



## **NanoDrop 2000/2000c Spectrophotometer**

V1.0 User Manual

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## 1. Introduction

### Instrument Description

Thermo Scientific NanoDrop™ 2000/2000c Spectrophotometers measure 0.5- 2  $\mu$ L samples with high accuracy and reproducibility. The NanoDrop 2000c model offers the convenience of both the NanoDrop patented sample retention technology and a traditional cuvette for sample measurements.

The sample retention system employs surface tension to hold the sample in place between two optical fibers. This enables the measurement of very highly concentrated samples without the need for dilutions. Using this technology, the full spectrum (190 – 840 nm) NanoDrop 2000/2000c Spectrophotometers have the capability to measure sample concentration up to 200 times more concentrated than samples measured using the standard cuvette.

### Instrument Specifications

#### NanoDrop 2000/2000c – pedestal mode

Instrument Type:	Spectrophotometer
Minimum Sample Size:	0.5 $\mu$ L
Pathlength:	1 mm (auto-ranging to 0.05 mm)
Light Source:	Xenon flash lamp
Detector Type:	2048-element linear silicon CCD array
Wavelength Range:	190-840 nm
Wavelength Accuracy:	$\pm 1$ nm
Spectral Resolution:	$\leq 1.8$ nm (FWHM @Hg 253.7 nm)
Absorbance Precision:	0.002 absorbance (1 mm path)
Absorbance Accuracy:	$\pm 2\%$ (at 0.76 absorbance at 257 nm)
Absorbance Range:	0.02 -300 (10 mm equivalent)
Detection limit:	2 ng/ $\mu$ L dsDNA
Maximum Concentration:	15,000 ng/ $\mu$ L (dsDNA)
Measurement Time:	< 5 seconds
Footprint:	14 cm x 20 cm
Weight:	2.0 kg
Sample pedestal Material of Construction:	303 stainless steel and quartz fiber
Operating Voltage:	12 VDC
Operating Power Consumption:	12-18 W, (max 30 W)
Software Compatibility:	Windows® XP and Vista (32 bit)

#### NanoDrop 2000c – cuvette mode

Beam height:	8.5 mm
Heating:	$37 \pm 0.5^\circ\text{C}$
Stirrer:	150-850 RPM
Pathlength:	10, 5, 2, 1 mm
Detection Limit:	0.4 ng/ $\mu$ L dsDNA
Maximum Concentration:	750 ng/ $\mu$ L (dsDNA)
Measurement Time:	< 3 seconds
Weight:	2.1 kg

All NanoDrop instruments are approved to CE and UL/CSA standards.

### Patents

The sample retention technology used in NanoDrop 2000/2000c Spectrophotometers is covered under US patents 6,628,382 and 6,809,826. Other patents are pending.

## Sample Retention Pedestal Measurements

A 1 - 2  $\mu\text{L}$  sample is pipetted onto a measurement pedestal. A smaller, 0.5  $\mu\text{L}$  volume sample, may be used for concentrated nucleic acid and protein A280 samples. A fiber optic cable (the receiving fiber) is embedded within this pedestal. A second fiber optic cable (the source fiber) is then brought into contact with the liquid sample causing the liquid to bridge the gap between the ends of the two fibers. A pulsed xenon flash lamp provides the light source and a spectrometer utilizing a linear CCD array analyzes the light passing through the sample. The instrument is controlled by PC based software, and the data is stored in workbook files (\*.twbk) on the PC.

### Pedestal Sample Size Requirements

Although sample size is not critical, it is essential that a liquid column is formed when using the pedestal option so that the pathlength between the upper and lower measurement pedestals is bridged with sample.

The dominant factor determining the surface tension of a droplet is the hydrogen bonding of the lattice of water molecules in solution. Generally, all additives (including protein, DNA, RNA, buffer salts and detergent-like molecules) can reduce the surface tension by interfering with the hydrogen bonding between water molecules. Although 1  $\mu\text{L}$  volumes are usually sufficient for most sample measurements, increasing the sample size to 2  $\mu\text{L}$  will ensure proper column formation for samples with reduced surface tension.

Field experience indicates that the following volumes are sufficient to ensure reproducibility:

Aqueous solutions of nucleic acids: 1  $\mu\text{L}$

Purified protein: 2  $\mu\text{L}$

Bradford, BCA, Lowry or Protein Pierce 660 nm assays: 2  $\mu\text{L}$

Microbial cell suspensions: 2  $\mu\text{L}$

It is best to use a precision pipettor (0-2  $\mu\text{L}$ ) with precision tips to ensure that sufficient sample (1-2  $\mu\text{L}$ ) is delivered. Lower precision pipettors (0-10  $\mu\text{L}$  and larger) are not as good at delivering 1  $\mu\text{L}$  volumes to the measurement pedestal. If the user is unsure about the sample characteristics or pipettor accuracy, a 2  $\mu\text{L}$  sample volume is recommended.

### Pedestal Basic Use

1. Raise the sampling arm and pipette the sample onto the lower measurement pedestal.

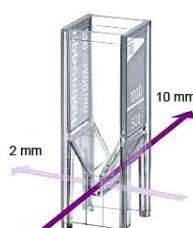


2. Lower the sampling arm and initiate a spectral measurement using the software on the PC. The sample column is automatically drawn between the upper and lower pedestals and the measurement is made.
3. When the measurement is complete, raise the sampling arm and wipe the sample from both the upper and lower pedestals using a dry, lint-free laboratory wipe. Simple wiping prevents sample carryover in subsequent measurements for samples varying by more than 1000 fold in concentration.



## Cuvette Measurements

The NanoDrop 2000c will accept 10 mm cuvettes up to 48 mm tall. When measuring samples using micro, semi-micro, or ultra-micro cuvettes, we recommend using masked cuvettes. Masked cuvettes ensure that all light hitting the detector has passed through the sample. Unmasked cuvettes can allow light to hit the detector that has not passed through the sample. Expected differences between unmasked plastic cuvettes can lead to significant measurement error, especially at low concentrations.



Un-masked Eppendorf Uvlette®



Starna masked micro cuvette

When measuring samples with analytical wavelengths in the UV (<340 nm), use cuvettes made of quartz, as these pass UV wavelengths. Although some manufacturers offer "UV-transparent" plastic disposable cuvettes, even the best of these are not transparent at wavelengths below 220 nm; most plastic and glass cuvettes block UV wavelengths entirely.

While matched cuvettes have been recommended for single-beam instruments, many cuvette manufacturers have sufficient controls over their manufacturing process to deliver excellent cuvette-to-cuvette performance without requiring matching or certification. These cuvettes should be sufficient for measurements with the NanoDrop 2000c.

## Cuvette Sample Size Requirements

It is essential to ensure that the sample volume in the cuvette is adequate to allow the light to pass through a representative portion of the sample when making a measurement. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

## Cuvette Basic Use

1. Add the sample to the cuvette and ensure that the volume is sufficient to cover the light path.
2. Raise the sampling arm and insert the cuvette into the instrument. Insert the cuvette noting the direction of the light path indicated by the etched arrow.



3. The sampling arm must be lowered into the down position for cuvette measurements.
4. Initiate a spectral measurement using the software on the PC.
5. When the measurement is complete, remove the cuvette, rinse thoroughly and dry between samples.

## Blanking and Absorbance Calculations

When a NanoDrop 2000/2000c spectrophotometer is blanked, a spectrum is taken of the reference solution (blank) and stored in memory as an array of light intensities by wavelength. When a measurement of a sample is taken, the intensity of light that was transmitted through the sample is recorded. The sample intensities along with the blank intensities are used to calculate the sample absorbance according to the following equation:

$$Absorbance = -\log \left[ \frac{Intensity_{sample}}{Intensity_{blank}} \right]$$

Thus, the measured light intensity of both the sample and of the blank are required to calculate the absorbance at a given wavelength.

The Beer-Lambert equation is used to correlate the calculated absorbance with concentration:

$$A = \epsilon * b * c$$

**A** = the absorbance represented in absorbance units (A)

**ε** = the wavelength-dependent molar absorptivity coefficient (or extinction coefficient) with units of liter/mol-cm

**b** = the pathlength in cm

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

The reference, or blank solution, generally is the buffer that the molecule of interest is suspended or dissolved in. This solution should be the same pH and of a similar ionic strength as the sample solution.

## Blanking Cycle for Pedestal Option

It is recommended that an aliquot of the blanking buffer be measured as if it were a sample. This confirms that the instrument is working well and that dried-down sample on the pedestals is not a concern. To run a blanking cycle, perform the following:

1. Open the desired application. Load an aliquot of the blank onto the lower measurement pedestal and lower the sampling arm.
2. Click **Blank** to measure and store the reference spectrum.
3. Analyze a fresh replicate of the blank as though it were a sample by choosing **Measure**. The result should be a spectrum that varies no more than 0.04 A (10 mm absorbance equivalent).
4. Wipe the blank from both measurement pedestal surfaces with a dry laboratory wipe, and repeat the process until the spectrum is within 0.04 A (10 mm path).

Although it is not necessary to blank between each sample, it is recommended that a new blank be taken every 30 minutes when measuring many samples. After 30 minutes, the time since the last blank measurement will be displayed in the bottom status bar.

A Quick Start Guide that includes a blanking procedure can be accessed from the "Appendices" chapter of the *NanoDrop 2000 User Manual PDF*. It is recommended that this guide be printed and posted near the instrument as a reference.

