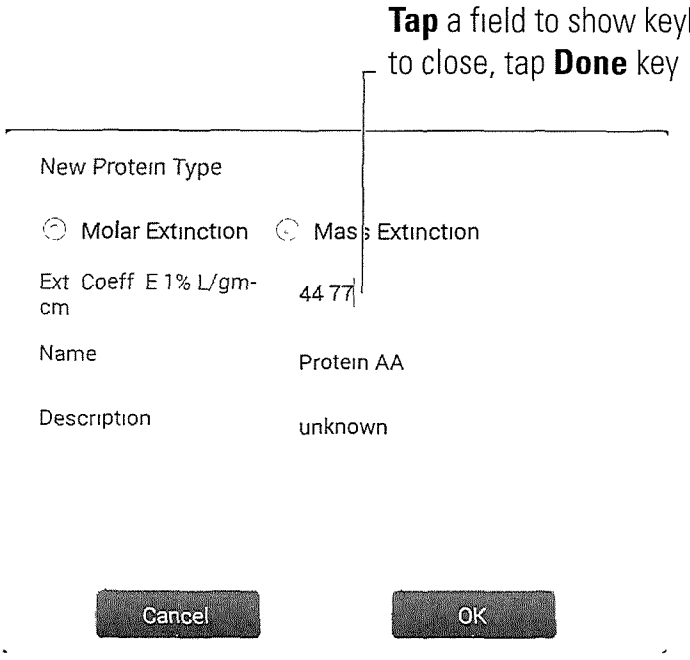
- from the Home screen, tap  > **Protein Editor**
- from the Protein A280 or Proteins & Labels measurement screen, tap  >  **Settings > Protein Editor**



These operations are available from the Protein Editor

Add custom protein

- in Protein Editor, tap ⊕ to show New Protein Type box
- enter unique **Name** for new protein (tap field to display keyboard, tap **Done** key to close keyboard)
- enter **Description** for new protein
- specify whether to enter **Molar Extinction** coefficient or **Mass Extinction** coefficient for custom protein
  - if **Mass Extinction** coefficient is selected
    - enter mass extinction coefficient in L/gm-cm for 10 mg/mL (**ε**1%) protein solution



- if **Molar Extinction** is selected
  - enter 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
  - enter molecular weight (MW) in kiloDaltons (kDa)
- tap **OK** to close New Protein Type box

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

Edit custom protein

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

Delete custom protein

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

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

Related Topics

- Instrument Settings

Detection Limits for Protein A280 Measurements

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

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

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

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

For example, if the sample's mass extinction coefficient at 280 nm is 6 67 for a 1% (10 mg/mL) solution, the equation looks like this

(550 / 6.67) * 10 = 824 6 (or ~825)

Related Topics

- Detection Limits for All Applications

Calculations for Protein A280 Measurements

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

Beer-Lambert Equation (solved for concentration)

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

where

A = UV absorbance in absorbance units (AU)

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

b = pathlength in cm

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

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

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

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

Extinction Coefficients for Proteins

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

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

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

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

If the extinction coefficient of the sample is known, choose the **ε** + MW (molar) or **ε**1% (mass) option and enter the value Otherwise, calculate the extinction coefficient or choose the option that best matches the sample solution

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

Available Options for Extinction Coefficient

- **1 Abs = 1 mg/mL**, where sample type and/or ext. coefficient is unknown (produces rough estimate of protein concentration)
- **BSA** (Bovine Serum Albumin, 6 67 L/gm-cm)
- **IgG** (any mammalian antibody, 13 7 L/gm-cm)
- **Lysozyme** (egg white lysozyme, 26 4 L/gm-cm)
- **Other protein** (**ε** + MW), user-specified molar ext coefficient
- **Other protein** (**ε**1%), 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 (**ε**1%) 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 (**ε**1%)

Conversions Between ε_{molar} and ε1%

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

Example To determine percent solution extinction coefficient (**ε**1%) 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

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

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·

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

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

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

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

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

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

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

Measured Values

A280 absorbance

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

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

A260 absorbance

- Normalized and baseline-corrected (if selected) absorbance value at *260 nm is also reported*

Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1 0 mm and 0 03 mm) based on sample absorbance at the analysis wavelength.
- For cuvette measurements, pathlength is determined by the cuvette Pathlength setting in the software (see General Settings)
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent
-

Reported Values

- **Protein concentration.** Reported in selected unit (mg/mL or µg/mL) Calculations are based on Beer-Lambert equation using corrected protein absorbance value
- **A260/A280 purity ratio** Ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm An A260/A280 purity ratio of ~0 57 is generally accepted as "pure" for proteins

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

Measure Proteins and Labels

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

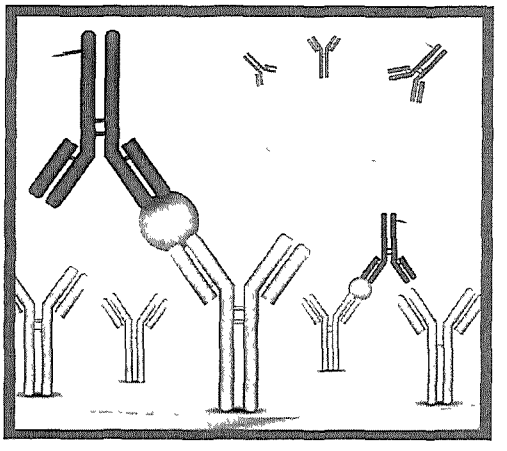
Measure Labeled Proteins

Reported Results

Settings

Detection Limits

Calculations



Measure Labeled Protein Samples

Use the Proteins and Labels application to quantify proteins and fluorescent dyes for protein array conjugates, as well as metalloproteins such as hemoglobin, using wavelength ratios This application reports protein concentration measured at 280 nm, an A269/A280 absorbance ratio, and the concentrations and measured absorbance values of the dyes, allowing detection of dye concentrations as low as 0 2 picomole per microliter This information is useful for evaluating protein/dye conjugation (degree of labeling) for use in downstream applications

To measure labeled protein samples

NOTICE

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

Before you begin...

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

❖ To measure a labeled protein sample

1 From the Home screen, select the **Proteins** tab and then tap **Protein & Labels**

2. Specify the sample type and the type of dye(s) used.

   Tip Select a dye from the pre-defined list or add a custom dye using the Dye/Chromophore Editor

3 Pipette 1–2 µL of the blanking solution onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder

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

4 Tap **Blank** and wait for the measurement to complete

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

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

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

7 Start the sample measurement

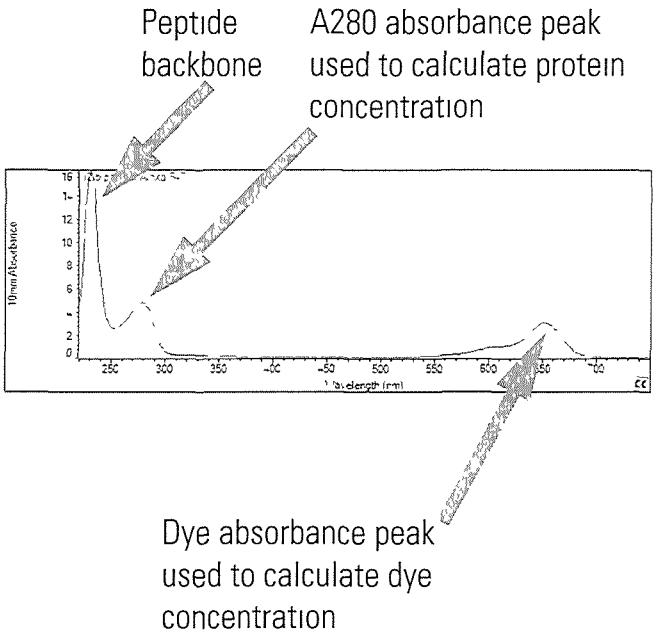
   – Pedestal If Auto-Measure is On, lower arm, if Auto-Measure is off, lower arm and tap **Measure**

   – Cuvette Tap **Measure**

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

8 When you are finished measuring samples, tap **End Experiment**

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



Typical sample spectrum measured with Proteins & Labels application

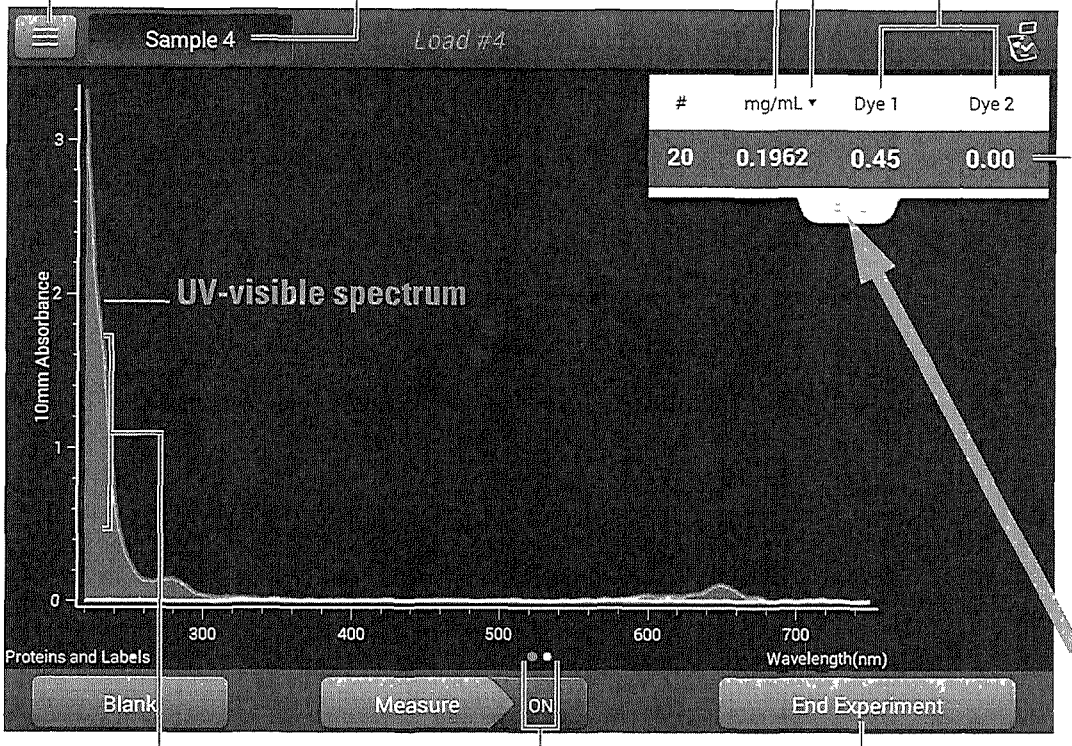
Related Topics

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

Proteins & Labels Reported Results

Proteins & Labels measurement screen

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



Note

- A baseline correction is performed at 750 nm (absorbance value at 750 nm is subtracted from absorbance values at all wavelengths in sample spectrum)
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10 0 mm pathlength equivalent

Proteins & Labels reported values

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

Reported values for Proteins & Labels application

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

Related Topics

- Basic Instrument Operations
- Proteins & Labels calculations

Settings for Proteins and Labels Measurements

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

Setting	Available Options	Mass Ext Coefficient (L/gm-cm)	Description
Sample type[a]	1 Abs = 1 mg/mL	General reference	Tap here for detailed description of each available setting
	BSA	6 67	Each sample type applies a unique extinction coefficient to the protein calculations If the extinction coefficient of the sample is known, choose the **ε** + MW (molar) or **ε**1% (mass) option and enter the value Otherwise, calculate the extinction coefficient or choose the option that best matches the sample solution If you only need a rough estimate of protein concentration and the sample extinction coefficient is unknown, select the 1 Abs=1 mg/mL sample type option
	IgG	13 7	
	Lysozyme	26 4	
	Other protein (**ε** + MW)	user-entered molar extinction coefficient/molecular weight	
	Other protein (**ε**1%)	User entered mass extinction coefficient	**Tip** Ideally, the extinction coefficient should be determined empirically using a solution of the study protein at a known concentration using the same buffer
Analysis Correction[b]	On or off Enter analysis correction wavelength in nm or use default value (340 nm)	N/A	Corrects sample absorbance measurement for any offset caused by light scattering particulates by subtracting absorbance value at specified analysis correction wavelength from absorbance value at analysis wavelength Corrected value is used to calculate sample concentration **Tip** If the sample has a modification that absorbs light at 340 nm, select a different correction wavelength or turn off Analysis Correction
Dye 1/Dye 2 Type[c]	Cy3, 5, 3 5, or 5 5, Alexa Fluor 488, 546, 555, 594, 647, or 660	See Dye/Chromophore Editor for specific values for each dye	Select pre-defined dye used to label sample material, or one that has been added using Dye/Chrom Editor
Dye 1/Dye 2 Unit	picomoles/microliter (pmol/uL), micromoles (uM), or millimoles (mM)	not applicable	Select unit for reporting dye concentrations
Sloping Dye Correction[d]	On or off		Corrects dye absorbance measurements for any offset caused by light scattering particulates by subtracting absorbance value of a sloping baseline from 400 nm to 750 nm from absorbance value at dye's analysis wavelength

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

[b] Analysis Correction affects calculation for protein concentration only

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

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

Related Topics

- Instrument Settings
- Protein Editor
- Dye/Chromophore Editor

Detection Limits for Proteins and Labels Measurements

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

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

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

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

For example, if the sample's mass extinction coefficient at 280 nm is 6 67 for a 1% (10 mg/mL) solution, the equation looks like this

(550 / 6 67) * 10 = 824 6 (or ~825)

Related Topics

- Detection Limits for All Applications

Calculations for Proteins and Labels Measurements

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

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

If the extinction coefficient of the sample is known, choose the **ε** + MW (molar) or **ε**1% (mass) option and enter the value Otherwise, calculate the extinction coefficient or choose the option that best matches the sample solution

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

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

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

Available Options for Extinction Coefficient

- **1 Abs = 1 mg/mL**, where sample type and/or ext coefficient is unknown (produces rough estimate of protein concentration)
- **BSA** (Bovine Serum Albumin, 6 67 L/gm-cm)
- **IgG** (any mammalian antibody, 13 7 L/gm-cm)
- **Lysozyme** (egg white lysozyme, 26 4 L/gm-cm)
- **Other protein** (**ε** + MW), user-specified molar ext coefficient
- **Other protein** (**ε**1%), user-specified mass ext coefficient

Note See Sample Type for details

Measured Values

A280 absorbance

Note. The absorbance value at 750 nm is subtracted from all wavelengths in the spectrum. As a result, the absorbance at 750 nm is zero in the displayed spectra Also, for micro-volume absorbance measurements and measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent

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

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

Dye absorbance

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

Dye correction

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

Sample Pathlength

- For micro-volume measurements, the software selects the optimal pathlength (between 1 0 mm and 0 03 mm) based on sample absorbance at the analysis wavelength
- For cuvette measurements, pathlength is determined by the cuvette Pathlength setting in the software (see General Settings)
- Displayed spectra and absorbance values are normalized to a 10 mm pathlength equivalent

Reported Values

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

Related Topics

- Beer-Lambert Equation
- Protein A280 Calculations

Measure Protein A205

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

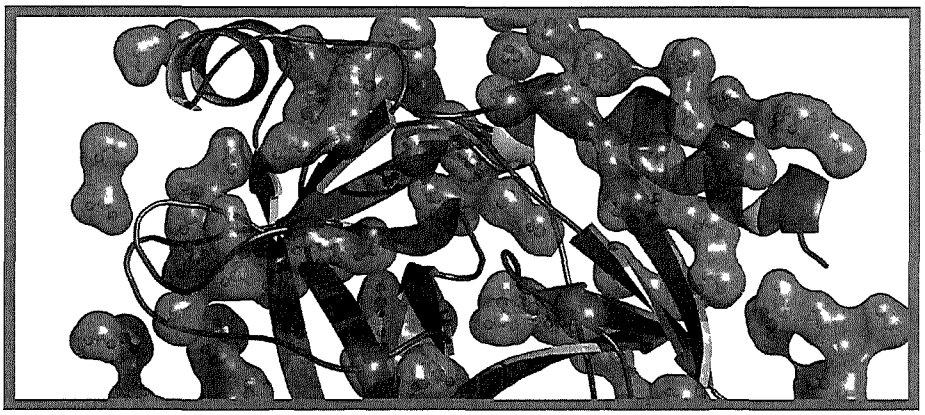
Measure A205 Proteins

Reported Results

Settings

Detection Limits

Calculations



Measure Protein Concentration at A205

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

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

To measure Protein A205 samples

NOTICE

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

Before you begin...