## Fluorescent Dyes

The software uses the general form of the Beer-Lambert equation to calculate fluorescent dye concentrations in the Micro Array and Proteins & Labels applications. The user can choose to enter new dye types using the Dye/Chrom. Editor or use one of the predefined dyes. A table of extinction coefficients for the predefined dyes is shown below:

Dye list

	Show	Dye	Unit	Coeff. (l/mole-cm)	Analysis Wavelength (nm)	260nm Correction	280nm Correction
		None		0	0	0	0
		Cy3	µM	1.5E+5	550	0.04	0.05
		Cy5	µM	2.5E+5	650	0.00	0.05
		Alexa Fluor 488	µM	7.1E+4	495	0.30	0.11
		Alexa Fluor 546	µM	1.04E+5	556	0.21	0.12
		Alexa Fluor 555	µM	1.5E+5	555	0.04	0.08
		Alexa Fluor 594	µM	7.3E+4	590	0.43	0.56
		Alexa Fluor 647	µM	2.39E+5	650	0.00	0.03
		Alexa Fluor 660	µM	1.32E+5	663	0.00	0.10
		Cy3.5	µM	1.5E+5	581	0.08	0.24
▶		Cy5.5	µM	2.5E+5	675	0.05	0.18
*							

## 2. Software

### Computer Requirements

- Microsoft Windows XP or Vista (32 bit) operating system
- 1.5GHz or higher processor
- CD ROM drive
- 1GB or more of RAM (2GB if running Vista)
- 40 MB of free hard disk space
- Available USB port (the instrument uses a USB cable to connect to the computer)

### Software Installation

The system software must be loaded onto the PC before the USB cable is connected. Administrator access on the PC is required to install the software. To properly install the operating software, follow the steps below:

1. Close all programs and make sure the USB cable is unplugged.
2. Insert the software CD in the CD drive. The software installation menu should appear automatically. If the installation does not begin, click **Start** and select **Run**. In the Run dialog box, type **x:\Set-up**, where "x" designates your computer's CD-Rom drive. Click **OK**.
3. Follow the on-screen prompts to complete the software installation, then connect the USB cable. If the Found New Hardware Wizard appears, the Windows XP SP2 operating system will ask to allow it to search the internet for the proper software as shown. Select - **No, not this time**. Follow the prompts for automatic installation of the software.



Intro Page: Windows XP- SP2



Vista Operating system

The NanoDrop 2000/2000c Spectrophotometer should now be ready for operation. If the software does not start properly, refer to ["Diagnostics and Troubleshooting"](#) for possible solutions.

Periodic upgrades are made to the software and are available for download. See our [website](#) for the latest software.

### Thermo Software IQ Overview

The Thermo Software IQ program performs Installation Qualification (IQ) for the NanoDrop 2000/2000c software. IQ verifies that the correct software files were installed and can also be used to verify that these files have not been changed, deleted or overwritten since they were installed.

To start the Thermo Software IQ program:

1. Open the Start menu by choosing **Start** on the desktop.
2. Choose **All Programs>Thermo>Thermo Software IQ**.
3. Follow the prompts to qualify your system software installation.
4. Use the **Help** menu to access the *Thermo IQ User Guide PDF*.

## Cable Connections

To make measurements with the instrument, connect the USB cable to the instrument and the PC, plug in the 12V power supply and connect to the power input at the back of the instrument.

The power supply can remain plugged into the NanoDrop 2000/2000c while the instrument is not in use. When the unit is in this “standby” mode, power consumption is ~5 W and the flash lamp is not energized. The instrument has an LED located on the top of the DC power connector which indicates that the unit is receiving 12 V of power.

## Registering Your Instrument

Please register your instrument! We periodically update our software and add new features free of charge. We keep our user list updated so that we can alert you to these updates. All information supplied is completely confidential. Please register your instrument on our [website](#).

## Software Features

The NanoDrop 2000/2000c software interface is divided into a left pane and a right pane. Task bars and action buttons are located in the left pane while the right pane displays either the main menu or data acquisition screens. A single page [Software Feature Overview](#) can be accessed from “Appendices” in the *NanoDrop 2000 User Manual* PDF.

### Left Pane

#### Task Bars

Task bar options displayed upon software startup include:

- **Home** - displays the main menu with the applications that are available for specific groups. The default group is **Classic**.
- **My Data** - manages data archiving and retrieval. Sample data is stored in workbooks determined by the user. Refer to “[Data and Account Management](#)” for additional details.
- **Diagnostics** - accesses the intensity check and calibration check screens. Refer to “[Diagnostics and Troubleshooting](#)” for additional information.
- **Options** - includes four tabs described in detail under “[Data and Account Management](#)”.

Task bar options available upon the opening of specific applications include:

- **Measure (specified application)** - displays the most recently selected application.
- **Reports** - includes the following three tabs:
  - **Report** - displays a table of accumulated sample data with spectral profile of selected samples.
  - **Configuration** - selects the report columns to display and the order in which the columns will appear.
  - **Print** - selects the information that will be included in the printed report, defines the page set up and how the printed graphs will display within the report.
- **Oligo Calc** (Nucleic Acid and Micro Array applications only) - calculates molecular weight, extinction coefficients, concentration factors and melting points for specific nucleic acid sequences. Refer to “Oligo Calc” within “Nucleic Acid” and “Micro Array” for additional details.

- **Dye/ Chrom. Editor** (Micro Array and Protein & Labels only) - enables the user to enter and edit new dyes in addition to predefined dyes. Refer to "Dye/Chrom. Editor" within "Micro Array" and "Protein & Labels" for additional details.
- **Editor Options** (within Method Editor) – defines the available options for editing methods. Refer to "[Method Editor](#)" for additional information.

## Action Buttons

The following four action icons are displayed at the top of the left pane when an application is open:

- **Measure** - initiates the measurement of a sample. This button is initially grayed-out upon opening an application. It becomes active after a blank has been established.
- **Print Screen** - prints a copy of the spectrum and associated sample data to the default printer.
- **Blank** - initiates the measurement of the buffer or carrier liquid in which the sample is suspended. A blank must be measured and stored before a sample can be measured. Refer to "[Overview](#)" for more details.
- **Re-Blank-** establishes a new reference (blank) that is used for the absorbance calculations of subsequent samples. The Re-Blank feature recalculates the concentration for the most recent sample and displays the revised spectrum on the screen.

The following four action buttons are displayed at the top of the pane only when Method Editor or Kinetics Editor is selected:

- **New** - initiates a new method wizard to sequentially step through the process of creating a custom method.
- **Save** - saves the new method to the list under the currently selected group.
- **Measure** - opens a newly created method.
- **Delete** - deletes a method.

## Additional Left Pane Feature Selections

### Menu Bar Options

- **File** - menu bar drop down list which includes the following selections:
  - **New Workbook** - opens a new workbook. The current workbook will automatically be closed and saved.
  - **Close Workbook and go Home** - closes the current workbook and returns to the home page. All data is automatically saved when a workbook is closed.
  - **Close All Workbooks and go Home** - closes all open workbooks (all applications and methods) and returns to the home page.
  - **Print Report** - prints the current report to default printer.
  - **Use current settings as default** - sets all current application feature selections such as: sample type, units type, baseline correction wavelength and pedestal/cuvette mode selection as defaults, whenever a new workbook for that application is opened. When selected, the current report configuration fields are also set as defaults. This option is a useful tool which can be used to set individual user preferences within each application or method.

- **E-mail current workbook** - automatically attaches the current workbook to a new email message. When sending data to NanoDrop products technical support, please use the **My Data** task bar to locate the appropriate autosave data file. Open the file with the NanoDrop 2000/2000c software and send to [Technical Support](#).

**Note:** Although all Report fields may not be displayed when opening an autosave file, all fields are recoverable by the Technical Support team.

- **Help** - searchable help files may be accessed from any screen using the [Ctrl]-m, [Ctrl]-h or the F1 key. Refer to “Preferences” under [Data and Account Management](#) for more information about shortcut keys. A PDF version of the *NanoDrop 2000 User Manual* is available under the Windows Start menu.

The About option under the Help menu provides information specific to the current software version and instrument.

### Left Pane Instrument Settings

- **Add to Report** - indicates whether sample data should be added to the current report. Although all data is archived to an autosave workbook and can be retrieved at a later date, **Add to Report** should be selected on a routine basis for easy data retrieval. Select Add to report prior to a measurement to save the sample to a report and a workbook.
- **Overlay spectra** - displays multiple spectra. A crosshair cursor is displayed and linked to the most recent sample measured. When Overlay Spectra is selected, click a spectrum to link the crosshairs to the spectrum.
  - The overlaid sample IDs are listed on the top left side of the graph display. The last sample measured appears at the top of the legend and will be labeled red. Clicking on a different spectrum will cause the corresponding sample ID to appear in red.
  - Un-checking **Overlay Spectra** will clear the display of all spectra when a new measurement is made.
- **Small sample volume** – (Nucleic Acid and Protein A280 applications only) –allows measurements of just 0.5  $\mu$ L when samples have 10 mm equivalent absorbance values of 3.0 or higher (e.g. > 150 ng/ $\mu$ L dsDNA).
- **Use Cuvette** (NanoDrop 2000c model only) - enables cuvette measurements. Options available for use with cuvette measurements include:
  - **Pathlength** - includes pathlengths of 10, 5, 2, and 1 mm.
  - **Stir speed** - speed settings range from 1-10. The default setting is Off.
  - **Heat to 37 °C** - heats the cuvette holder to 37  $\pm$ 0.5 °C. When selected, the current temperature of the cuvette will be displayed at the bottom of the software screen. It may take between 1-10 minutes for the cuvette holder to reach 37 °C. The time for the sample to reach 37 °C will depend on the starting temperature of the liquid. The heater is turned off as the default setting.

**Note:** If moving to a different application, the heater will turn off. However, the heater will continue to warm the cuvette block if pedestal measurements are made within the same application.

## Right Pane

The right pane displays the main menu which includes the following:

- Group drop-down box** - selects the preferred main menu display and associated applications. The default selection is the Classic NanoDrop group of applications.
- Predefined application buttons** - and additional main menu selections.
- User-Defined list** - empty until user defined methods or kinetics methods are created. Once created, quantification methods have a scale icon to their left while kinetics methods have a clock icon to their left.

Once an application is opened, the right pane displays the sample spectra as well as drop-downs and data fields relevant to the specific application. A pedestal or a cuvette icon is displayed in the user guidance text box above the graph. The icon shown indicates which mode will be used to make the next measurement.

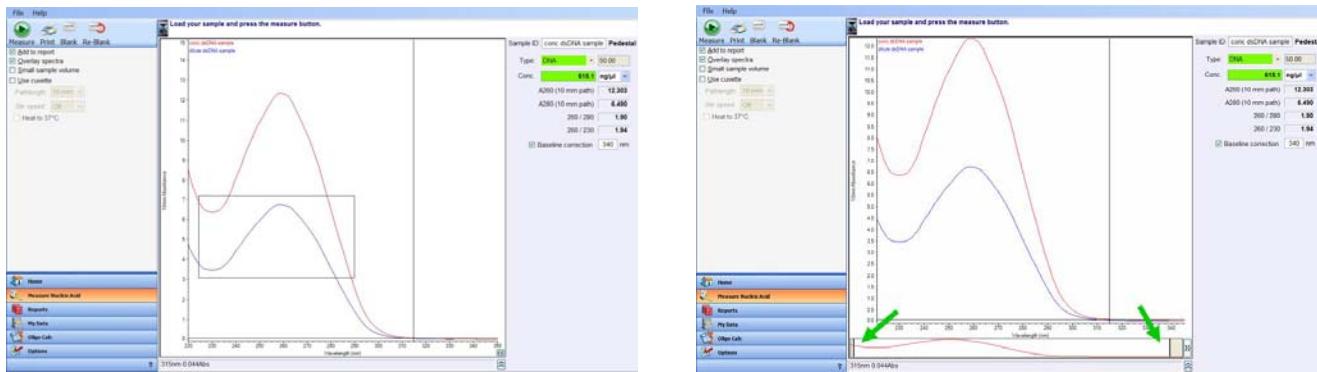
## Graph displays

The graph panel displays the most recent sample spectrum. When Overlay Spectra is selected, the data from multiple samples is displayed. The sample ID is shown at the top left of the display panel. The most recent sample measured will be at the top of the list and the text and associated spectrum are shown in red.

**Note:** Clicking a previously measured sample turns that sample ID text and associated spectrum red but does not highlight that particular sample in the report view at the bottom of the page.

Although there is no practical limit to the number of sample spectra that can be displayed, the software can only display approximately 28 sample IDs in the left hand legend when viewed in a full screen window. Once the display limit is reached, the list becomes a drop down box as indicated by a black arrow icon at the top left of the display. Use the arrow to display the previous sample IDs. Right-click in the graph display to bring up a menu box which includes an option to turn off the sample legend.

A single region can be enlarged by simply drawing a box around the area of interest and then clicking inside the box. Alternatively, a chevron at the bottom of the graph display opens up the view finder which displays a single sample spectrum. The bold lines at the ends of the view finder are movable (click and drag) and define the wavelengths to display on the graph.

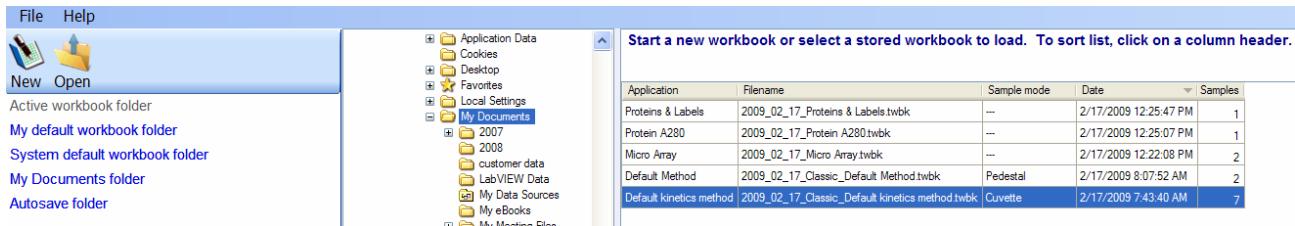


- **Sample labels** (UV-Vis only) - selects whether absorbance values for specific wavelengths are displayed on the graph for the most recent sample measurement.
- **Sample legend** - determines whether or not the samples IDs are displayed on the top left hand side of the graph.

## Data and Account Management

### My Data

Sample measurement data is recorded in workbooks that can be filed in a user-specified location. The left pane My Data task bar is used to access saved workbooks. Use the navigator pane to find the folder where the workbook of interest is stored.



Two icons are available at the top of the left pane once the My Data task bar is selected. Selecting **New** opens a new workbook for the highlighted application in the right pane. Selecting **Open** opens the selected workbook and initiates the associated application. Additional measurements can be appended to the opened workbook.

The five quick links below the New and Open icons are shortcuts to archived workbooks then displayed in the right pane. Double-clicking on a workbook opens the file and allows for additional measurements to be appended.

**Note:** The Add to Report button must be selected prior to measurement for a sample to be added to a workbook.

### Reports

Reports are user configurable tables that display sample data. A report can be accessed by clicking the Reports task bar, which appears once an application or a custom method is opened.

Three tabs appear in the right pane when the Reports task bar is selected:

- **Report** - displays the sample data archived in the opened workbook. A graph displaying the spectrum of the high-lighted sample in the report table is shown at the bottom of the page. If a sample is not highlighted, the spectrum displayed is that of the first sample in the table. Multiple spectra can be displayed by highlighting the sample data in the report table.
- **Configuration** - selects the report columns to display and the order in which the columns should appear. Only the information associated with selected column fields will be included in exported files. Refer to the information on exporting reports below.
- **Print** - adds a title and subtitle to a report. Method information and spectral graphs can be printed along with report tables.

**Note:** A view-only copy of the active report can be accessed by clicking the chevron located under the application or method measurement display graph.

Three icons appear at the top of the left pane when the Reports task bar is selected:

- **Preview** – allows review of the report prior to printing. The preview displays the selected header and footer parameters using the Options task bar or the Print icon. Refer to “Options” in “[Data and Account Management](#)” for additional information.
- **Print** - sends a report to the default system printer.
- **Export** - saves a report as either an .xml file, .tsv or .twbk file. The specific options include:

- **Report, Excel XML Spreadsheet (\*.xml)** - saves a report in a format which can be opened in Excel. Only the columns displayed in the report will be saved using this feature. It is important to configure the report to display the information desired prior to exporting.
- **Report, Tab Separated Values (\*.tsv)** - saves a report in a format which can be opened in Notepad or Excel. Only the columns displayed in the report will be saved using this feature. It is important to configure the report to display the information desired prior to exporting.
- **Spectrum, Excel XML Spreadsheet (\*.xml)** - saves the absorbance values along with the corresponding wavelengths for a highlighted sample. If multiple samples are highlighted, the corresponding absorbance values for each wavelength will be saved onto separate worksheets within one Excel file.
- **Spectrum, Tab Separated Values (\*.tsv)** - saves the absorbance values along with the corresponding wavelengths for highlighted samples in a format which can be opened in Notepad or Excel. Multiple samples are saved sequentially in one column.
- **Spectra, New Workbook (\*.twbk)** - saves the selected sample data into a new workbook that can be reopened with the NanoDrop 2000/2000c software.
- **ND Legacy (\*.tsv)** - saves the absorbance values for corresponding wavelengths along with the specific fields configured in the report. This option will display multiple sample data within one worksheet when opened with Notepad or Excel. It is important to configure the report to display the information desired prior to exporting.

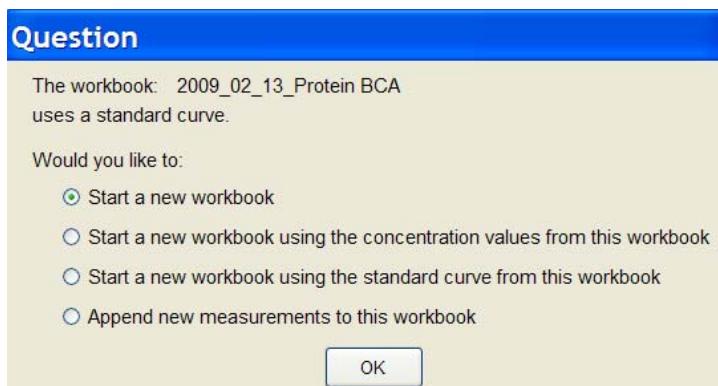
**Note:** The Spectra, New Workbook (\*.twbk) and ND Legacy (\*.tsv) options are not available for kinetics measurements.

If the PC is not mapped to recognize that .xml files should be opened with Excel, it will be necessary to open the exported file from within the Excel application.