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

❖ To measure a Protein A205 sample

1 From the Home screen, select the **Proteins** tab and then tap **Protein A205**

2 Specify a sample type and baseline correction if desired

3. Pipette 1–2 µL of the blanking solution onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder

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

4 Tap **Blank** and wait for the measurement to complete

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

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

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

7 Start the sample measurement·

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

   – Cuvette Tap **Measure**

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

8 When you are finished measuring samples, tap **End Experiment**.

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

Related Topics

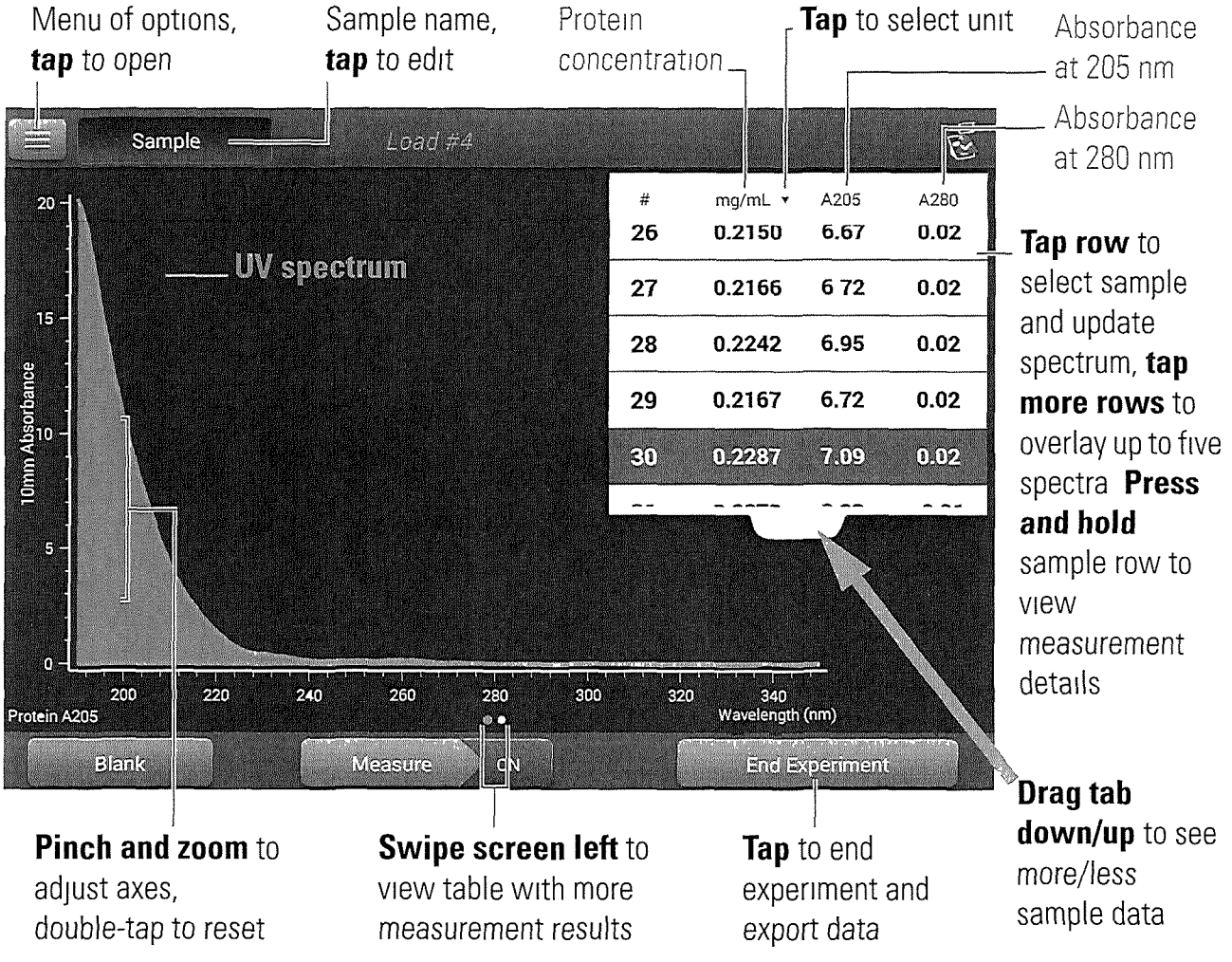
- Best Practices for Protein Measurements
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette

- Prepare Samples and Blanks
- Basic Instrument Operations

Protein A205 Reported Results

Protein A205 measurement screen

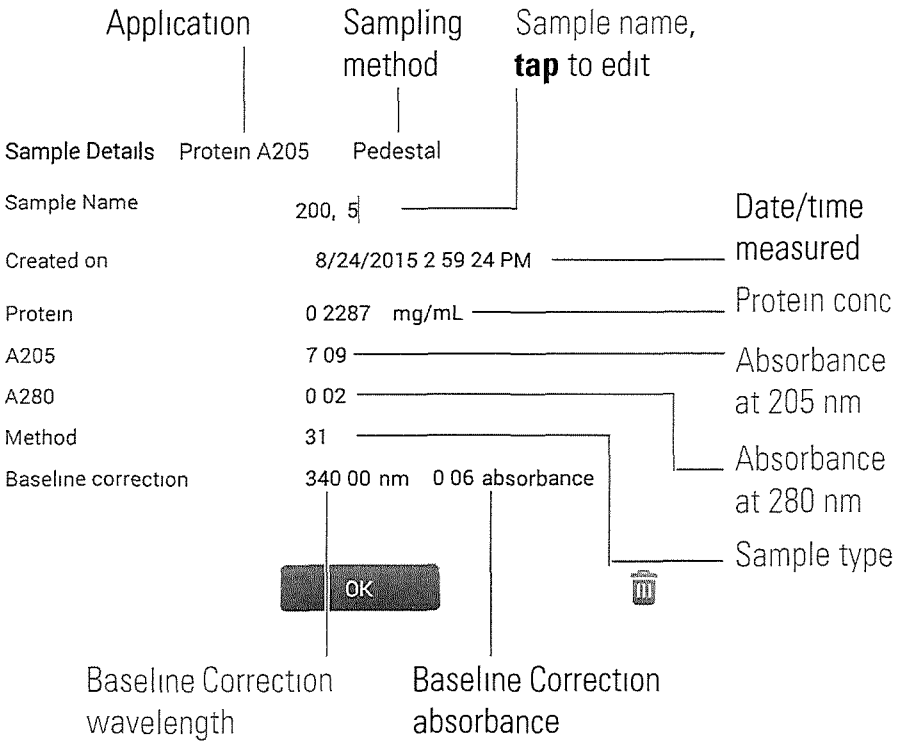
For each measured sample, this application shows the absorbance spectrum and a summary of the results Here is an example



Note Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10 0 mm pathlength equivalent

Protein A205 reported values

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



Related Topics

- Basic Instrument Operations
- Protein A205 Calculations

Settings for Protein A205 Measurements

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

Setting	Available Options	Mass Ext. Coefficient (L/gm-cm)	Description
Sample type	31	31	Assumes ε0 1% (1 mg/mL) at 205 nm = 31
	Scopes	27 + 120 * (A280/A205)	Assumes ε0 1% (1 mg/mL) at 205 nm = 27 + 120 * (A280/A205)
	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

Related Topics

- Instrument Settings

Calculations for Protein A205 Measurements

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

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

If the extinction coefficient of the sample is known, choose the **ε**1% (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 **ε**0 1% (1 mg/mL) at 205 nm = 31
- **Scopes**, assumes **ε**0.1% (1 mg/mL) at 205 nm = 27 + 120 * (A280/A205)
- **Other protein**, enter mass extinction coefficient in L/gm-cm for 1 mg/mL (**ε**0 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

Related Topics

- Beer-Lambert Equation
- Protein A280 Calculations

Measure Protein BCA

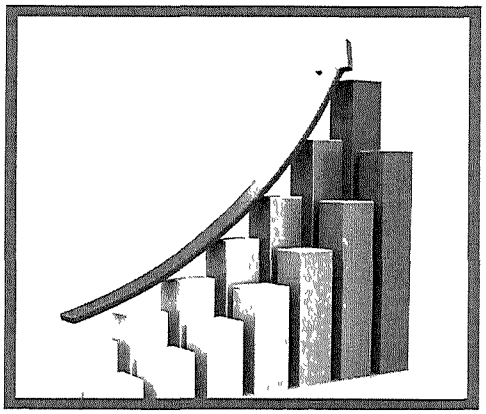
Measures total protein concentration of unpurified protein samples using a bicinchoninic acid colorimetric detection reagent

Measure Total Protein

Reported Results

Settings

Detection Limits



Measure Total Protein Concentration

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

Theory of Protein BCA assay

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

Protein assay kits and protocols

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

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

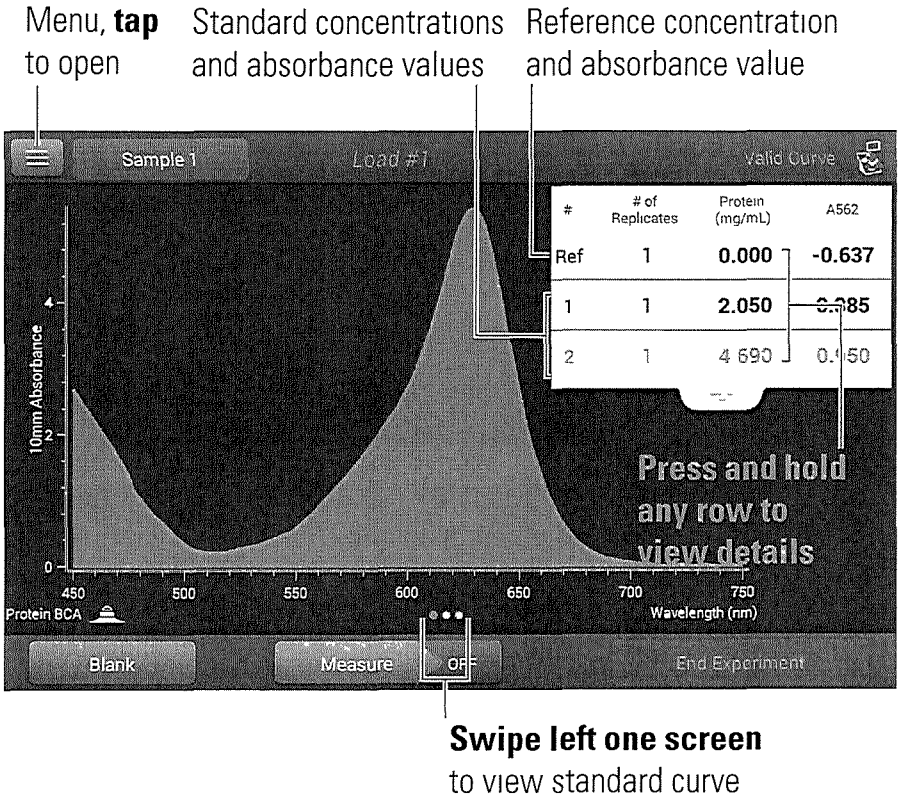
Working with standard curves

A standard curve is required for colorimetric protein analysis

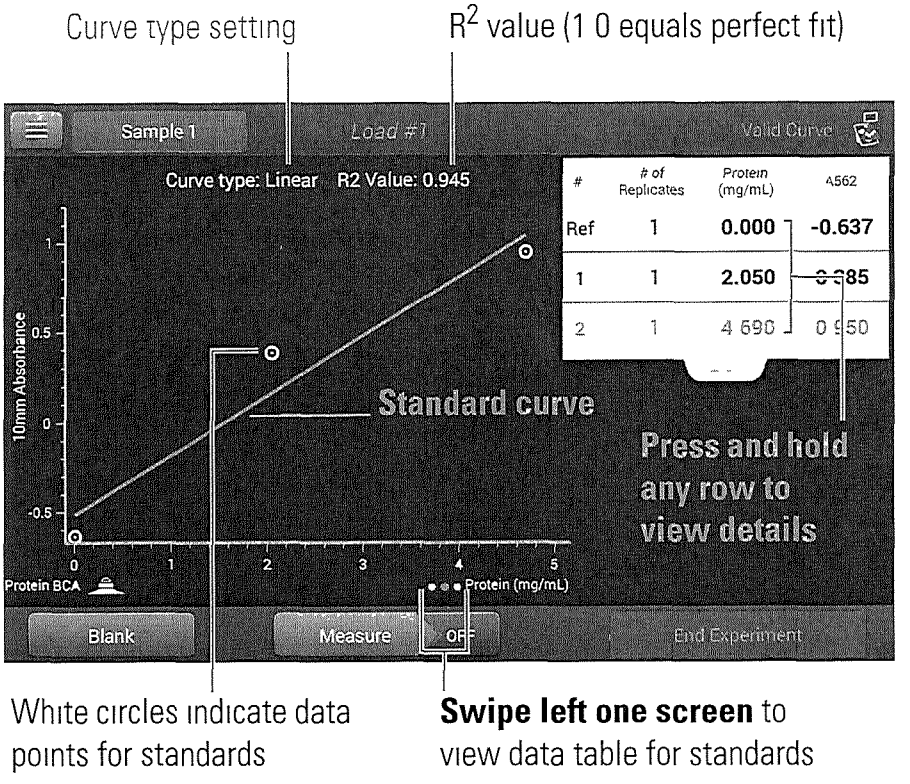
- Each experiment requires a new standard curve
- Prepare standards and unknown samples the same way See the kit manufacturer's guidelines and recommendations.
  - **All reference and standards solutions** should be the same buffer used to resuspend the samples plus the same volume of reagent added to the samples
  - **First standard** is a reference measurement The reference solution should contain none of the analyte of interest. (The reference measurement is not the same as a blank measurement This application requires both)
  - **Concentration range of the standards** must cover the dynamic range of the assay and the expected range of the unknown samples Sample analyte concentrations are not extrapolated beyond the concentration of the highest standard
- Use the application setup screen to enter concentration values for the standards and to specify how standards and samples will be measured (number of replicates, etc)
  - Depending on the Curve Type setting, a standard curve can be generated using two or more standards
  - The software **requires one reference measurement** and allows **up to 7 standards**
  - ***Concentration values for standards*** can be entered in any order but the standards must be measured in the order in which they were entered, however, best practice dictates that standards be measured from the lowest concentration of the standard analyte stock to the highest

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

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

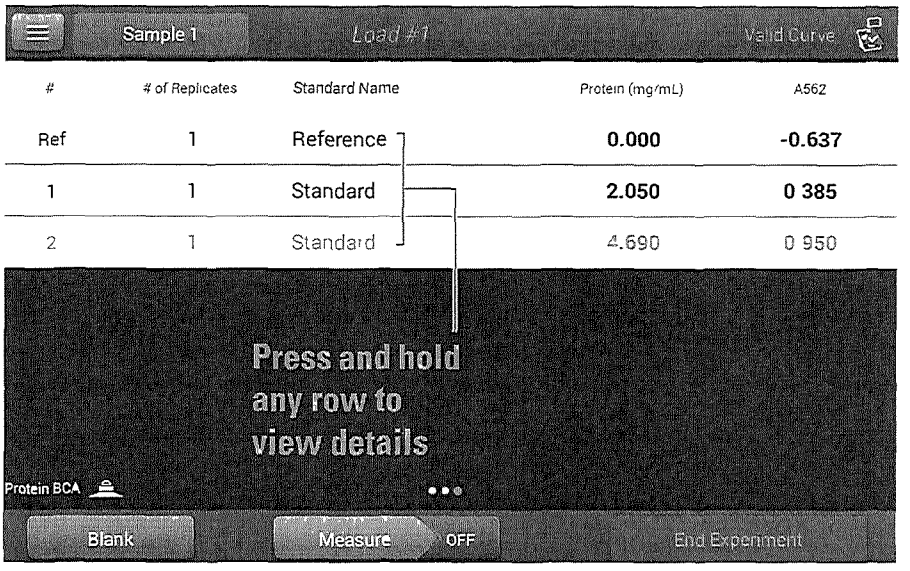


Swipe left one screen to see the standard curve as you build it Here is an example

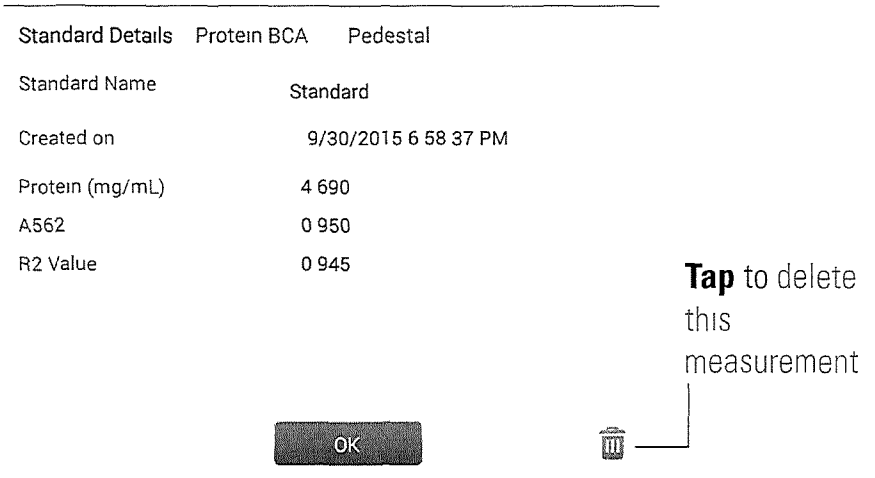


The R^2 value indicates how well the standard curve fits the standard data points (1 0 is a perfect fit, all points lie exactly on the curve).