### Appending data to previous workbooks

If the software is closed while a workbook is open, a message asking if the user wants to append data to that workbook will be displayed the next time that application is opened. Append data to a workbook that was closed prior to the software being closed by opening the workbook through the My Data task bar.

Colorimetric applications offer several options regarding the use of standard curves when appending sample data to previous workbooks. An example of the message displayed when opening a colorimetric workbook is shown below:



It is recommended that the user follow the protein assay manufacturer's guidelines for generating standard curves before appending new data to a workbook.

## Reprocess

The Reprocess feature is available in the top left pane of the reports page for some applications. This feature recalculates a sample concentration based upon the selection of alternative baseline correction wavelengths, concentration units and/or sample types. This feature is not used to rename samples. A Reprocess field is available as a report configuration column option.

**Note:** Reprocessed sample data will appear in the current report as a new data entry. Reprocessed data is not archived in an autosave workbook.

## Renaming a sample

A sample ID can be modified at any time by highlighting the name in the report and overwriting the sample ID.

**Note:** Sample name changes are not reflected in autosave files.

## Autosave files

All data except kinetics measurements are automatically archived as Autosave files (\*.twbk) to the following locations depending on the PC operating system:

Vista: C:\Users\Public\Public Documents\Thermo\Autosave\ (Software Application)

XP: C:\Documents and Settings\All Users\Shared Documents\Thermo\NanoDrop2000\Autosave\ (Software Application)

Autosave files contain data for all samples measured within a 24 hour period and are separated by application and method. Autosave files are not workbooks that can be modified by the user. Due to the fact that data can be appended to user-defined workbooks, the data in user-defined workbooks may not always directly correspond to the data in autosave files.

If there is a need to retrieve data not saved in a user-defined workbook, open the associated autosave file with the NanoDrop 2000/2000c software, highlight the samples of interest and export using the Spectra, New Workbook (\*.twbk) option. Use the My Data taskbar to navigate to the new workbook.

If an autosave workbook has been opened, it must first be closed within the software before new measurements for that application type can be made.

## Options

There are four tabs within the **Options** Task bar:

- **Applications** - adds new groups and defines which applications and methods are associated with each group. To add a new group, enter the desired group name and click **Add**. Drag one or more of the applications to any of the empty buttons to customize the appearance of a group.

There are two ways to display custom methods under a group other than Classic:

- Drag the custom method to a specific menu button location.
- Include List of Methods as a menu button option. The actual methods will not be shown on the Options screen but will be displayed on the home page under the appropriate group. Due to the size of the box, List of Methods can only be dragged to one of the top 9 menu buttons.

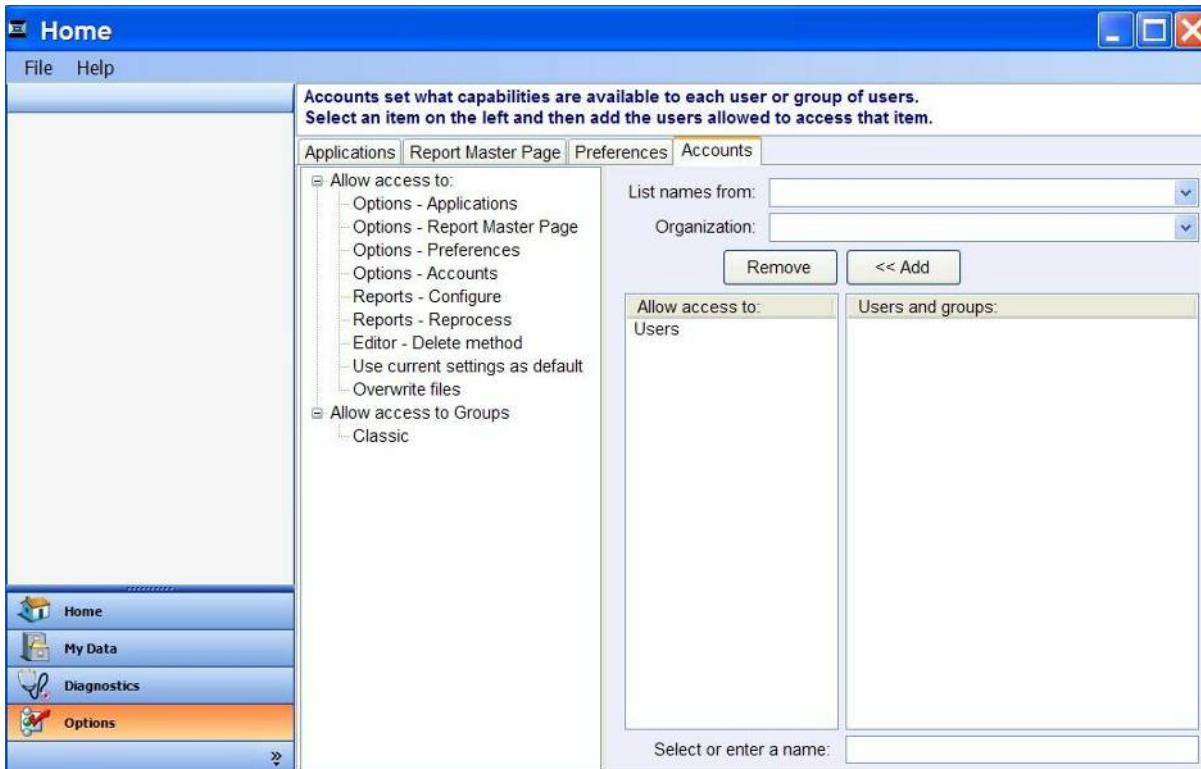
Use the **Delete group** button to remove an entire group. Use **Clear App Buttons** at the bottom of the page to reset application button(s) without deleting the group.

- **Report Master Page** - defines the layout and appearance of headers and footers for printed pages. Using this tab, parameters such as whether or not to include an organization name, a logo, the date and/or the time in a header are selected. Footer options include text additions and page numbers. Selections will be applied to printed reports for all applications and user-defined methods. The Page Setup icon brings up the window used for determining paper size and printer margins.
- **Preferences** - determines whether default settings for applications and reports are utilized for each user or are shared by all users. Selecting **Support Dymo Label printer** utilizes the stand alone device to print out reports. Keyboard Shortcut preferences are also selected under this tab. Refer to the table below for a list of keys associated with the two options available. The default is the NanoDrop Classic shortcut keys.

Command	NanoDrop Classic	Standard
<b>Exit</b>	[Ctrl]-q	[Alt]-F4
<b>Help</b>	[Ctrl]-m, [Ctrl]-h	F1
<b>Blank</b>	F3	F3, [Ctrl]-b
<b>Measure</b>	F1	F5, [Ctrl]-m
<b>Re-Blank</b>	F2	F2, [Ctrl]-r
<b>Print Screen</b>	F4	F4, [Ctrl]-p
<b>Print Report</b>	F5, [Ctrl]-r	[Shift]-F4
<b>Show Report</b>	F7	F7
<b>New Workbook</b>	[Ctrl]-n	[Ctrl]-n
<b>Reset (Calibration check)</b>	-	F11
<b>Home</b>	-	[Ctrl]-h
<b>Close Workbook</b>	[Ctrl]-e	[Ctrl]-e
<b>Close All Workbooks</b>	-	[Ctrl]-q
<b>Configure Report</b>	[Ctrl]-f	[Ctrl]-f
<b>Layout Report</b>	[Ctrl]-l	[Ctrl]-l

The Re-Blank icon has been replaced with a Stop icon for kinetic methods.

- **Accounts** - manages which users or group of users have permission to change or configure reports, define default settings and modify standard curves. In addition, the ability to overwrite files or change directories in which files are saved is controlled from the Accounts tab.



The left side of the Accounts page displays a list of access control categories. Each of these categories includes software features to which specified users, or groups of users, can be granted access. The two categories of access include:

- **Allow access to:**
  - **Options** - enables users or groups to have access to: Applications, Report Master Page, Preferences, and Accounts.
  - **Reports** - enables users or groups to have access to the Configuration tab and Reprocess button on the Reports screen.
  - **Editor** - allows users or groups to delete user defined methods and saved kinetic methods.
  - **Use Current Settings as default** - enables users or groups to make an application's current settings the default.
  - **Overwrite files** - enables a user to save a new workbook under the name of an existing workbook.
- **Allow access to Groups:**
  - Enables users to access, add, delete, and configure Groups.

To allow specific access as described above:

1. Highlight the feature of interest in the left most box. Access must be granted individually for each sub category.
2. Use the **List names from** and **Organization** drop downs to select specific users and groups.
3. Click **Add** to move the user or group to the middle box. Only names in the middle box will have access to the software feature highlighted in the left most box. By default, the users group is added to the Allow access to list for every application.

Drop downs and list boxes:

- **List names from** - displays a list of available domains from which to choose.
- **Organization** - displays a list of organizations within selected domain. (This list is not available if a local domain is selected.)
- **Allow access to** - the list of users and groups selected to be granted access to specified software features as described above.
  - To add a user or group that doesn't appear in the users and groups list, type a valid user or group name into the **Select or enter a name** field at the bottom of the page and click the **<< Add** button.
  - The name must include the domain name. If the name is valid, it appears in the Allow access to list. If the name is invalid, a warning message will appear that states that the name is not a valid user account or group.
- **Users and Groups** – the list of users available within a selected domain.

### 3. Applications

#### Overview

UV-VIS spectrophotometry is easy for micro-volume samples using NanoDrop 2000/2000c spectrophotometers. The NanoDrop 2000/2000c is ideally suited for measuring:

- Nucleic acid concentration and purity of nucleic acid samples  $\leq 15,000 \text{ ng}/\mu\text{L}$  (dsDNA) without dilution
- General UV-Vis spectrophotometry
- Purified protein analysis (A280)
- Fluorescent dye corporation for Micro array and Proteins & Labels applications
- Expanded spectrum measurement and quantitation of fluorescent dye labeled proteins, conjugates, and metalloproteins
- BCA Assay analysis of protein
- Bradford Assay analysis of protein
- Lowry Assay analysis of protein
- Pierce 660 nm Assay analysis of protein
- Microbial cell culture measurements
- Kinetic methods
- Custom methods

#### Quick Start

1. Double-click the software icon and select the software application of interest from the right pane.
    - Select **Add to report** prior to a measurement to save the sample data to a workbook.
  2. Establish a blank using the appropriate buffer.
    - Pedestal Option: Pipette 1-2  $\mu\text{L}$  of the appropriate blanking solution onto the bottom pedestal, lower the arm and click **Blank**.
    - Cuvette Option (Model 2000c only): Select **Use Cuvette**. Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

**Note:** The arm must be down for all measurements, including those made with cuvettes. It is recommended that cuvettes be removed from the instrument prior to making a pedestal measurement to ensure that the pedestal arm can move to the proper starting position.
  3. Wipe away the blank from the measurement pedestals using a dry, lint free laboratory wipe. Enter the sample ID in the appropriate field. Pipette 1  $\mu\text{L}$  of sample and click **Measure**.
- Note:** A fresh aliquot of sample should be used for each measurement.

#### After the measurement:

- Simply wipe the upper and lower pedestals using a dry lint free-laboratory wipe and the unit is ready for the next sample.
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples.

Although it is not necessary to blank between each sample, it is recommended that a new blank be taken every 30 minutes when measuring many samples in one measurement session. After 30 minutes, the time since the last blank measurement will be displayed in the bottom status bar.

A Quick Start Guide that includes a blanking procedure can be accessed from “Appendices” of the *NanoDrop 2000 User Manual PDF*. It is recommended that this guide be printed and posted near the instrument as a reference.

## Measurement Ranges

Sample Type	Lower Detection Limit	Approximate Upper Detection Limit	Typical Reproducibility (5 replicates, SD = ng/uL; CV = %)
Nucleic Acids	2 ng/µL (pedestal) 0.4 ng/µL (cuvette)	≤ 15,000 ng/ µL (dsDNA)	2 - 100 ng/uL: ± 2 ng/uL >100 ng/uL: ± 2%
Microarray	2 ng/ µL (pedestal) 0.4 ng/ µL (cuvette)	750 ng/ µL (dsDNA)	2 - 100 ng/uL: ± 2 ng/uL >100 ng/uL: ± 2%
Protein A280	0.10 mg/mL (purified BSA - pedestal) 0.010 mg/mL (purified BSA- cuvette )	400 mg/mL (purified BSA)	0.10 - 10 mg/mL: ± 0.10mg/mL >10 mg/mL: ± 2%
Proteins & Labels	0.10 mg/mL (purified BSA - pedestal) 0.010 mg/mL (purified BSA- cuvette )	20 mg/mL (purified BSA)	0.10 - 10mg/mL: ± 0.10mg/mL
BCA	0.2 mg/mL (20:1 reagent.sample volume) .01 mg/mL (1:1 reagent.sample volume)	8.0 mg/mL 0.20 mg/mL	2% over entire range 0.01 mg/mL over entire range
Modified Lowry	0.2 mg/mL	4.0 mg/mL	2% over entire range
Bradford	100 ug/mL (50:1 reagent.sample volume)  15 ug/mL (1:1 reagent.sample volume)	8000 ug/mL  100 ug/uL	100-500 ug/mL: ± 25ug/mL 500-8000 ug/mL: ± 5%  15 - 50 ug/mL: ± 4 ug/mL 50 - 125 ug/mL: ± 5%
Pierce 660 nm	50 ug/ml (15:1 reagent.sample volume)  25 ug/ml (7.5:1 reagent.sample volume)	50-125 ug/ml > 125 ug/ml  25-125 ug/ml > 125 ug/ml	50-125 ug/mL: ± 3 ug/ml >125 ug/mL: 2%  25-125 ug/mL: ± 3 ug/ml >125 ug/mL: 2%

Cy3, Cy3.5, Alexa Fluor 555 and Alexa Fluor 660	0.2	100	sample range 0.20-4.0 pmol/ul: ± 0.20 pmol/ul sample range >4.0 pmol/ul: ± 2%
Cy5, Cy5.5 and Alexa Fluor 647	0.12	60	sample range 0.12-2.4 pmol/ul: ± 0.12 pmol/ul sample range >2.4 pmol/ul: ± 2%
Alexa Fluor 488 and Alexa Fluor 594	0.4	215	sample range 0.40-8.0 pmol/ul: ± 0.40 pmol/ul sample range >8.0 pmol/ul: ± 2%
Alexa Fluor 546	0.3	145	sample range 0.30-6.0 pmol/ul: ± 0.30 pmol/ul sample range >6.0 pmol/ul: ± 2%

**Note:** Measurement ranges noted for dyes in the above are specific to pedestal measurements.

## Nucleic Acid

### Overview

Nucleic acid samples can be easily checked for concentration and quality using the NanoDrop 2000/2000c spectrophotometer. To measure nucleic acid samples select the Nucleic Acid application from the home page.

### Nucleic Acid Calculations

For nucleic acid quantification, the Beer-Lambert equation is modified to use a factor with units of ng-cm/microliter. The modified equation used for nucleic acid calculations is the following:

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

**c** = the nucleic acid concentration in ng/microliter

**A** = the absorbance in AU

**ε** = the wavelength-dependent extinction coefficient in ng-cm/microliter

**b**= the pathlength in cm

The generally accepted extinction coefficients for nucleic acids are:

- Double-stranded DNA: 50 ng-cm/ $\mu$ L
- Single-stranded DNA: 33 ng-cm/ $\mu$ L
- RNA: 40 ng-cm/ $\mu$ L

When the pedestal mode is selected, the NanoDrop 2000/2000c spectrophotometer uses short pathlengths between 1.0 mm to 0.05 mm to enable measurement of concentrated samples without dilution.

**Note:** Absorbance data shown in reports is archived as displayed on the software screen. The Nucleic Acid application absorbance values are normalized to a 1.0 cm (10.0 mm) path for all pedestal and cuvette measurements.

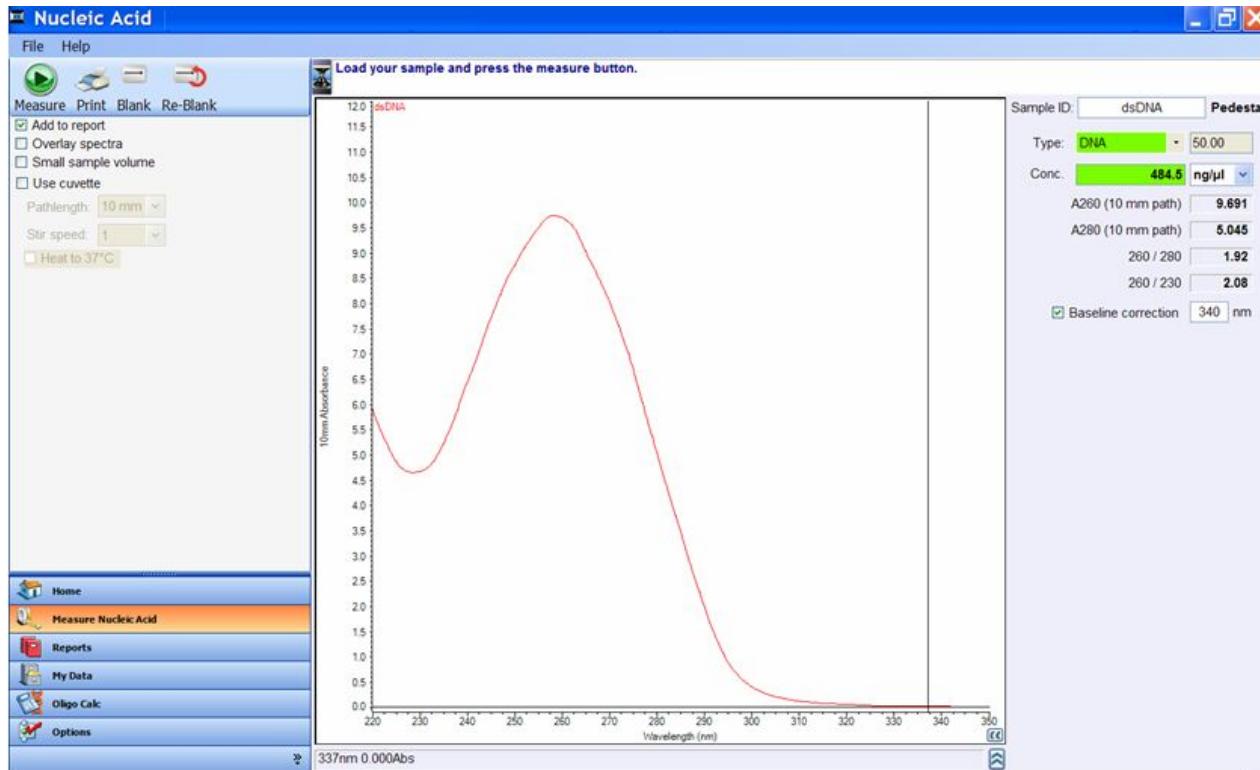
### Measurement Concentration Ranges

The NanoDrop 2000/2000c will accurately measure purified dsDNA samples  $\leq$ 15,000 ng/ $\mu$ L without dilution. The software automatically utilizes the optimal pathlength to measure the absorbance of each sample. Refer to "[Measurement Ranges](#)" for additional information.