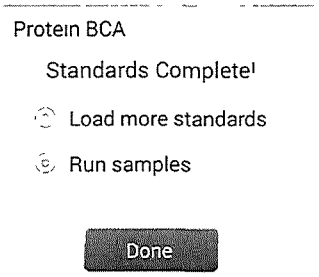
Swipe left one screen to see the data table for the standards Here is an example



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



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



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

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

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

Add standard

- from standards measurement screen, tap > **[application name] Setup**
- tap the next empty Concentration field and enter the concentration value for the new standard
- tap **Done**

Edit standard

- from standards measurement screen, tap > **[application name] Setup**
- tap the Concentration field and edit the concentration value
- tap **Done**

Delete standard

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

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

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

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

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

To measure Protein BCA standards and samples

NOTICE

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

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Before you begin...

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

❖ To measure Protein BCA standards and samples

1 From the Home screen, select the **Proteins** tab and tap **Protein BCA**

2 Specify a curve type and number of replicates for each standard and enter the concentration of each standard.

  Tip For this assay, we recommend setting **Curve Type** to "Linear"

3 Measure blank
  - pipette 2 µL DI H_2O onto lower pedestal and lower arm, or insert DI H_2O blanking cuvette into cuvette holder

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

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

4 Measure reference standard

- pipette 2 µL reference solution onto pedestal, or insert reference cuvette (reference solution should contain none of the standard protein stock, see Working With Standard Curves for details)
- lower arm to start measurement (or tap **Measure** if Auto-Measure is off)
- lift arm and clean both pedestals with new wipe, or remove cuvette
- if Replicates setting is greater than 1, repeat measurement

5 Measure remaining standards

- pipette 2 µL standard 1 onto pedestal, or insert standard 1 cuvette
- lower arm to start measurement (or tap **Measure** if Auto-Measure is off)
- lift arm and clean both pedestals with new wipe, or remove cuvette
- if Replicates setting is greater than 1, repeat measurement
- repeat substeps above for each additional standard (when specified number of standards and replicates have been measured, a message asks whether to load more standards or begin measuring samples)
- if finished measuring standards, tap **Done** (swipe left to view standard curve)

6 Measure samples

- pipette 2 µL sample 1 onto pedestal, or insert sample 1 cuvette
- lower arm to start measurement (or tap **Measure** if Auto-Measure is off)
- lift arm and clean both pedestals with new wipe, or remove cuvette
- if Replicates setting is greater than 1, repeat measurement

7 When you are finished measuring samples, tap **End Experiment**

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

Related Topics

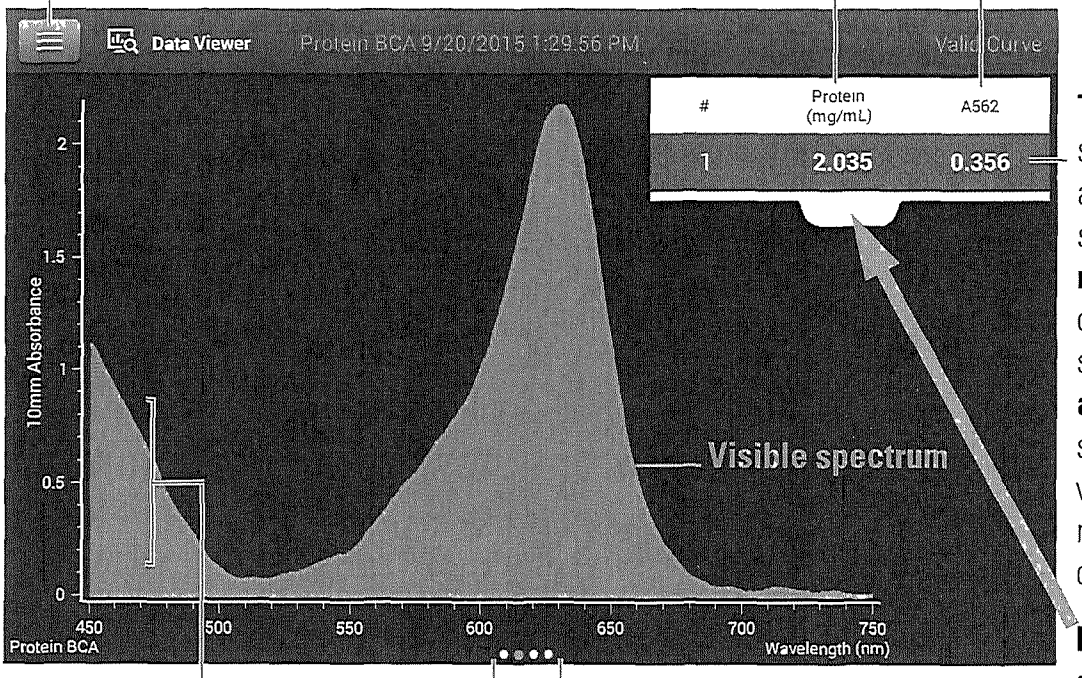
- Best practices for protein measurements
- Measure a Micro-Volume Sample
- Measure a Sample Using a Cuvette

- Prepare Samples and Blanks
- Basic Instrument Operations

Protein BCA Reported Results

Protein BCA measurement screen (shown from Data Viewer)

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



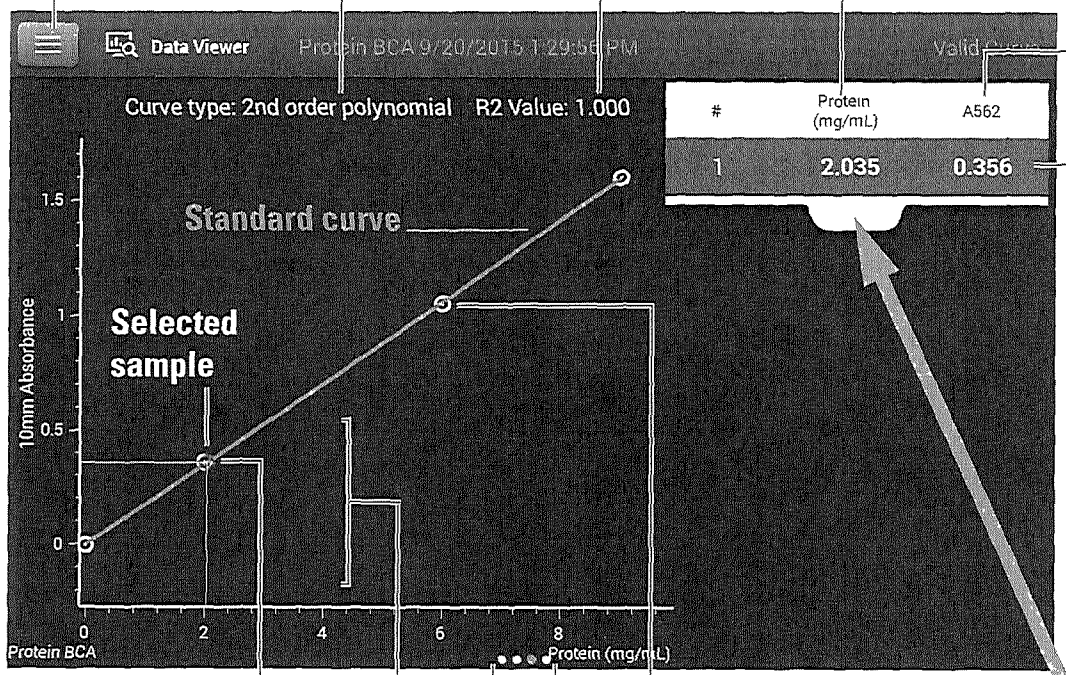
Note

- A baseline correction is performed at 750 nm (absorbance value at 750 nm is subtracted from absorbance values at all wavelengths in sample spectrum)
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10 0 mm pathlength equivalent

Protein BCA standard curve screen

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

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



Menu of options, **tap** to open

Curve type

R^2 value (1.0 equals perfect fit)

Protein concentration for selected sample

Absorbance at 562 nm

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

Red squares indicate sample data points

White circles indicate standard data points

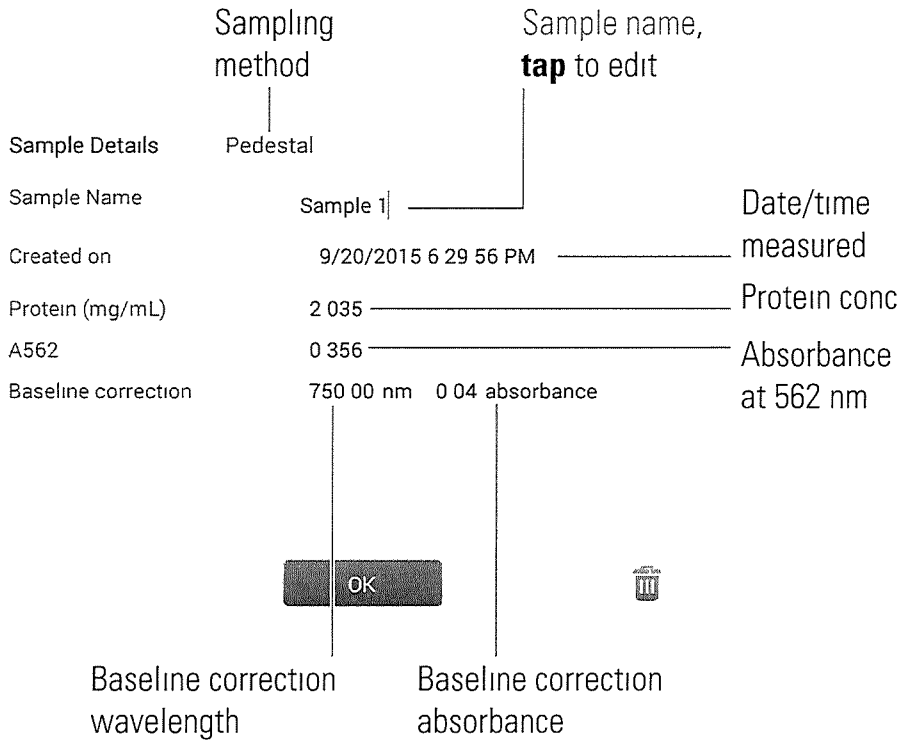
Drag tab down/up to see more/less sample data

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

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

Protein BCA reported values

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



Related Topics

- Basic Instrument Operations
- Protein A280 Calculations

Settings for Protein BCA Measurements

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

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

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

Related Topics

- *Instrument Settings*

Measure Protein Bradford

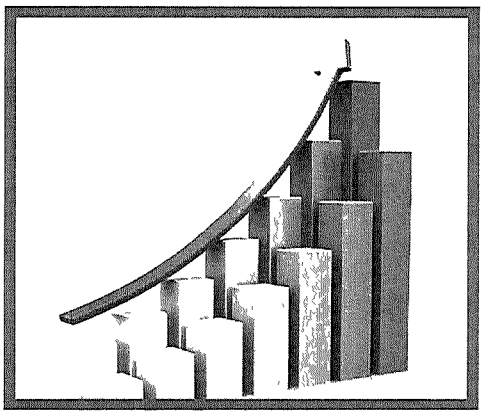
Measures total protein concentration of unpurified protein samples using a Coomassie Blue dye colorimetric detection reagent

Measure Total Protein

Reported Results

Settings

Detection Limits



Measure Total Protein Concentration

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

Theory of Protein Bradford assay

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

To maximize reliability with the Protein Bradford assay

- **Work quickly and do not allow prepared standards or samples to sit longer than necessary** Coomassie dye-dye and Coomassie dye-protein aggregates can form particulates with increasing development time, resulting in significant fluctuations in absorbance readings
- **Measure standards and samples in triplicate** using a new aliquot for each measurement For pedestal measurements, the total analyte (protein-dye) signal at 595 nm is limited to ~0-0 150A due to the pedestal's 1 0 mm pathlength, the Coomassie dye concentration, and the acidic pH

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

Protein assay kits and protocols

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

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

To measure Protein Bradford standards and samples

NOTICE

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

Before you begin...

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

❖ To measure Protein Bradford standards and samples

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

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

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

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

4 Measure reference standard

- pipette 2 µL reference solution onto pedestal, or insert reference cuvette (reference solution should contain none of the standard protein stock, see Working With Standard Curves for details)
- lower arm to start measurement (or tap **Measure** if Auto-Measure is off)
- lift arm and clean both pedestals with new wipe, or remove cuvette
- if Replicates setting is greater than 1, repeat measurement

5 Measure remaining standards

- pipette 2 µL standard 1 onto pedestal, or insert standard 1 cuvette
- lower arm to start measurement (or tap **Measure** if Auto-Measure is off)
- lift arm and clean both pedestals with new wipe, or remove cuvette
- if Replicates setting is greater than 1, repeat measurement
- repeat substeps above for each additional standard (when specified number of standards and replicates have been measured, a message asks whether to load more standards or begin measuring samples)
- if finished measuring standards, tap **Done** (swipe left to view standard curve)

6 Measure samples

- pipette 2 µL sample 1 onto pedestal, or insert sample 1 cuvette
- lower arm to start measurement (or tap **Measure** if Auto-Measure is off)
- lift arm and clean both pedestals with new wipe, or remove cuvette
- if Replicates setting is greater than 1, repeat measurement

7 When you are finished measuring samples, tap **End Experiment**

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

Related Topics

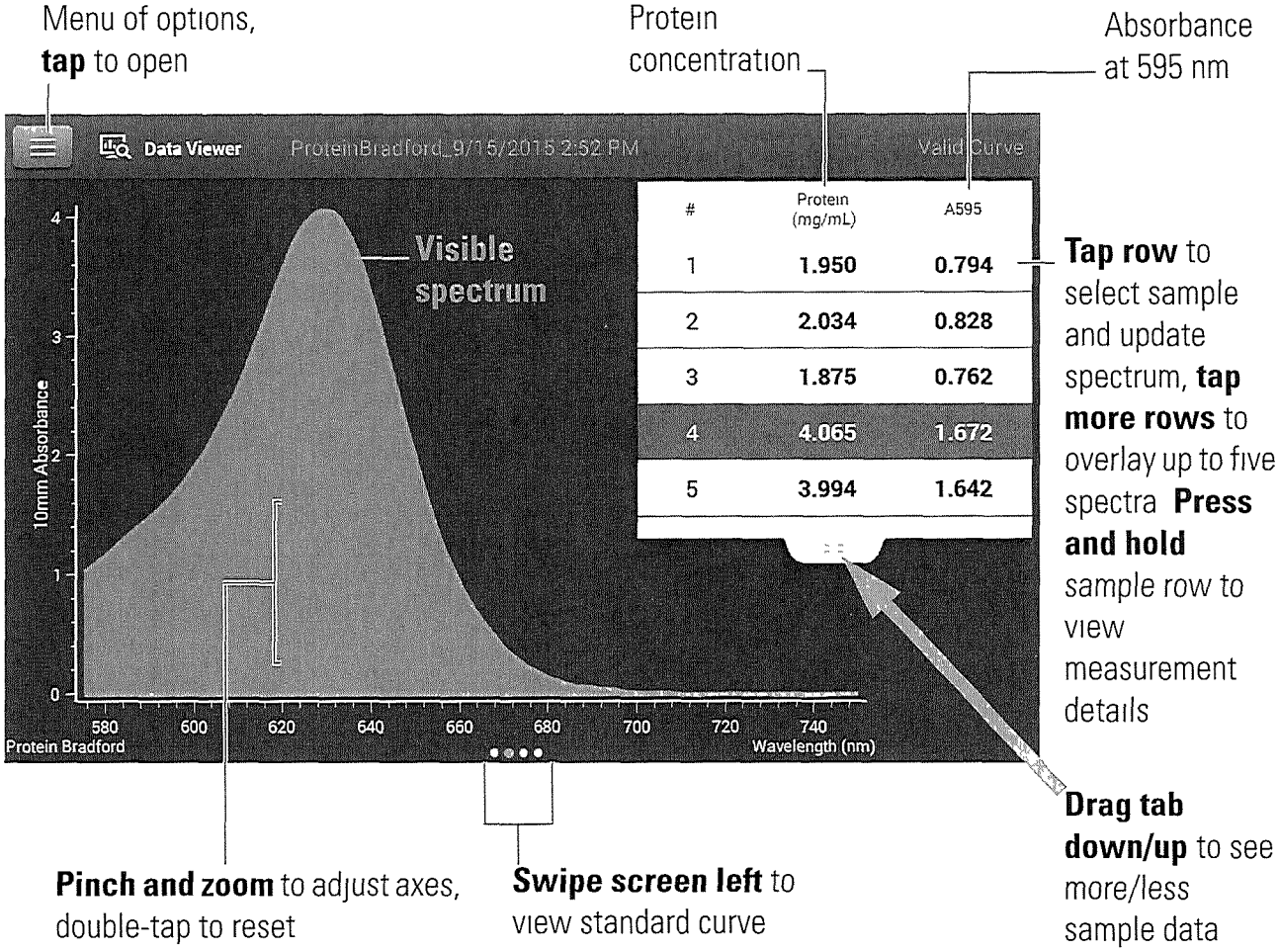
- Working with standard curves
- Best practices for protein measurements
- Measure a Micro-Volume Sample