The small sample volume option is available when samples have 10 mm equivalent absorbance values of 3.0 or higher ( $>150$  ng/ $\mu$ L dsDNA.)

## Unique Screen Features

The right pane displays features specific to the Nucleic Acid application. Task bars in the left pane not described below are described in “[Software Overview](#).”



The spectral display shows data for the current sample normalized to a 10 mm path for all measurements including measurements made with any cuvette pathlength.

The following features are to the right of the spectral display:

- **Sample ID** - field into which a sample ID is entered. The appropriate sample ID should be entered prior to each measurement.
- **Type** - a drop down list from which the user may select the (color-keyed) type of nucleic acid being measured. Options include DNA-50 for dsDNA, RNA-40 for RNA, and ssDNA-33 for single-stranded DNA. Additional options include Oligo DNA and Oligo RNA which utilize the appropriate extinction coefficient based upon user-defined base sequences. The Custom option allows the user to enter an extinction coefficient between 15 and 150.
- **Conc** - concentration based on absorbance at 260 nm and the default or user defined extinction coefficient. Concentration units may be selected from the adjacent drop-down box. Refer to “[Nucleic Acid Calculations](#)” for more details.
- **A260** - displays absorbance at 260 nm normalized to a 10 mm pathlength.
- **A280** - displays absorbance at 280 nm normalized to a 10 mm pathlength.
- **260/280** - ratio of absorbance at 260 nm and 280 nm. The ratio of absorbance at 260 and 280 nm is used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as “pure” for DNA; a ratio of ~2.0 is generally accepted as “pure” for RNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm. See “260/280 Ratio” in “[Diagnostics and Troubleshooting](#)” for more details on factors that can affect this ratio.

- **260/230** - ratio of absorbance at 260 nm and 230 nm. This is a secondary measure of nucleic acid purity. The 260/230 values for a “pure” nucleic acid are often higher than the respective 260/280 values and are commonly in the range of 1.8-2.2. If the ratio is appreciably lower, this may indicate the presence of co-purified contaminants.
- **Baseline correction** - if selected, the default wavelength for the bichromatic normalization is 340 nm. The user can manually enter a different wavelength for the bichromatic normalization of the absorbance data. In either case, the baseline is automatically set to the absorbance value of the sample at the selected wavelength. All wavelength data will be referenced off this value.

**Note:** If a baseline correction is not selected, the spectra may be offset from the baseline and the calculated concentration will change accordingly.

## Making Nucleic Acid Measurements

1. Select the **Nucleic Acid** application from the main menu. If the wavelength verification window appears, ensure the arm is down and click **OK**.
2. Select the type of sample to be measured from the Type drop-down list. The default setting is DNA-50.
3. Choose the concentration units from the drop-down list adjacent to the color coded concentration box. The default units are ng/ $\mu$ L.
4. A default wavelength of 340 nm is automatically used for a bichromatic normalization. Select an alternative reference wavelength or choose not to have the spectrum normalized by de-selecting the **baseline correction** box.
  - Select the file drop-down option **Use current settings as default** as a convenient way to limit set-up time for each new workbook.
5. Select **Add to report** to automatically include all measurements in the current report. The default setting is for all samples to be added to reports. The **Add to report** checkbox must be selected prior to a measurement to save the sample data to a workbook.
6. Select **Overlay spectra** to display multiple spectra at a time.
7. Establish a blank using the appropriate buffer. The blank solution generally is the buffer that the molecule of interest is suspended or dissolved in. This solution should be the same pH and of a similar ionic strength as the sample solution.
  - Pedestal Option: Pipette 1-2  $\mu$ L of the appropriate blanking solution onto the bottom pedestal, lower the arm and click the **Blank** button.
  - Cuvette Option (Model 2000c only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

**Note:** The arm must be down for all measurements, including those made with cuvettes. It is recommended that cuvettes be removed from the instrument prior to making a pedestal measurement to ensure that the pedestal arm can move to the proper starting position.

8. Enter a Sample ID in the appropriate field, load the first sample as described for the blank above and click **Measure**.

**Note:** A fresh aliquot of sample should be used for each measurement.

## After the measurement:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe and the instrument is ready to measure the next sample.
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples.

## Oligo Calc

Oligo Calc is used to calculate molecular weights, extinction coefficients, concentration factors and melting points for specific nucleic acid sequences. Selecting this Task bar will bring up two tabs:

- **Oligo Calc** - used to enter sequences of interest and select appropriate sample type variables.
- **Melting Points** - displays the calculated melting points of a DNA strand. This tab is available only for DNA sequences.

## To use the Oligo Calc:

1. Use one of the following options to enter in a base sequence:
  - The buttons below the Base Sequence display.
  - The keyboard. (Only the A, C, G, T, and U keys will work to enter bases.)
  - Copy and paste a base sequence into the display from another application. (Only the letters A, C, G, T, and U can be pasted into the Base Sequence display.)
  - To clear the base sequence display, click **Clear** to the right of the display. Individual bases can only be manually deleted.
2. Select degree of phosphorylation, if applicable: Mono-phosphate for DNA, mono or tri-phosphate for RNA.
3. Select **Double-Stranded** if applicable. The complementary base sequence will be included in the analysis.
4. From the drop down list, select the type of nucleic acid to be analyzed. The default is DNA.
5. For additions to the base sequence select **Modification** and enter the molecular weight associated with the addition.

## Oligo Calc analysis result fields include:

- **Molecular Weight** - displays calculated base sequence molecular weight
- **Extinction Coefficient** - displays the 260 nm wavelength-dependent extinction coefficient in ng-cm/ $\mu$ L.
- **Concentration Factor** - the constant, based on the extinction coefficient that is used to calculate the concentration of the base sequence.
- **Number of bases** - displays how many bases have been entered.
- **% GC-** shows the percentage of the total number of bases made up by guanine and cytosine.

**To calculate the melting point of a DNA sequence:**

1. Enter the base sequence as described above. If a base sequence has already been entered in the Oligo Calc Tab, then the base sequence box in the Melting Points tab will be auto-populated with that sequence.
2. Enter the appropriate values in the each of the boxes as described below:
  - **Oligo Molarity**- enters the oligo molarity of the sample. A default value of 10 uM is displayed, but can be changed to a value more appropriate for the sample.
  - **Cation Molarity**- enters the concentration of cations in the sample. A default value of 50 mM is displayed, but can be changed to a value more appropriate for the sample.
  - **% Formamide** - enters the percentage concentration of formamide in the sample. A default value of 0.00 % is displayed, but can be changed to a value more appropriate for the sample.

**Melting Point analysis result fields include:**

- **Salt-Adjusted** - calculates the melting point of the base sequence without accounting for the effect of interaction between neighboring bases.
- **Nearest-Neighbor** - displays the melting point of the base sequence when the effect of interaction between neighboring bases is taken into account.

## Micro Array

### Overview

The capability to pre-select viable fluorescently-tagged hybridization probes for use on micro arrays can eliminate potentially flawed samples and improve research effectiveness. The NanoDrop 2000/2000c measures the absorbance of the fluorescent dye, allowing detection at dye concentrations as low as 0.2 picomole per microliter. The software automatically utilizes the optimal pathlength to measure the absorbance of each sample.

### Measurement Concentration Ranges

The NanoDrop 2000/2000c will accurately measure fluorescent-dye and nucleic acid concentrations up to 100 pmol/ $\mu$ l (Cy3) and 750 ng/ $\mu$ l (DNA) respectively without dilution. Refer to "Measurement Ranges" for additional information.