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

Protein Bradford Reported Results

Protein Bradford measurement screen (shown from Data Viewer)

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



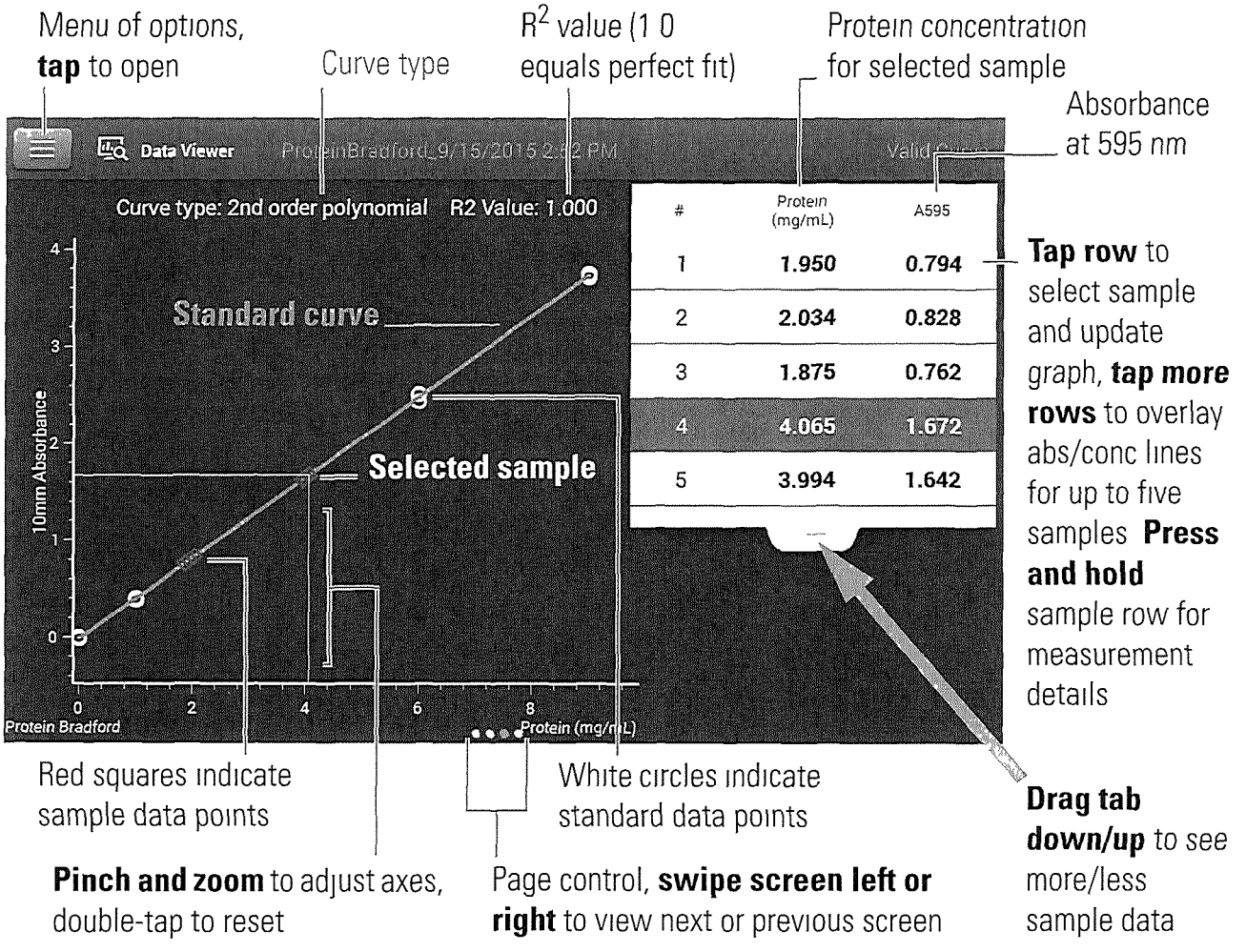
Note

- A baseline correction is performed at 750 nm (absorbance value at 750 nm is subtracted from absorbance values at all wavelengths in sample spectrum)
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10 0 mm pathlength equivalent.

Protein Bradford standard curve screen

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

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

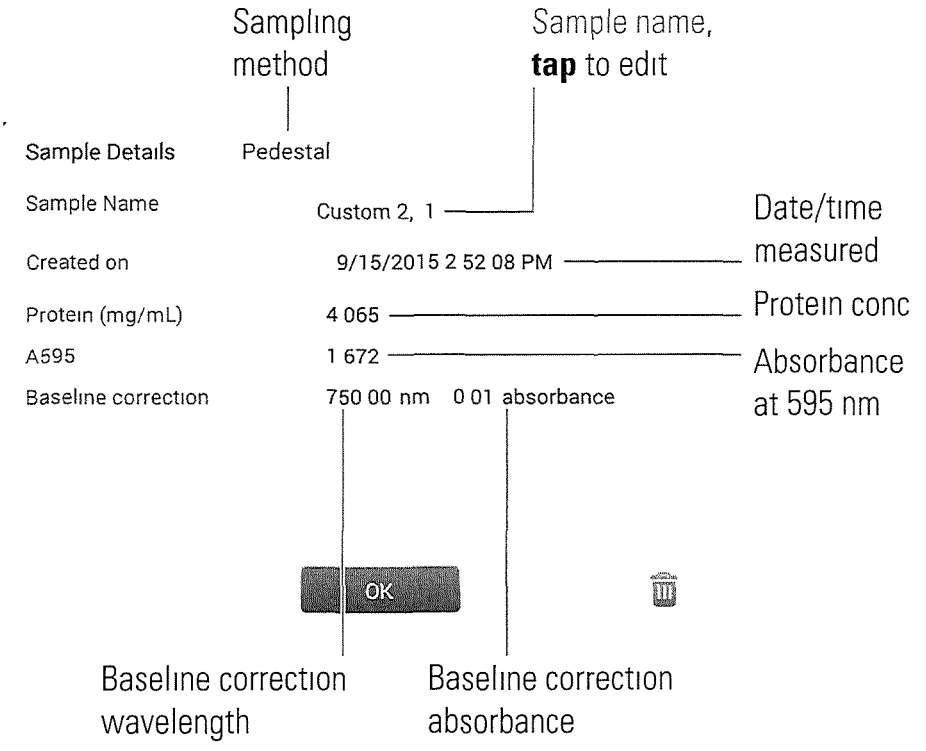


Note

- A baseline correction is performed at 750 nm (absorbance value at 750 nm is subtracted from absorbance values at all wavelengths in sample spectrum)
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10 0 mm pathlength equivalent

Protein Bradford reported values

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



Related Topics

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

Settings for Protein Bradford Measurements

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

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

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

Related Topics

- Instrument Settings

Measure Protein Lowry

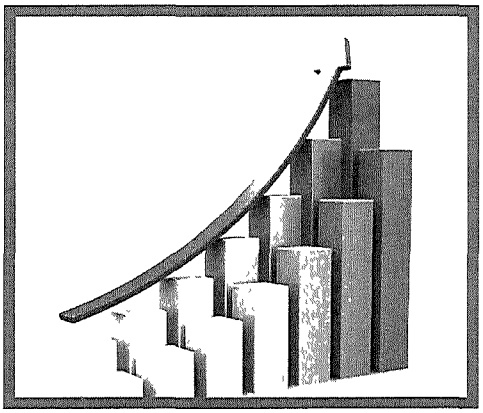
Measures total protein concentration of unpurified protein samples using a Folin-Ciocalteu colorimetric detection reagent

Measure Total Protein

Reported Results

Settings

Detection Limits



Measure Total Protein Concentration

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

Theory of Protein Lowry assay

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

Protein assay kits and protocols

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

To measure Protein Lowry standards and samples

NOTICE

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

Before you begin...

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

❖ **To measure Protein Lowry standards and samples**

1 From the Home screen, select the **Proteins** tab and tap **Protein Lowry**

2 Specify a curve type and number of replicates for each standard and enter the concentration of each standard

Tip For this assay, we recommend setting **Curve Type** to "2nd Order Polynomial"

3 Measure blank

– pipette 2 µL DI H_2O onto lower pedestal and lower arm, or insert DI H_2O blanking cuvette into cuvette holder

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

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

4 Measure reference standard

- pipette 2 µL reference solution onto pedestal, or insert reference cuvette (reference solution should contain none of the standard protein stock, see Working With Standard Curves for details)
- lower arm to start measurement (or tap **Measure** if Auto-Measure is off)
- lift arm and clean both pedestals with new wipe, or remove cuvette
- if Replicates setting is greater than 1, repeat measurement

5 Measure remaining standards

- pipette 2 µL standard 1 onto pedestal, or insert standard 1 cuvette
- lower arm to start measurement (or tap **Measure** if Auto-Measure is off)
- lift arm and clean both pedestals with new wipe, or remove cuvette
- if Replicates setting is greater than 1, repeat measurement
- repeat substeps above for each additional standard (when specified number of standards and replicates have been measured, a message asks whether to load more standards or begin measuring samples)
- if finished measuring standards, tap **Done** (swipe left to view standard curve)

6 Measure samples

- pipette 2 µL sample 1 onto pedestal, or insert sample 1 cuvette
- lower arm to start measurement (or tap **Measure** if Auto-Measure is off)
- lift arm and clean both pedestals with new wipe, or remove cuvette
- if Replicates setting is greater than 1, repeat measurement

7. When you are finished measuring samples, tap **End Experiment**

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

Related Topics

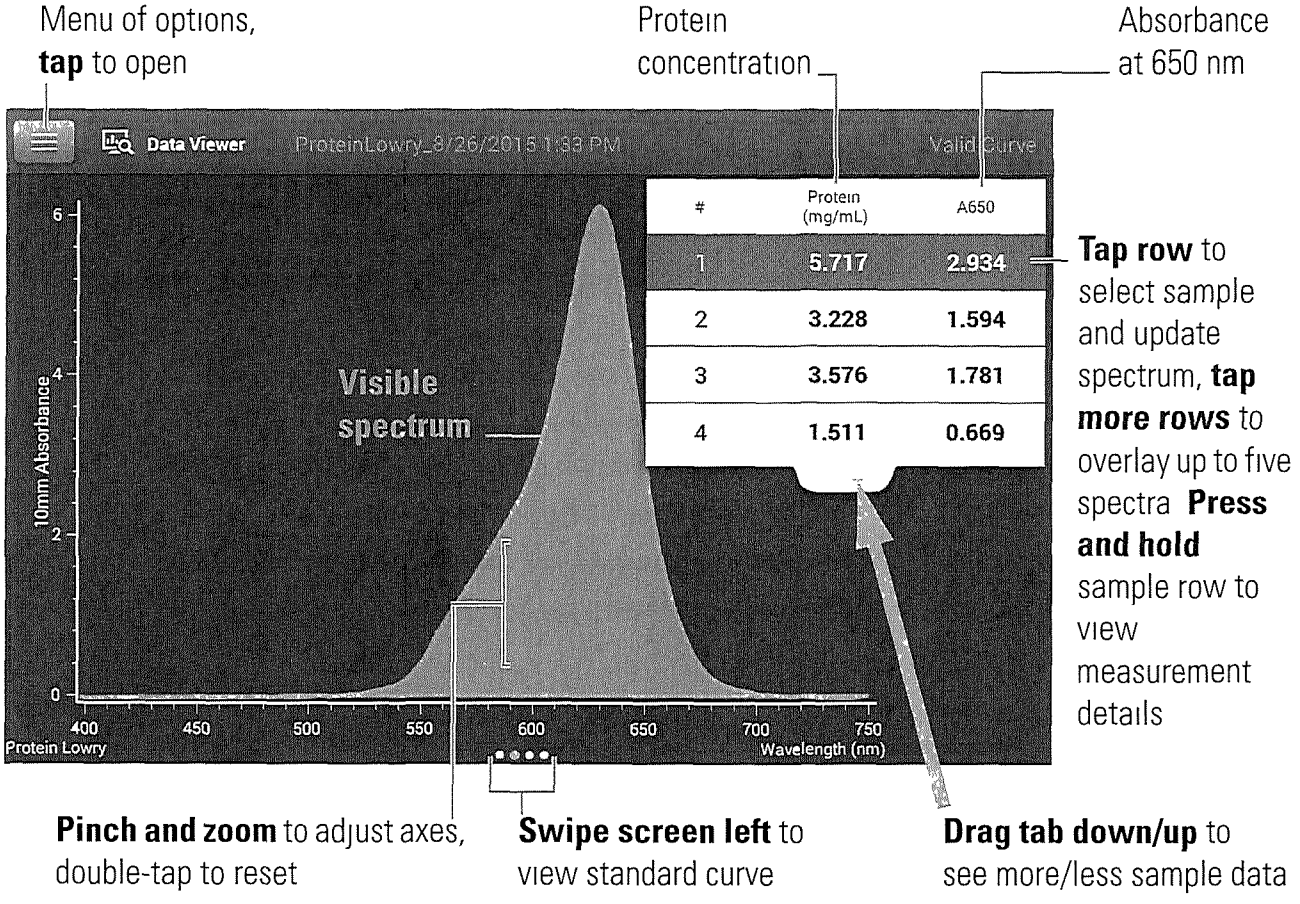
- Working with standard curves
- Best practices for protein measurements
- Measure a Micro-Volume Sample

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

Protein Lowry Reported Results

Protein Lowry measurement screen (shown from Data Viewer)

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



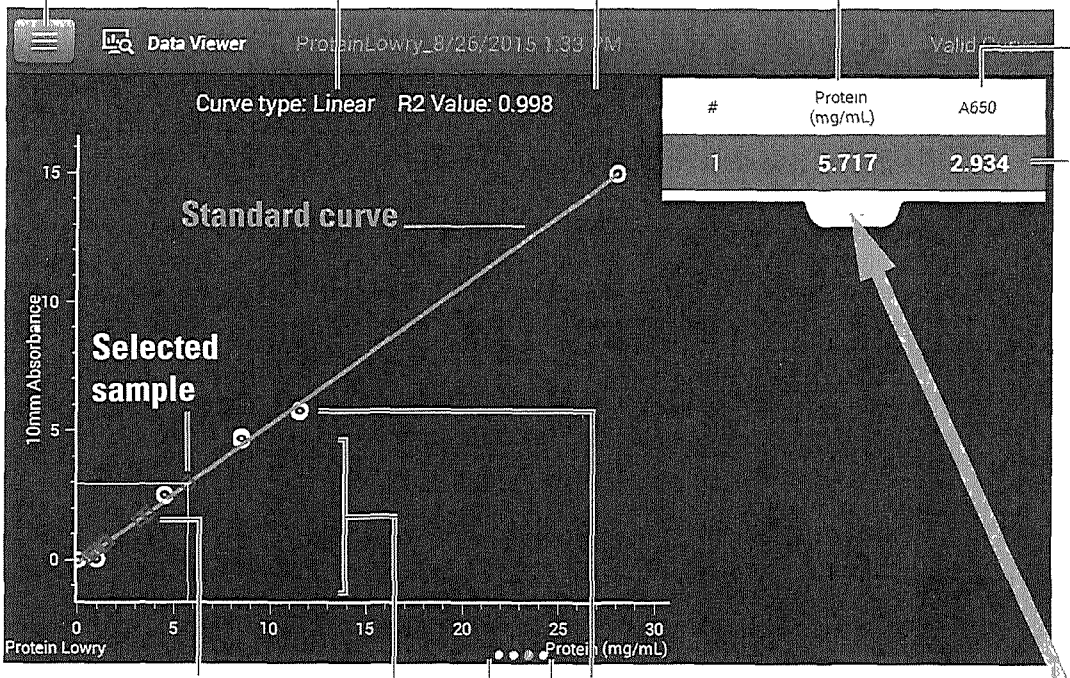
Note

- A baseline correction is performed at 405 nm (absorbance value at 405 nm is subtracted from absorbance values at all wavelengths in sample spectrum)
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10 0 mm pathlength equivalent

Protein Lowry standard curve screen

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

The R^2 value indicates how well the standard curve fits the standard data points (1 0 is a perfect fit, that is, all points lie exactly on the curve).



Menu of options, **tap** to open

Curve type

R^2 value (1 0 equals perfect fit)

Protein concentration for selected sample

Absorbance at 650 nm

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

Red squares indicate sample data points

White circles indicate standard data points

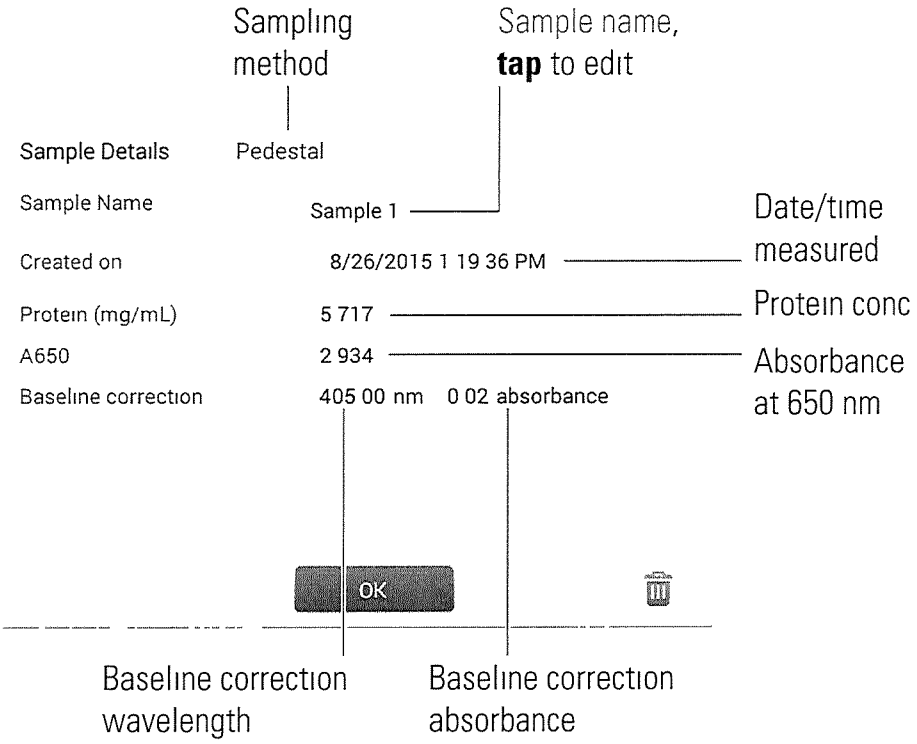
Drag tab down/up to see more/less sample data

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

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

Protein Lowry reported values

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



Related Topics

- Example standard curve
- Basic Instrument Operations

Settings for Protein Lowry Measurements

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

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

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

Related Topics

- Instrument Settings

Measure Protein Pierce 660

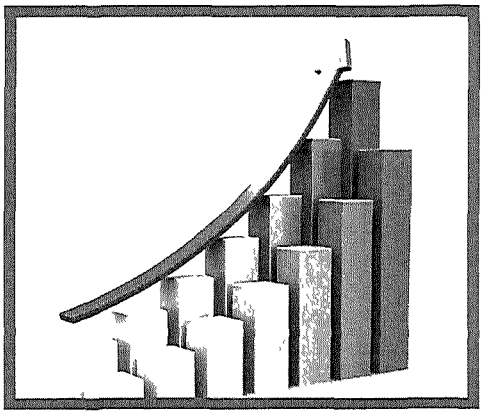
Measures total protein concentration of unpurified protein samples using a proprietary colorimetric detection reagent

Measure Total Protein

Reported Results

Settings

Detection Limits



Measure Total Protein Concentration

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

Theory of Protein Pierce 660 assay

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

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

Protein assay kits and protocols

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

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

To measure Protein Pierce 660 standards and samples

NOTICE

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

Before you begin...

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

❖ To measure Protein Pierce 660 standards and samples

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

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

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

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

4 Measure reference standard

- pipette 2 µL reference solution onto pedestal, or insert reference cuvette (reference solution should contain none of the standard protein stock, see Working With Standard Curves for details)
- lower arm to start measurement (or tap **Measure** if Auto-Measure is off)
- lift arm and clean both pedestals with new wipe, or remove cuvette
- if Replicates setting is greater than 1, repeat measurement

5 Measure remaining standards·

- pipette 2 µL standard 1 onto pedestal, or insert standard 1 cuvette
- lower arm to start measurement (or tap **Measure** if Auto-Measure is off)
- lift arm and clean both pedestals with new wipe, or remove cuvette
- if Replicates setting is greater than 1, repeat measurement
- repeat substeps above for each additional standard (when specified number of standards and replicates have been measured, a message asks whether to load more standards or begin measuring samples)
- if finished measuring standards, tap **Done** (swipe left to view standard curve)

6 Measure samples

- pipette 2 µL sample 1 onto pedestal, or insert sample 1 cuvette
- lower arm to start measurement (or tap **Measure** if Auto-Measure is off)
- lift arm and clean both pedestals with new wipe, or remove cuvette
- if Replicates setting is greater than 1, repeat measurement

7 When you are finished measuring samples, tap **End Experiment**

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

Related Topics

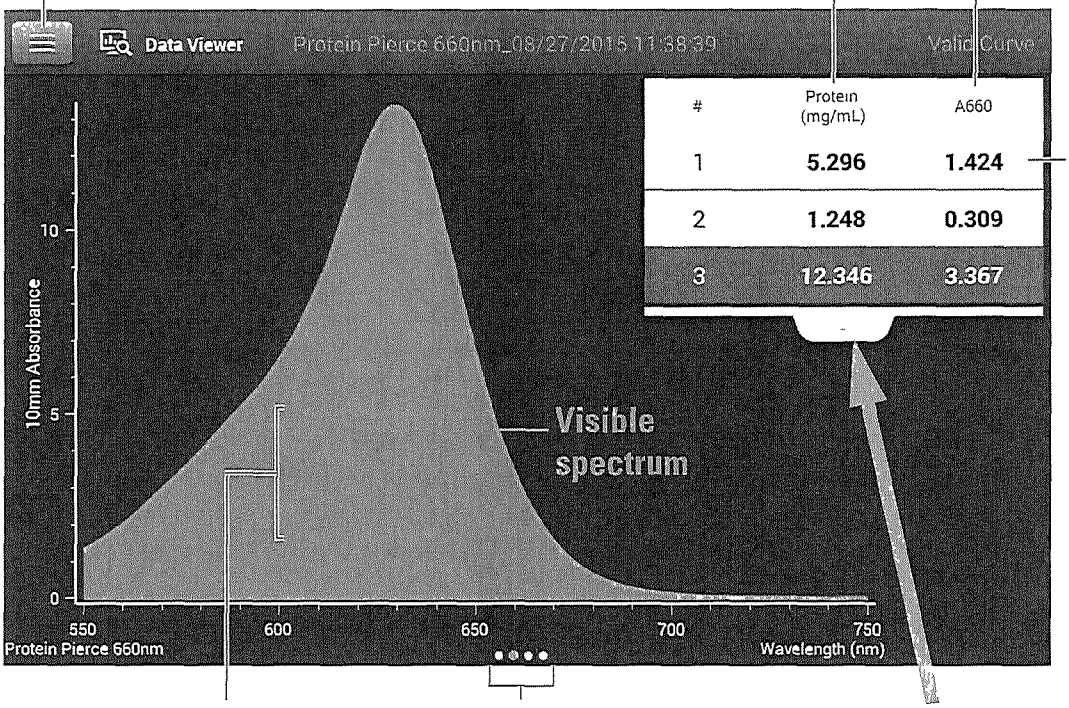
- Working with standard curves
- Best practices for protein measurements
- Measure a Micro-Volume Sample

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

Protein Pierce 660 Reported Results

Protein Pierce 660 measurement screen (shown from Data Viewer)

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



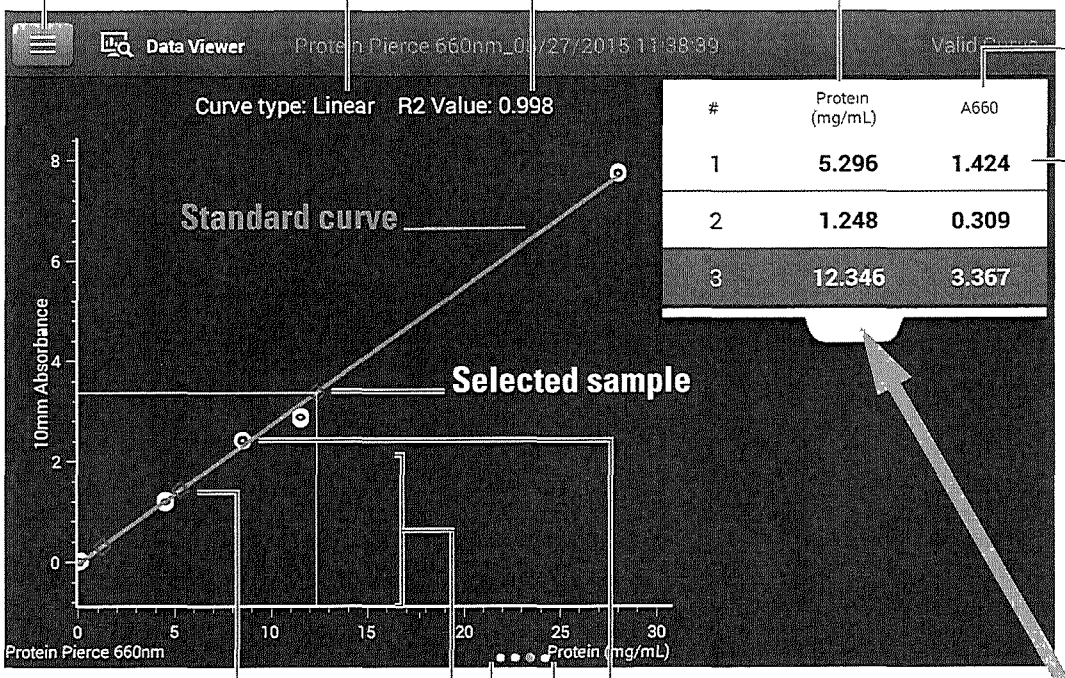
Note

- A baseline correction is performed at 750 nm (absorbance value at 750 nm is subtracted from absorbance values at all wavelengths in sample spectrum)
- Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10 0 mm pathlength equivalent

Protein Pierce 660 standard curve screen

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

The R^2 value indicates how well the standard curve fits the standard data points (1 0 is a perfect fit, that is, all points lie exactly on the curve)



Menu of options, **tap** to open

Curve type

R^2 value (1 0 equals perfect fit)

Protein concentration for selected sample

Absorbance at 660 nm

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

Red squares indicate sample data points

White circles indicate standard data points

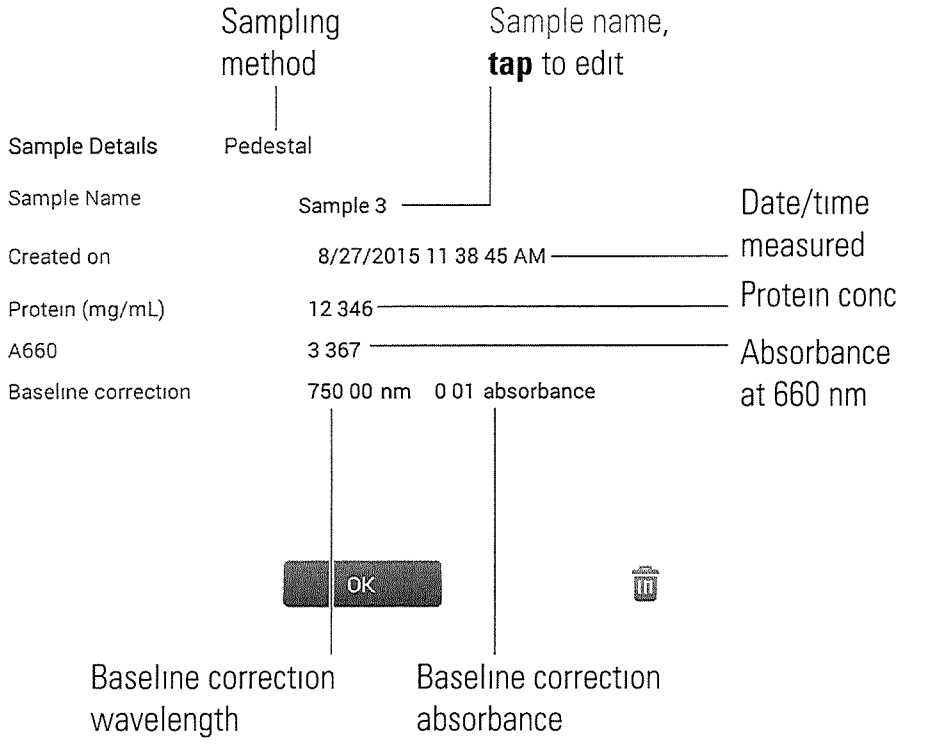
Drag tab down/up to see more/less sample data

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

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

Protein Pierce 660 reported values

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



Related Topics

- Example standard curve
- Basic Instrument Operations

Settings for Protein Pierce 660 Measurements

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

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

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

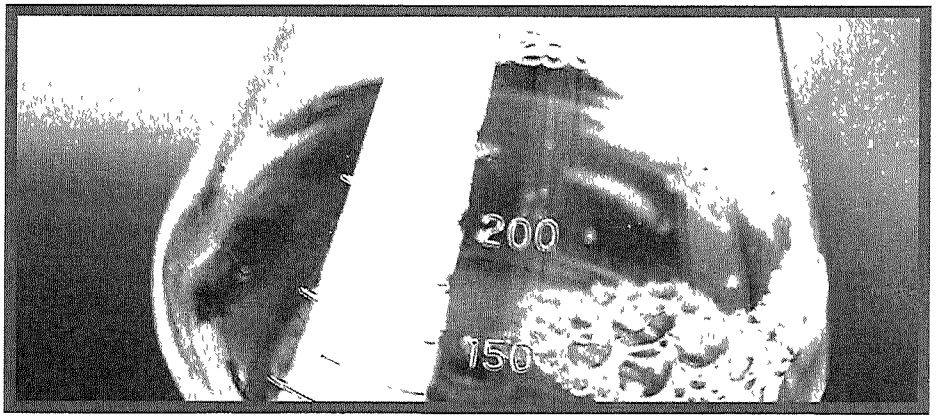
Measures the concentration of microbial cell cultures in solution by measuring scattered light at 600 nm.