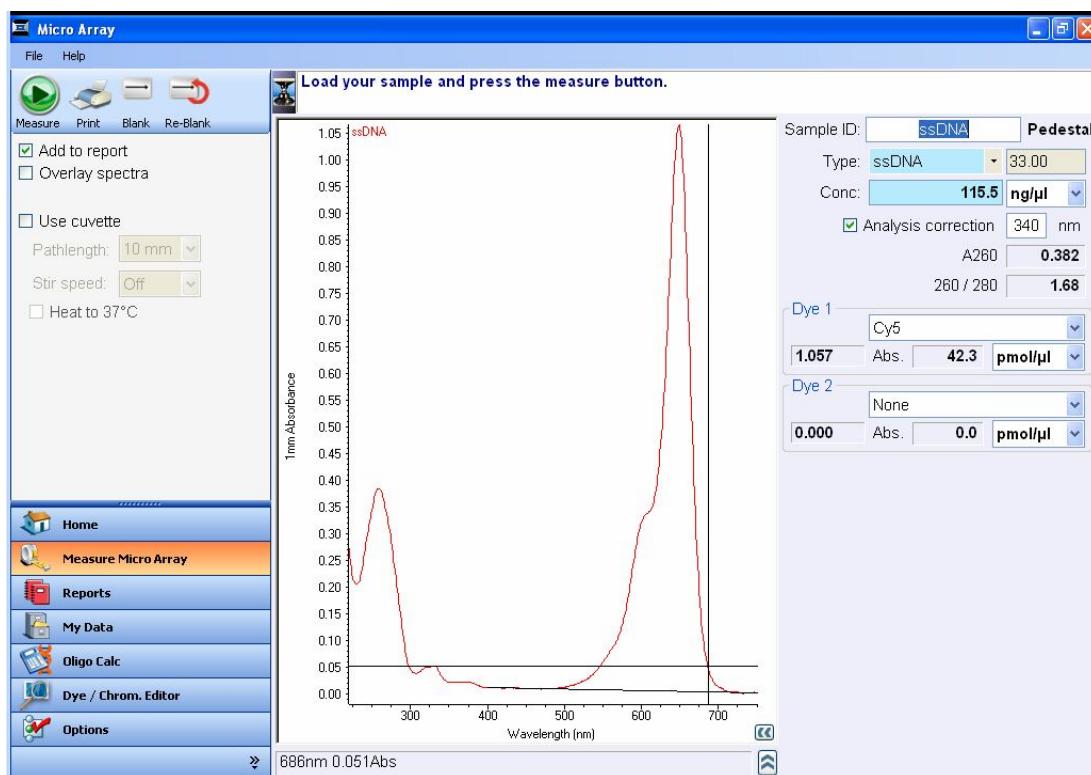
### Dye/Chromophore Editor

The NanoDrop 2000/2000c software allows the user to either select from a list of pre-defined dyes or enter new dyes using the Dye/Chromophore Editor. To enter a new dye, select the **Show** column (the first row). This will activate the fields for manual entry of the appropriate information. Refer to the dye manufacturer for the appropriate correction factors for user entered dyes. The 260 nm corrections will be automatically utilized for nucleic acid sample concentration calculations when the respective dye is selected. Once entered, the information is saved.

To delete a user defined dye, highlight the row of interest by clicking in the grey box to the left of the (+) icon and either use the keyboard delete key or right-click to bring up the delete option. Pre-defined dyes, identified by the lock icon adjacent to the dye entry in the editor, may not be edited or deleted. The default settings are Cy3 for Dye 1 and Cy5 for Dye 2.

### Unique Screen Features

The right pane displays features specific to the Micro Array application. Task bars in the left pane not described below are described in "Software Overview."



The spectral display shows data for the current sample normalized to a 1 mm path if measured using the pedestal mode or the pathlength selected if using the cuvette mode (model 2000c only). The pathlength information is indicated on the Y axis.

The following features are to the right of the spectral display:

- **Sample ID-** field into which a sample ID is entered. The appropriate sample ID should be entered prior to each measurement.
- **Type-** a drop down list from which the user may select the (color-keyed) type of nucleic acid being measured. Options include DNA-50 for dsDNA, RNA-40 for RNA, and ssDNA-33 for single-stranded DNA. Additional options include Oligo DNA and Oligo RNA which utilize the appropriate extinction coefficient based upon user defined base sequences. The **Custom** option allows the user to enter an extinction coefficient between 15 and 150.
- **Conc-** concentration based on absorbance at 260 nm and the default or user defined extinction coefficient. Concentration units may be selected from the adjacent drop-down box. Beer's law is used to calculate the nucleic acid concentration as described in '[Nucleic Acid Calculations](#)'.
- **A260-** displays absorbance at 260 nm normalized to a 10 mm path.

**Note:** The displayed A260 value is the difference in absorbance of the sample at 260 nm compared to the absorbance of the sample at 750 nm. The A260 value used to calculate the nucleic acid concentration takes into account any appropriate dye correction factors, the absorbance correction due to the selected analysis correction nm as well as the 750 nm baseline. Therefore, the displayed A260 value may not be the value used to calculate the nucleic acid concentration.

- **260/280-** ratio of sample absorbance at 260 and 280 nm. The ratio of absorbance at 260 and 280 nm is used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as "pure" for DNA; a ratio of ~2.0 is generally accepted as "pure" for RNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm. See "260/280 Ratio" in "[Diagnostic and Troubleshooting](#)" for more details on factors that can affect this ratio.
- **Dye 1 (or 2):** user selected dye(s). The default selections are Cy3 for Dye 1 and Cy5 for Dye 2.
  - **Abs** - the normalized absorbance of the respective dye at the 1 mm pathlength.
  - **pmol/ µL** - is the concentration based upon the respective dye's extinction coefficient. Concentration units may be selected from the adjacent drop-down list box.
- **Analysis correction** - the absorbance at the specified correction wavelength is subtracted from the absorbance at the analysis wavelength before calculating the concentration. This only affects the reported nucleic acid concentration. The default analysis wavelength is 340 nm.

**Note:** The software normalizes the visual spectrum display for all readings at 750 nm and will automatically calculate a baseline between 400 nm and 750 nm for dye concentration calculations.

## Making Micro Array Measurements

1. Select the **Micro Array** application from the main menu. If the wavelength verification window appears, ensure the arm is down and click **OK**.
2. Select the type of sample to be measured from the **Type** drop-down list. The default setting is ssDNA-33.
3. Choose the concentration units from the drop-down list adjacent to the color coded concentration box. The default units are ng/µL.

4. Use the drop-down lists in the Dye 1 or Dye 2 box to select the appropriate dye (or dyes). The default for Dye 1 is Cy3 and the default for Dye 2 is Cy5. If the nucleic acid has been labeled with only one dye, choose **None** as the dye type for Dye 2.
5. A default wavelength of 340 nm is automatically used for a bichromatic normalization for the nucleic acid component of the sample. Select an alternative reference wavelength or choose not to have the spectrum normalized by deselecting the **Analysis correction** box.
  - Select the file drop-down option **Use current settings as default** as a convenient way to limit set-up time for each new workbook.
6. Select **Add to report** to automatically include all measurements in the current report. The default setting is for all samples to be added to reports. The **Add to report** checkbox must be selected prior to a measurement to save the sample data to a workbook.
7. Select **Overlay spectra** to display multiple spectra at a time.
8. Establish a blank using the appropriate buffer. The blank solution generally is the buffer that the molecule of interest is suspended or dissolved in. This solution should be the same pH and of a similar ionic strength as the sample solution.
  - Pedestal Option: Pipette 1-2  $\mu$ L of the appropriate blanking solution onto the bottom pedestal, lower the arm and click **Blank**.
  - Cuvette Option (Model 2000c only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

**Note:** The arm must be down for all measurements, including those made with cuvettes. It is recommended that cuvettes be removed from the instrument prior to making a pedestal measurement to ensure that the pedestal arm can move to the proper starting position.

9. Enter a sample ID in the appropriate field, load the first sample as described for the blank above and click **Measure**.

**Note:** A fresh aliquot of sample should be used for each measurement.

#### After the measurement:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe and the instrument is ready to measure the next sample.
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples.

#### Oligo Calc

Oligo Calc is a software feature which enables the user to calculate molecular weight, extinction coefficients, concentration factors and melting points for specific nucleic acid sequences. Selecting this Task bar will bring up two tabs:

- **Oligo Calc** - used to enter sequences of interest and select appropriate sample type variables.
- **Melting Points** - displays the calculated melting point of a DNA strand. This tab is available only for DNA sequences.

**To use the Oligo Calc:**

1. Use one of the following options to enter in a base sequence:
  - The buttons below the Base Sequence display.
  - The keyboard. (Only the A, C, G, T, and U keys will work to enter bases.)
  - Copy and paste a base sequence into the display from another application. (Only the letters A, C, G, T, and U can be pasted into the Base Sequence display.)
  - To clear the Base Sequence display, click **Clear** to the right of the display. Individual bases can only be manually deleted.
2. Select degree of phosphorylation, if applicable: Mono-phosphate for DNA, mono or tri-phosphate for RNA.
3. Select **Double-Stranded** if applicable. The complementary base sequence will be included in the analysis.
4. From the drop down list, select the type of nucleic acid to be analyzed. The default is DNA.
5. For additions to the base sequence select **Modification** and enter the molecular weight associated with the addition.

**Oligo Calc analysis result fields include:**

- **Molecular Weight**- displays calculated base sequence molecular weight.
- **Extinction Coefficient**- displays the 260 nm wavelength-dependent extinction coefficient in ng-cm/ $\mu$ L.
- **Concentration Factor**- the constant, based on the extinction coefficient that is used to calculate the concentration of the base sequence.
- **Number of bases**- displays how many bases have been entered.
- **% GC**- shows the percentage of the total number of bases made up by guanine and cytosine.

**To calculate the melting point of a DNA sequence:**

1. Enter the base sequence as described above.

**Note:** If a base sequence has already been entered in the Oligo Calc Tab, then the base sequence box in the Melting Points Tab will be auto-populated with that sequence.

2. Enter the appropriate values in the each of the boxes as described below:

- **Oligo Molarity**- enters the oligo molarity of the sample. A default value of 10 uM is displayed, but may be changed to a value more appropriate for the sample.
- **Cation Molarity**- enters the concentration of cations in the sample. A default value of 50 mM is displayed, but may be changed to a value more appropriate for the sample.
- **% Formamide** - enters the percentage concentration of formamide in the sample. A default value of 0.00 % is displayed, but may be changed to a value more appropriate for the sample.

**Melting Point analysis result fields include:**

- **Salt-Adjusted** - calculates the melting point of the base sequence without accounting for the effect of interaction between neighboring bases.
- **Nearest-Neighbor** - displays the melting point of the base sequence when the effect of interaction between neighboring bases is taken into account.