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

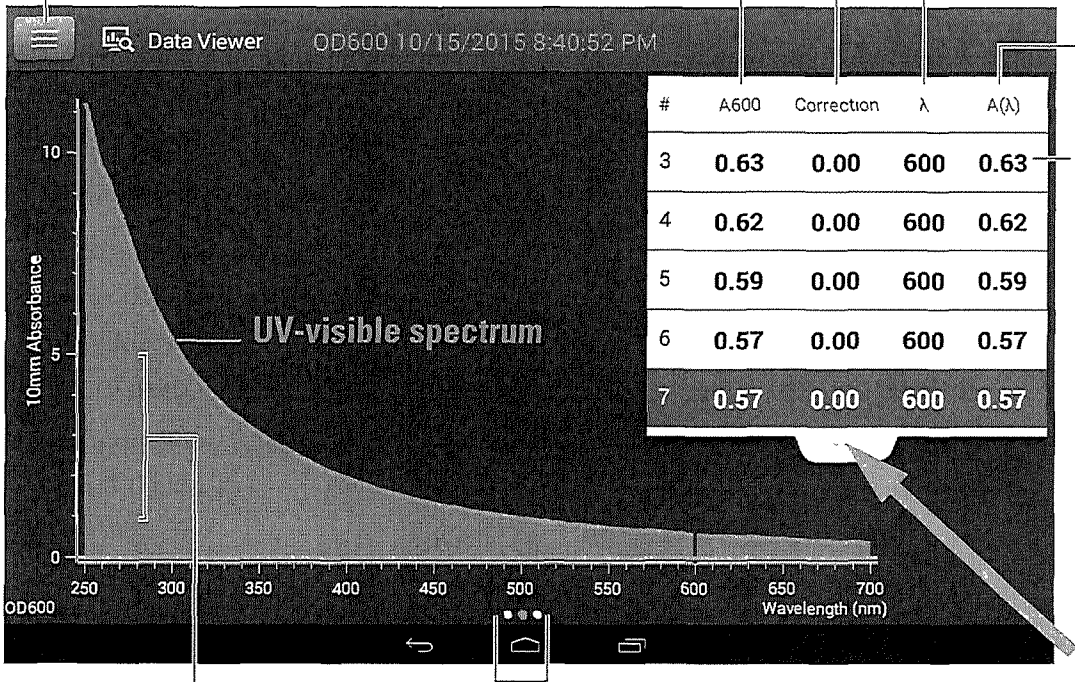
Related Topics

- 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 Data Viewer)

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

Corrected absorbance at 600 nm

Absorbance correction

Additional wavelength

Corrected absorbance at additional wavelength

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

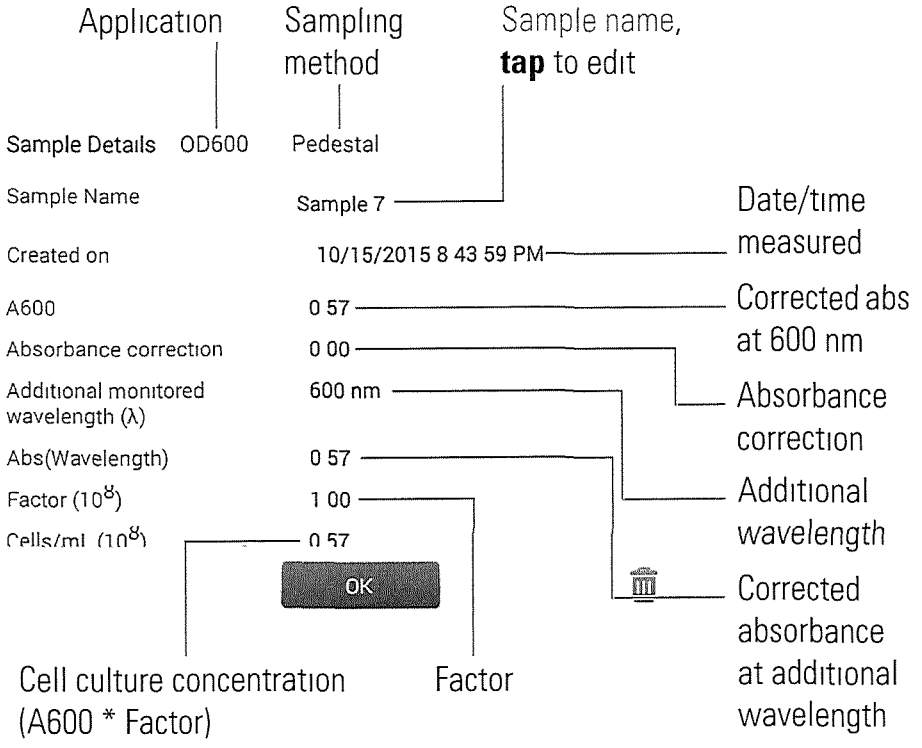
Pinch and zoom to adjust axes, double-tap to reset

Swipe screen left to view table with more measurement results

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.

Measure Custom

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

Measure Custom Method

Delete Custom Method

Reported Results

Measure using a Custom Method

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

To load a custom method

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

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

Load custom methods onto the instrument

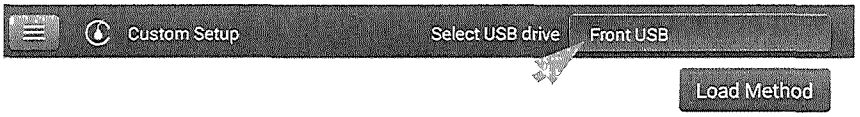
1 Export the method from the personal computer and copy the method file to the root of a portable USB device such as a memory stick

Method files have a " method" filename extension

2 Connect the USB device to one of the USB ports on the instrument

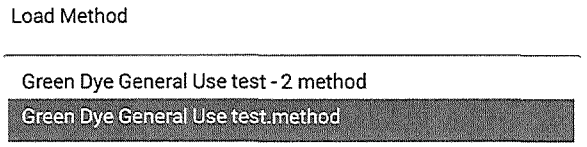
3 From the Home screen, select the **Custom** tab and tap **Custom**

4 Use the list box at the top of the screen to indicate the USB port used



5. Tap **Load Method**

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



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

7 Tap **Load**

To measure using a custom method

NOTICE

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

Before you begin...

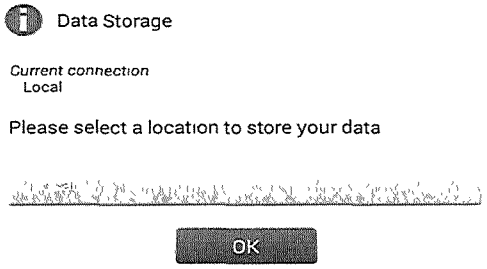
Before taking pedestal measurements with the NanoDrop One instrument, lift the instrument arm and clean the upper and lower pedestals. At a minimum, wipe the pedestals with a new laboratory wipe. For more information, see Cleaning the Pedestals.

❖ To measure a sample using a custom method

1 Make sure the method resides in same location as the database where you want to store the measurement results (see To Load a Custom Method for details)

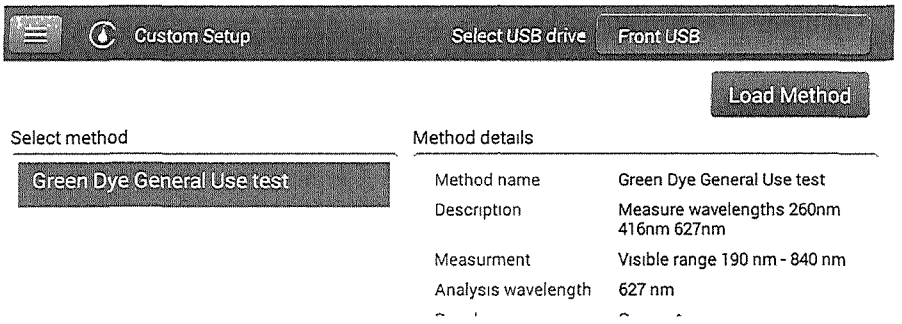
2 From the Home screen, select the **Custom** tab and tap **Custom**.

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



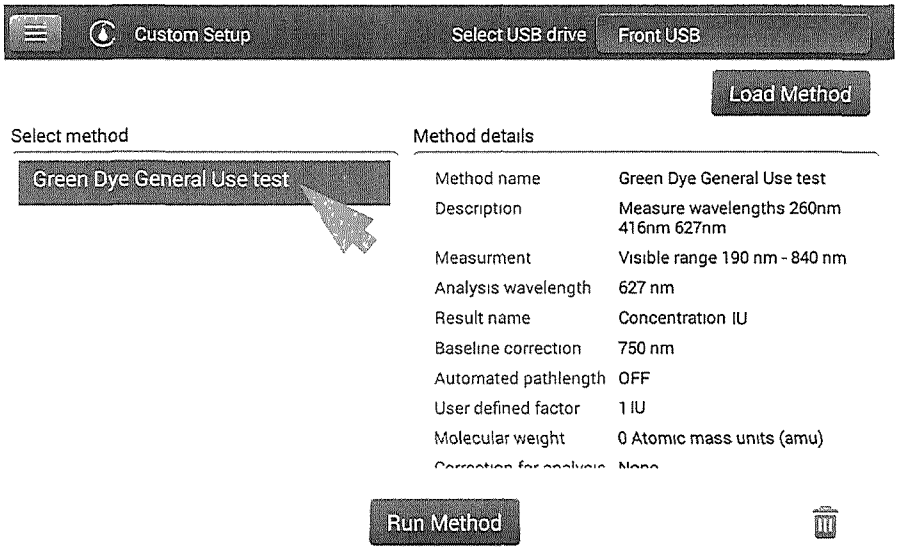
- To run a custom method that is loaded on the instrument and store all subsequently acquired measurement results in the database on the instrument, set Data Storage to **Local** (see example above)
- To run a custom method that resides on a personal computer connected to the instrument with an Ethernet cable and store all subsequently acquired measurement results in the database on that computer, set Data Storage to **Direct-Connect PC** (see "Set Up Ethernet Connection" in Set Up the Instrument for more information)
- To run a custom method that resides on a personal computer connected to the instrument through a wireless network and store all subsequently acquired measurement results in the database on that computer, set Data Storage to the **computer's assigned name** (see "Set Up Wi-Fi Connections" in Set Up the Instrument for more information)

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



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

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



Information about the selected method appears in the Method Details box

4 Tap **Run Method**

5 Follow the on-screen instructions to measure a sample

Related Topics

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

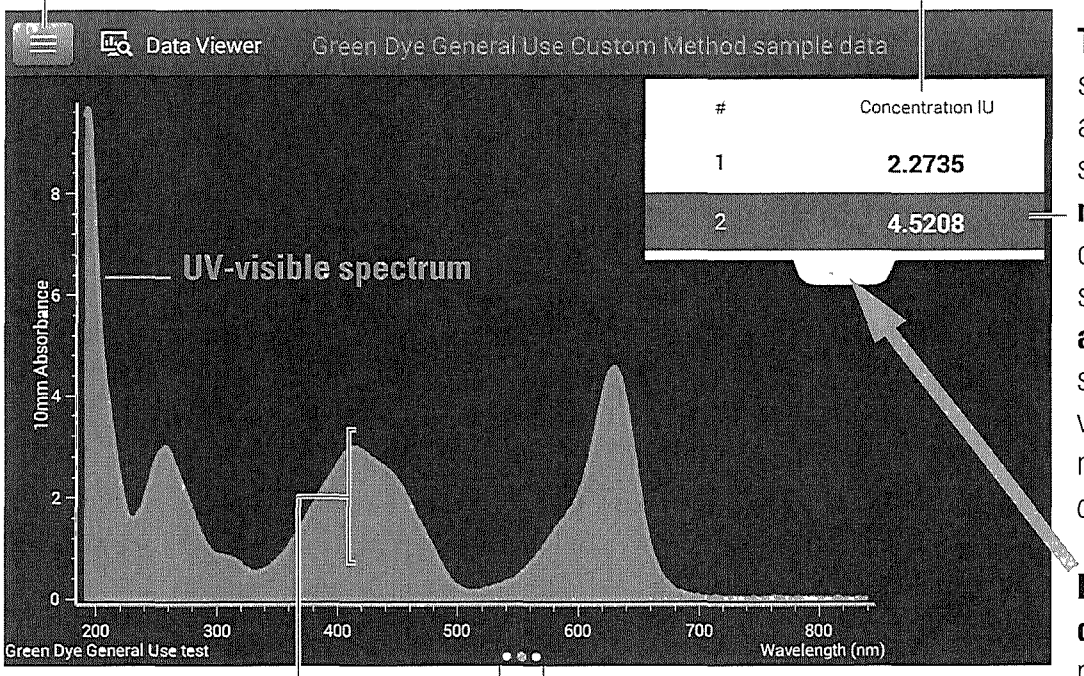
Delete Custom Method

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

Custom Method Reported Results

Custom method measurement screen (shown from Data Viewer)

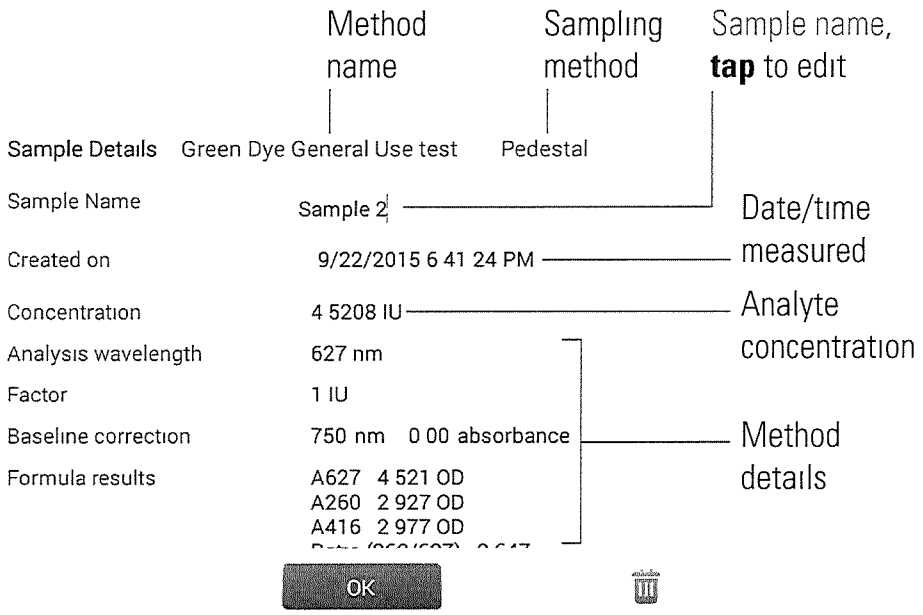
For each measured sample, this application shows the absorbance spectrum and a summary of the results. Here is an example



Note Micro-volume absorbance measurements and measurements taken with nonstandard cuvettes are normalized to a 10 0 mm pathlength equivalent

Custom method reported values

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



Related Topics

- Basic Instrument Operations

Measure UV-Vis

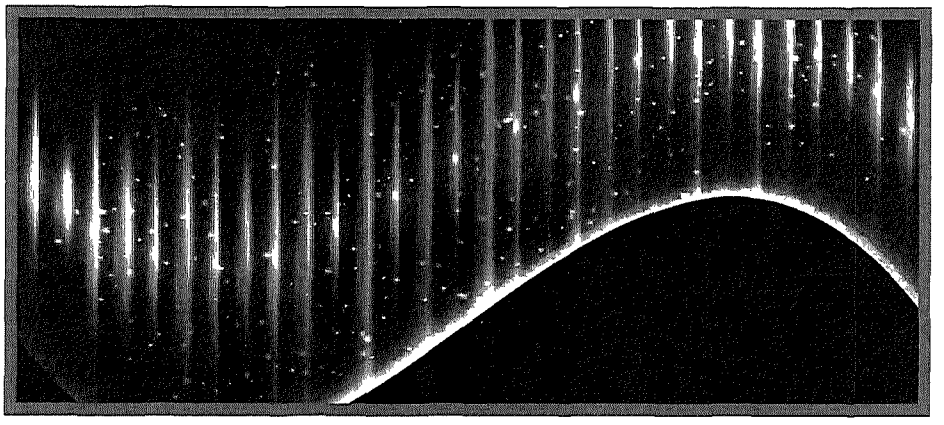
Measures the absorbance of any sample at up to 40 wavelengths across the ultra-violet (UV) and visible regions of the spectrum

Measure UV-Vis

Reported Results

Settings

Detection Limits



Measure UV-Vis

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

To make UV-Vis measurements

NOTICE

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

Before you begin...

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

❖ To measure a sample using the UV-Vis application

1 From the Home screen, select the **Custom** tab and tap **UV-Vis**

2 Specify up to 40 wavelengths to monitor (or you can specify them later if desired) and whether automated pathlength adjustment and baseline correction will be used

3 Pipette 1–2 µL blanking solution onto the lower pedestal and lower the arm, or insert the blanking cuvette into the cuvette holder

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

4 Tap **Blank** and wait for the measurement to complete

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

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

6 Pipette 1-2 µL sample solution onto the pedestal and lower the arm, or insert the sample cuvette into the cuvette holder

7 Start the sample measurement

- Pedestal If Auto-Measure is On, lower arm, if Auto-Measure is off, lower arm and tap **Measure**
- Cuvette Tap **Measure.**

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

8 When you are finished measuring samples, tap **End Experiment**

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

Best practices for UV-Vis measurements

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

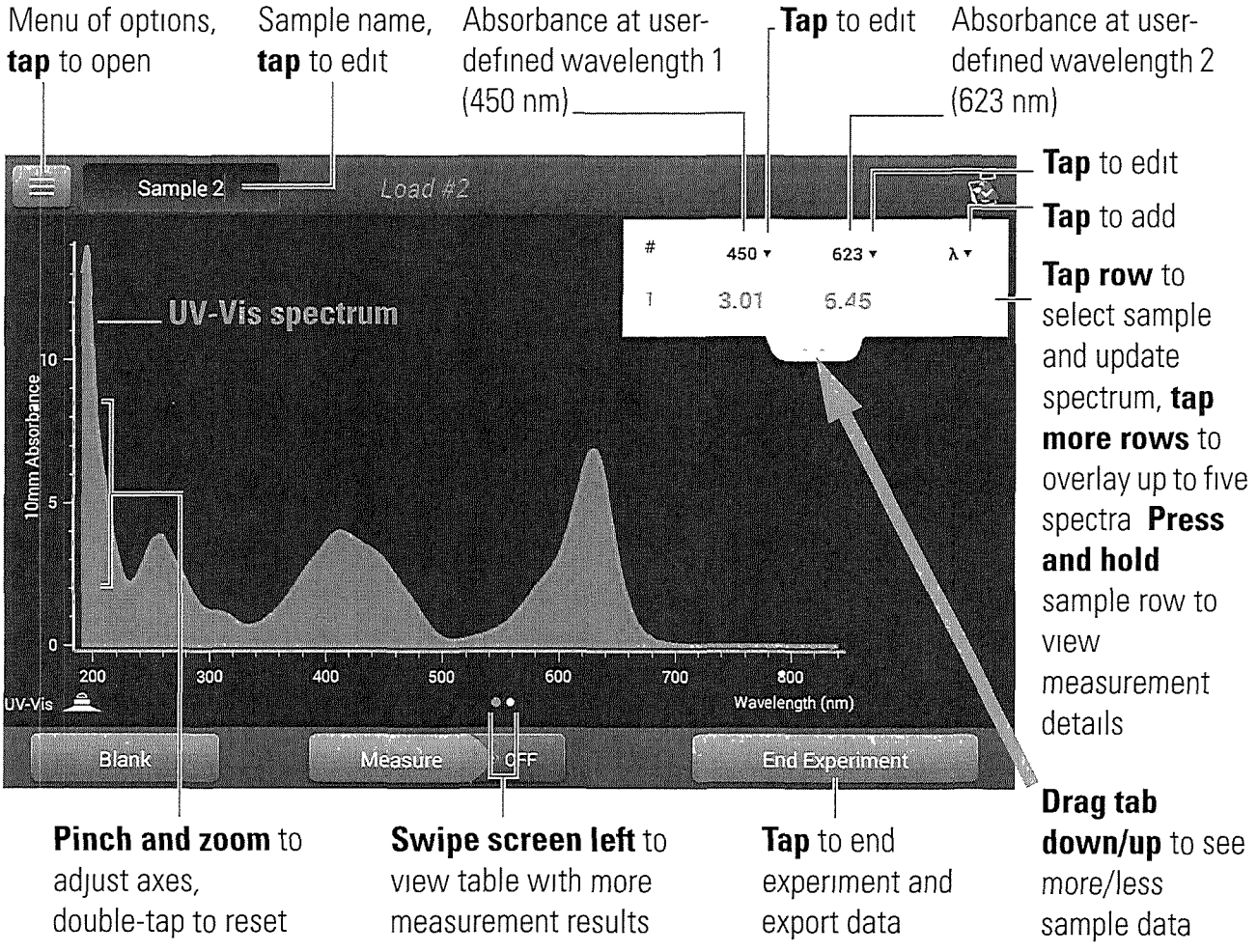
Related Topics

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

UV-Vis Reported Results

UV-Vis measurement screen

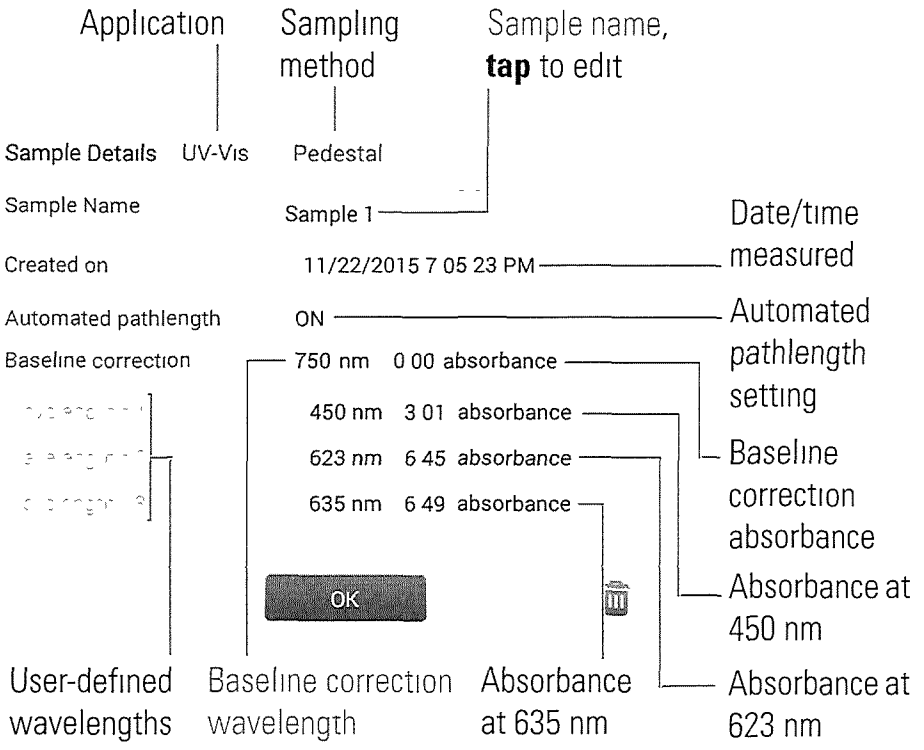
For each measured sample, this application shows the absorbance spectrum and a summary of the results Here is an example



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

UV-Vis reported values

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



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

Related Topics

- Basic Instrument Operations

Settings for UV-Vis Measurements

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

Setting	Available Options	Description
Monitored wavelengths	Enter up to 40 wavelengths between 190 nm and 850 nm	**User-defined wavelengths to be measured and reported at run time** Absorbance values for the first three entered wavelengths are displayed in the measurement screen To see absorbance values for 8 monitored wavelengths, swipe left in the measurement screen to show the Data table. To see all monitored wavelengths, press and hold a sample row to show the Sample Details screen (scroll up to display absorbance values for any additional user-defined wavelengths) **Note** If Baseline Correction is selected, all displayed absorbance values are the corrected values
Automated Pathlength	On or Off (affects pedestal measurements only)	**Optional automated pathlength selection** Allows the software to use the optimal (shorter) pedestal pathlength for high concentration samples to help prevent detector saturation (see Detection Limits for details) • When selected, the shorter pathlength is used when any wavelength between 220 nm and 850 nm has 10 mm equivalent absorbance value of 12 5 or higher For wavelengths between 190 nm and 219 nm the change to the shorter pathlength occurs when any wavelength in this range has a 10 mm equivalent absorbance value of 10 or higher • When deselected, the pedestal pathlength is restricted to 10 mm across all wavelengths **Note**· In either case, displayed absorbance values have been normalized to a 10 mm pathlength equivalent
Baseline Correction	On or off Enter baseline correction wavelength in nm or use default value (750 nm)	**Optional user-defined baseline correction** Can be used to correct for any offset caused by light scattering particulates by subtracting measured absorbance at specified baseline correction wavelength from absorbance values at all wavelengths in sample spectrum *As a result, absorbance of sample spectrum is zero at* specified baseline correction wavelength

Related Topics

- Instrument Settings

Measure Kinetics

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

Measure Kinetics

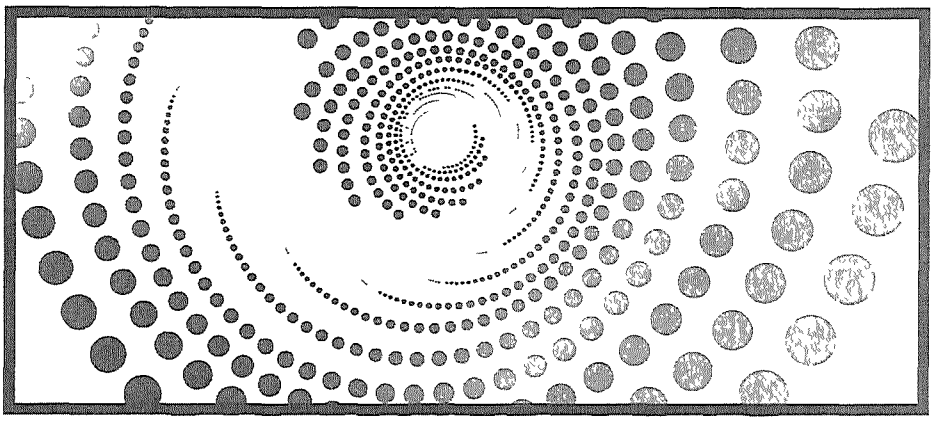
Create Kinetics Method

Edit Kinetics Method

Reported Results

Settings

Detection Limits



Measure Kinetics

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

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

To make kinetic measurements

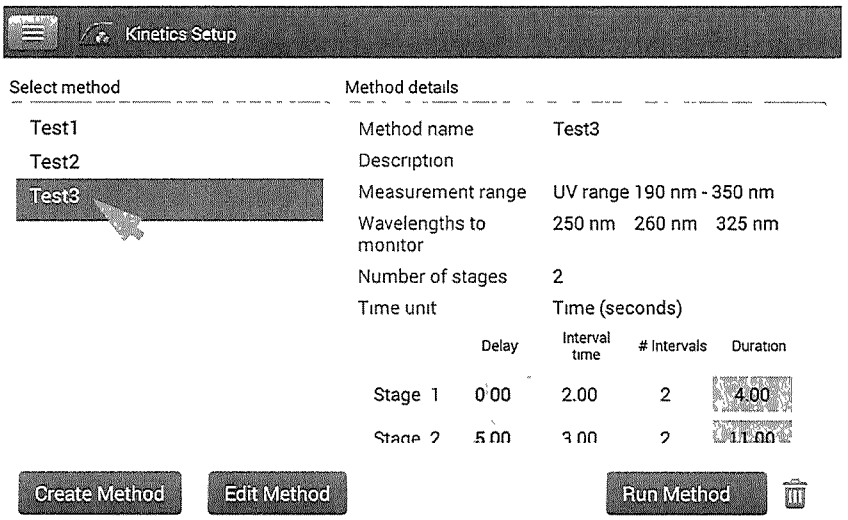
NOTICE

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

❖ To measure a sample using the Kinetics application

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

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



2 Select a method

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

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

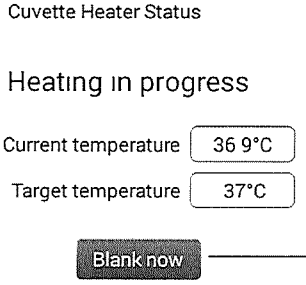
Note If your cuvette pathlength is not 10 mm, specify the correct pathlength in General Settings.

4 Tap **Run Method**

5 Measure a blank

- Fill clean, dry cuvette with enough blanking solution to cover instrument optical path
- Lift instrument arm and insert blanking cuvette into cuvette holder, making sure to align light path of cuvette with light path of instrument
- Tap **Blank**

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



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

- Wait for blank measurement to complete and then remove cuvette

  Note: The heater target temperature is not adjustable

6 Measure a sample:

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

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

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

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

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

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

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

Related Topics

- Measure a Sample Using a Cuvette

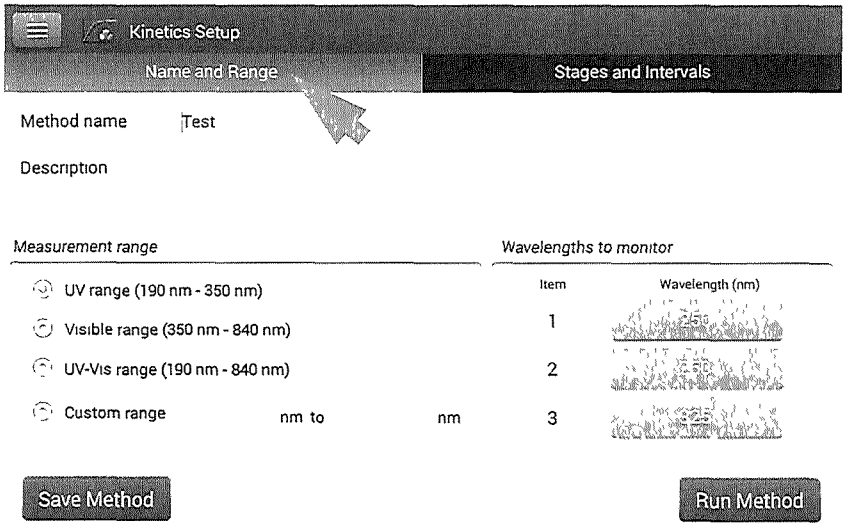
- Best Practices for Cuvette Measurements
- Prepare Samples and Blanks
- Basic Instrument Operations

Create Kinetics Method

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

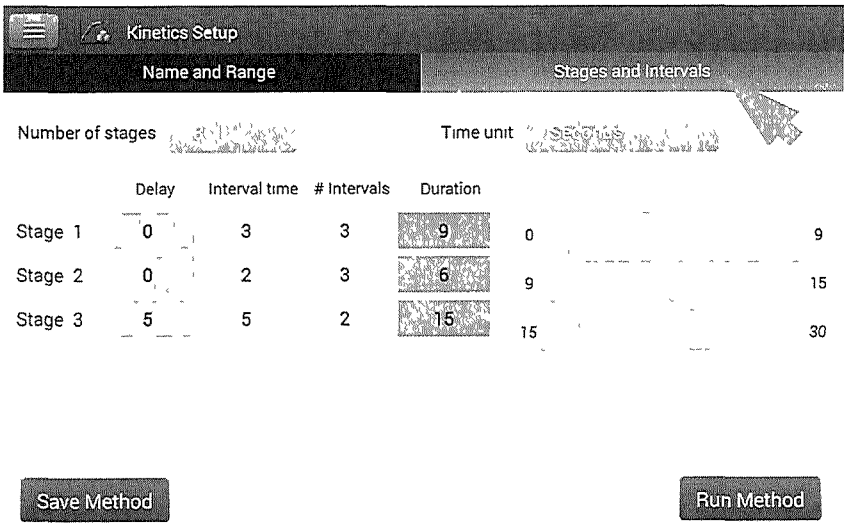
– from Home screen, tap **Kinetics** tab > **Kinetics** application

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



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

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



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

– for each stage, specify **# intervals**, **Interval times** and any **Delays** between stages

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

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

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

– to run the method, tap **Run Method**

Related Topics

- Edit Kinetics Method

Edit Kinetics Method

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

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

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

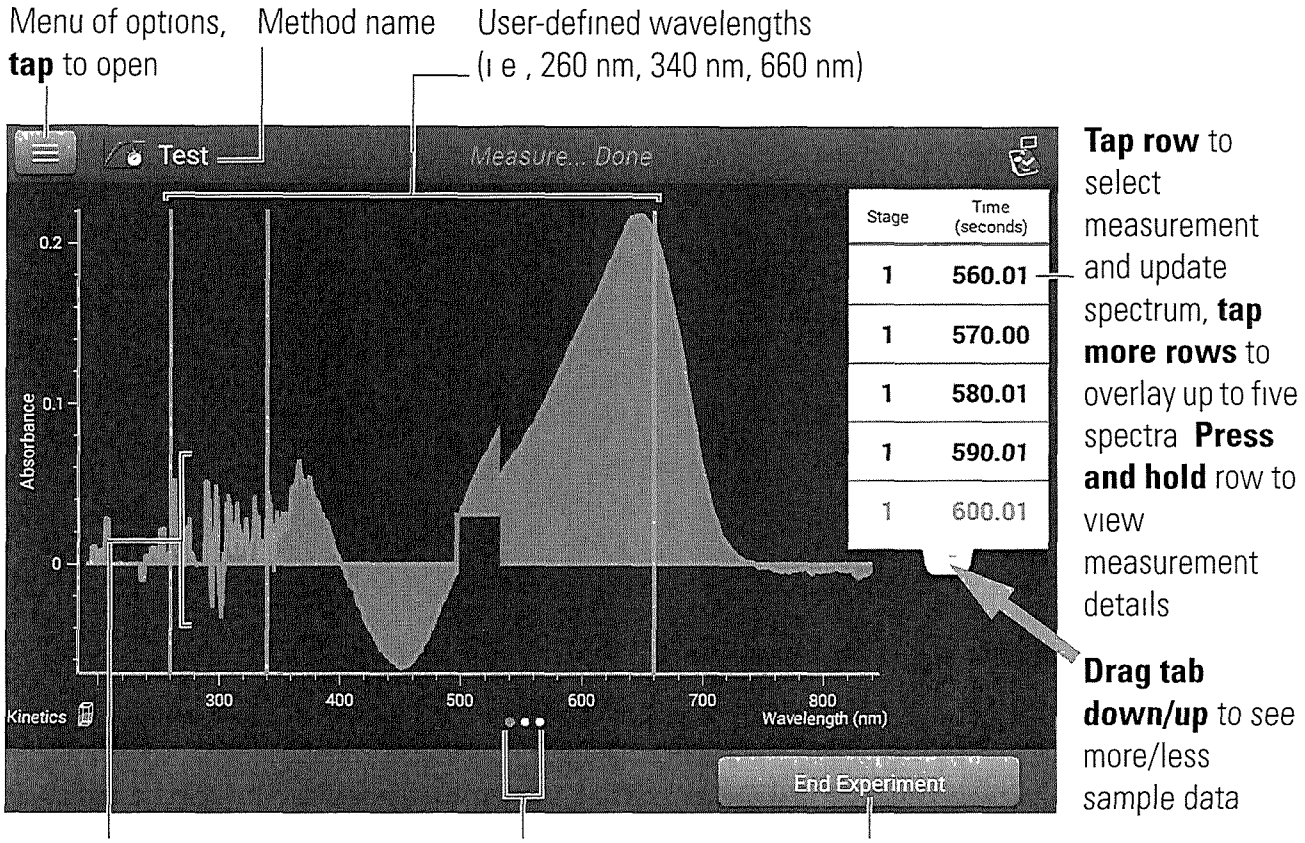
Related Topics

- Create Kinetic Method

Kinetics Reported Results

Absorbance measurement screen

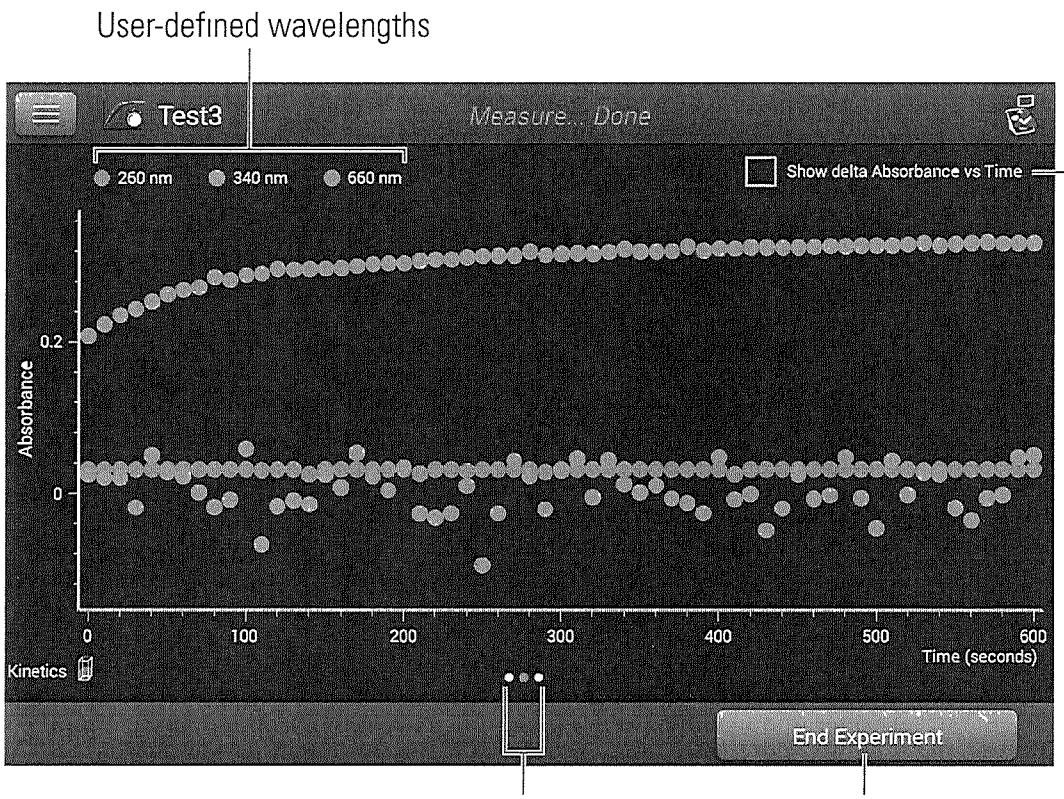
The absorbance measurement screen appears immediately after you tap Measure in the kinetics experiment This screen shows the absorbance spectrum for each measurement, with wavelength on the X-axis and absorbance on the Y-axis Vertical lines indicate the specified wavelengths to monitor The list at the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



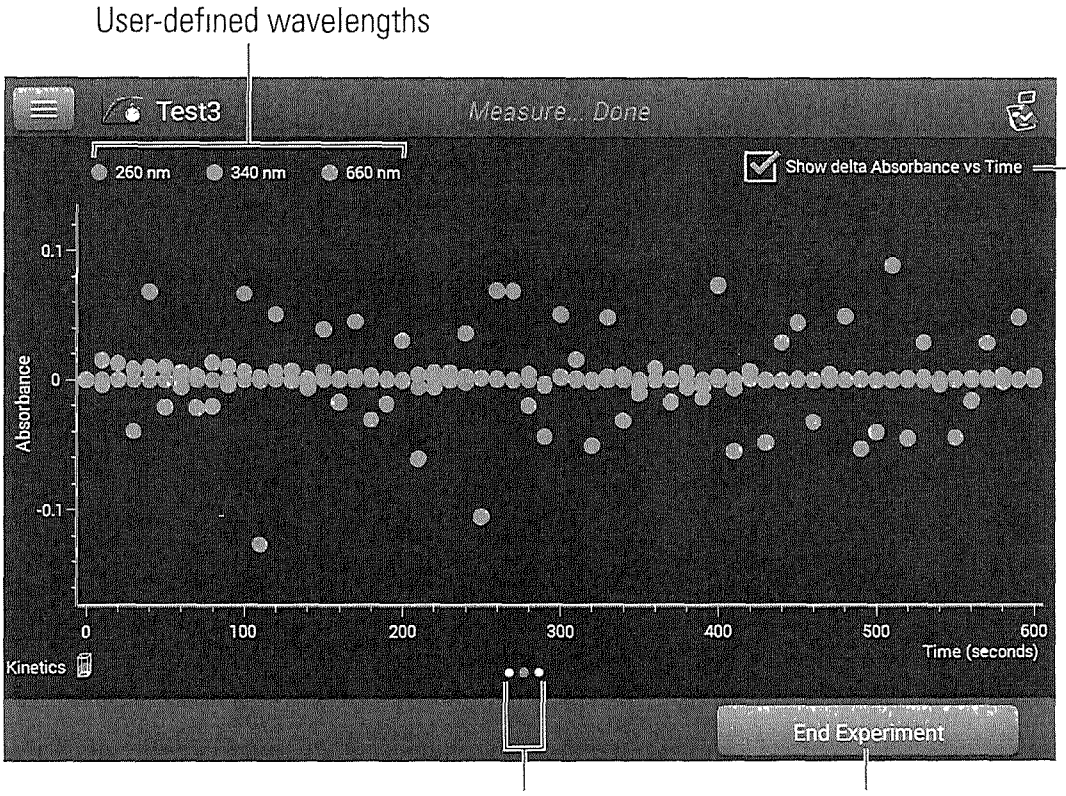
Note For measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent

Rate measurement screen

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

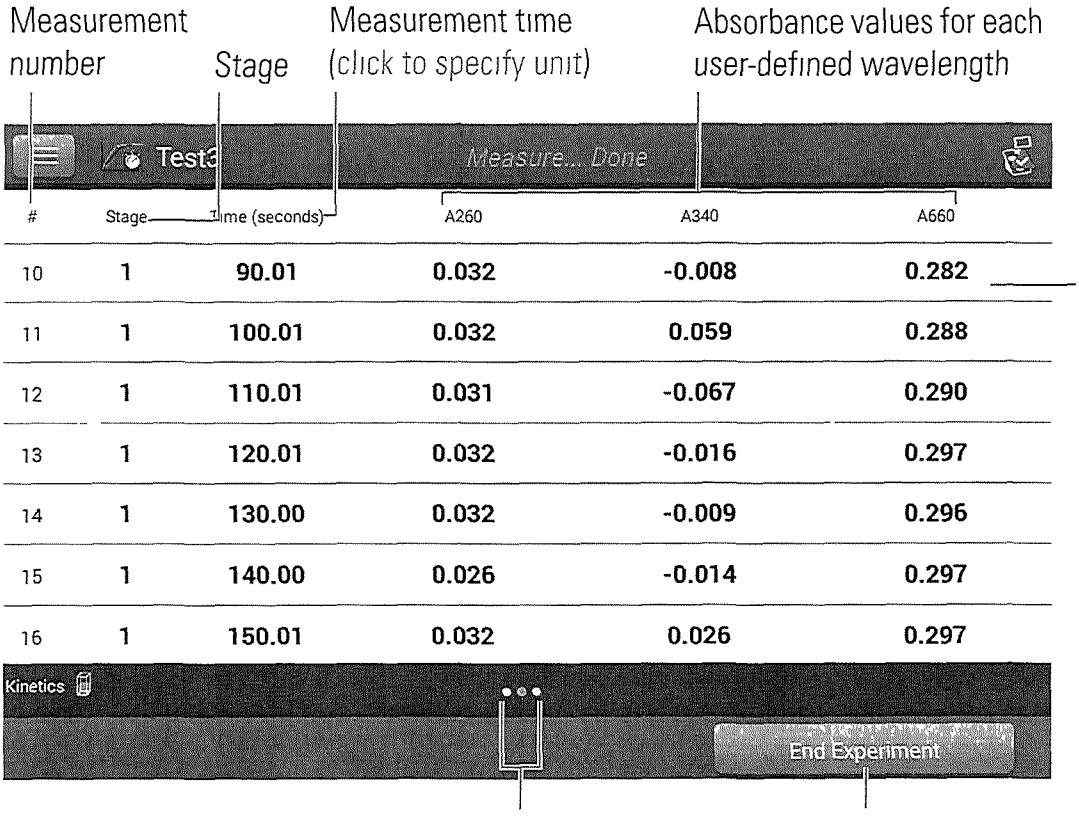


Tap **Show Delta Absorbance Vs Time** to show the change in measured absorbance over time, where each data point is the difference in absorbance from the previous measurement



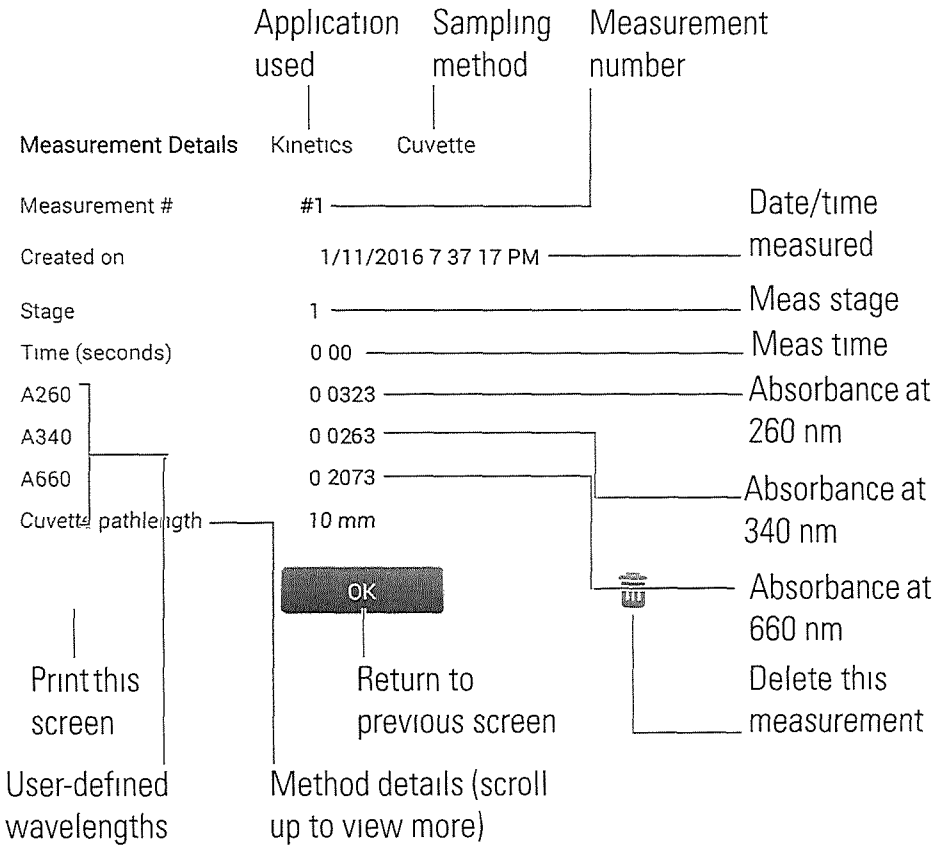
Data Table

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



Measurement Details

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



Related Topics

- Basic Instrument Operations

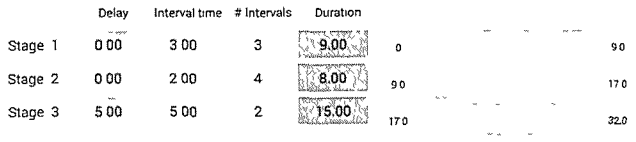
Settings for Kinetic Measurements

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

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

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

Tab	Setting	Description
Name and Range	Method name	**Enter a name** for this method (this name appears in the Kinetics Setup box after the method has been saved)
	Description	Enter a detailed **description** for this method, if desired, such as the type of samples, added reagents, etc
	Measurement range	**Select the spectral range** in which this method will acquire data Available options. • Ultra-violet only (190 nm - 350 nm) • Visible only (350 nm - 850 nm) • Ultra-violet and visible (190 nm - 850 nm) • Custom (specify starting and ending point in nanometers) **Note** For measurements taken with nonstandard (other than 10 mm) cuvettes, the spectra are normalized to a 10 mm pathlength equivalent
	Monitored wavelengths	**Enter up to 3 wavelengths** to be measured and reported at run time **Note** All specified wavelengths must fall within the selected measurement range.
Stages and Intervals	Number of Stages	**Specify up to 5 stages** for kinetic measurements Each stage can have unique Delay, Interval Time and # Intervals settings **Note** Many kinetic measurements include only one stage. Additional stages are necessary only when a variation in stage interval or duration is needed

Tab	Setting	Description
	Time Unit	Select the **unit** for time-based measurements (seconds or minutes)
	Stage 1, 2, etc	Specify the available settings for each stage • **Delay** Specify a delay before a stage starts • **Interval Time** Specify the length of time between measurements taken during this stage (minimum is 2 seconds) The first measurement occurs when the stage starts (or after the delay is completed if a Delay is specified) **Note** If two or more stages are specified with Delay set to zero, two measurements occur at the same time (the measurement at the beginning of the new stage directly overlaps the one at the end of the previous stage) • **# Intervals** Specify the number of absorbance measurements to take in this stage **Note** Since the first measurement is taken when the stage starts, the number of measurements reported for each stage will be the **# Intervals** setting plus 1 • **Duration** Readout shows the total time required for this stage, including any delay and all specified intervals
		The colored **rows** at the right (see image below) show the start and end times for each stage, the colored **boxes** at the right correspond with the specified delay and number of intervals for each stage  If no delay is specified, absorbance measurements are taken at the start and end of each stage and after each specified interval If a delay is specified, as in stage 3 above, the first measurement occurs at the start of the first interval If the unit is seconds in the example above, a total of 11 measurements are taken at the following times over a period of 32 seconds·

Tab	Setting	Description
		• Stage 1 0, 3, 6 and 9 seconds • Stage 2 9, 11, 13, 15 and 17 seconds • Stage 3 22, 27 and 32 seconds **Note** Kinetic experiments are limited to 1000 measurements This means the total number of measurements from all intervals in all stages must be less than 1000 Consider available instrument or computer disc space for lengthy experiments

Related Topics

- Instrument Settings