## UV-Vis

### Overview

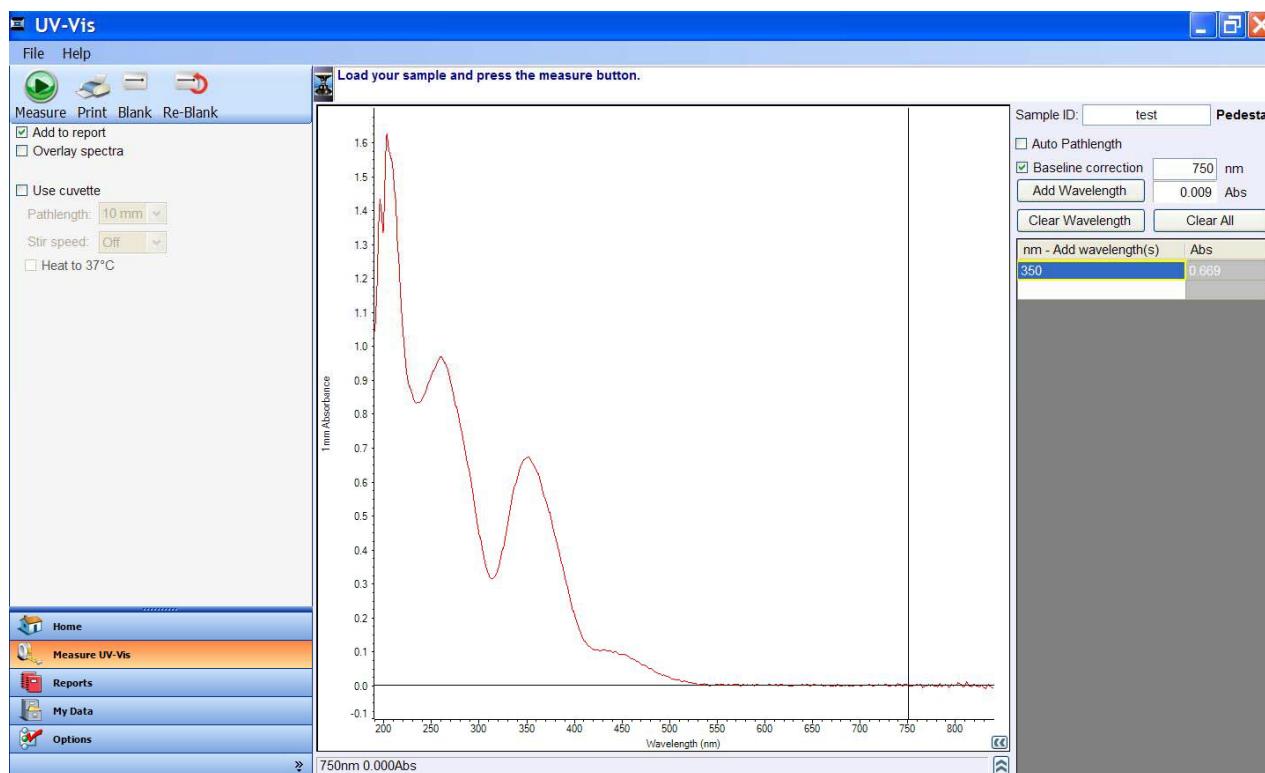
The UV-Vis application allows the NanoDrop 2000/2000c to function as a conventional spectrophotometer. Sample absorbance is displayed on the screen from 190 nm to 840 nm. Up to 40 wavelengths can be designated for absorbance monitoring and inclusion in the report.

### Measurement Concentration Ranges

The NanoDrop 2000/2000c can measure samples with 10 mm pathlength absorbance equivalent of 300 A when using the auto pathlength feature.

### Unique Screen Features

The right pane displays features specific to the UV-Vis application. Task bars in the left pane not described below are described in ['Software Overview.'](#)



The spectral display shows data for the current sample normalized to a 1 mm path if measured using the pedestal mode or the selected pathlength if using the cuvette mode (model 2000c only). The pathlength information is indicated on the Y axis.

The following features are to the right of the spectral display:

- **Sample ID** - field into which a sample ID is entered. The appropriate sample ID should be entered prior to each measurement.
- **Auto Pathlength** – automatically allows the software to use the optimal pedestal pathlength for high concentration samples. If selected, the shorter pathlength will be utilized when any wavelength between 220 nm to 840 nm has 1 mm equivalent absorbance value of 1.25 or higher. For wavelengths between 190 nm to 219 nm the change to the shorter pathlength occurs when any wavelength in this range has a 1 mm equivalent absorbance value of 1.0 or higher.

- The shorter pathlength is advantageous for highly concentrated samples. Although this is an automatic function in other applications, the user does have the option of using the auto pathlength feature or restricting the pedestal to a 1 mm pathlength in the UV-Vis application. In either case, the absorbance data will be displayed as 1 mm equivalent absorbance values.
- **Baseline Correction** - allows for specification of a wavelength for the bichromatic normalization of absorbance data for the entire spectrum. The baseline is automatically set to the absorbance value of the sample at the reference wavelength. All wavelength data will be referenced off of this value. The default wavelength is 750 nm. If the baseline correction is not utilized, the spectra may be offset from the baseline.
- **Add Wavelength** - adds cursor wavelength and cursor absorbance to the table. After a sample is measured, move the crosshair to the wavelength of interest and click **Add Wavelength** to add to the table. Alternatively, simply enter a wavelength in the highlighted field.
- **Clear Wavelength** – clears one entry from the wavelength table.
- **Clear All** – clears all entries from the wavelength table.

As with other applications, right-clicking within the graph display box generates a multi-option menu box. In the UV-VIS application, an additional Sample labels on/off option is available. The Sample labels option either shows or hides the absorbance values displayed at each selected wavelength. Right-clicking on a label allows editing, color/font changes, rotation, send to back/front and deletion.

## Making UV-Vis Measurements

1. Select the UV-Vis application from the main menu. If the wavelength verification window appears, ensure the arm is down and click **OK**.
2. A default wavelength of 750 nm is automatically used for a bichromatic normalization. Select an alternative reference wavelength or choose not to have the spectrum normalized by deselecting the **Baseline correction** box.
3. Enter up to 40 wavelengths to monitor in the **nm - Add wavelength(s)** box. Select the first row, enter the wavelength and click **Enter**. The next row will now be highlighted and additional wavelengths can be added as above. Alternatively, wavelengths can be added by positioning the crosshair cursor on the desired wavelength and clicking **Add Wavelength**. There are also optional buttons to clear one or all wavelengths.
  - Select the file drop-down option **Use current settings as default** as a convenient way to limit set-up time for each new workbook.
4. Select **Add to report** to include all measurements in the current report. The default setting is for all samples to be added to reports. The **Add to report** checkbox must be selected prior to a measurement to save the sample data to a workbook.
5. Select **Overlay spectra** to display multiple spectra at one time.
6. Establish a blank using the appropriate buffer. The blank solution generally is the buffer that the molecule of interest is suspended or dissolved in. This solution should be the same pH and of a similar ionic strength as the sample solution.
  - Pedestal Option: Pipette 1-2  $\mu$ L of the appropriate blanking solution onto the bottom pedestal, lower the arm and click **Blank**.
  - Cuvette Option (Model 2000c only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

**Note:** The arm must be down for all measurements, including those made with cuvettes. It is

recommended that cuvettes be removed from the instrument prior to making a pedestal measurement to ensure that the pedestal arm can move to the proper starting position.

7. Enter a sample ID in the appropriate field, load the first sample as described for the blank above and click **Measure**.

**Note:** A fresh aliquot of sample should be used for each measurement.

**After the measurement:**

- Simply wipe the upper and lower pedestals using a dry laboratory wipe and the instrument is ready to measure the next sample.
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples.

## Protein A280

### Overview

Proteins, unlike nucleic acids, can exhibit considerable diversity. The Protein A280 application is applicable to purified proteins that contain Trp, Tyr residues or Cys-Cys disulphide bonds and exhibit absorbance at 280 nm. This method does not require generation of a standard curve and is ready for protein sample quantitation at software startup. Colorimetric assays such as BCA, Pierce 660 nm, Bradford, and Lowry are more commonly used for uncharacterized protein solutions and cell lysates. If you are currently using a colorimetric assay to measure proteins, it is recommended that you use one of the pre-programmed colorimetric methods available on the NanoDrop 2000/2000c.

The Protein A280 application displays the UV spectrum, measures the protein's absorbance at 280 nm (A280) and calculates the concentration (mg/mL). Like Nucleic Acid, Protein A280 records and displays 10 mm (1 cm) equivalent data on the screen and in the archived workbooks.

### Measurement Concentration Range

The NanoDrop 2000/2000c will accurately measure protein samples up to 400 mg/mL (BSA) without dilution using the pedestal mode. The software automatically utilizes the optimal pathlength to measure the absorbance of each sample. Refer to "Measurement Ranges" for additional information.

The **small sample volume** option is available when samples have 10 mm equivalent absorbance values of 3.0 or higher (> 4.5 mg/mL BSA.)

### Sample Volume Requirements

Although sample size is not critical, it is essential that a liquid column is formed when using the pedestal option so that the gap between the upper and lower measurement pedestals is bridged with sample.

The dominant factor determining the surface tension of a droplet is the hydrogen bonding of the lattice of water molecules in solution. Generally, all additives including protein, buffer salts and detergent-like molecules may reduce the surface tension by interfering with the hydrogen bonding between water molecules. Although 1  $\mu\text{L}$  volumes are usually sufficient for most sample measurements, increasing the sample size to 2  $\mu\text{L}$  for protein measurements will ensure proper column formation for samples with reduced surface tension.

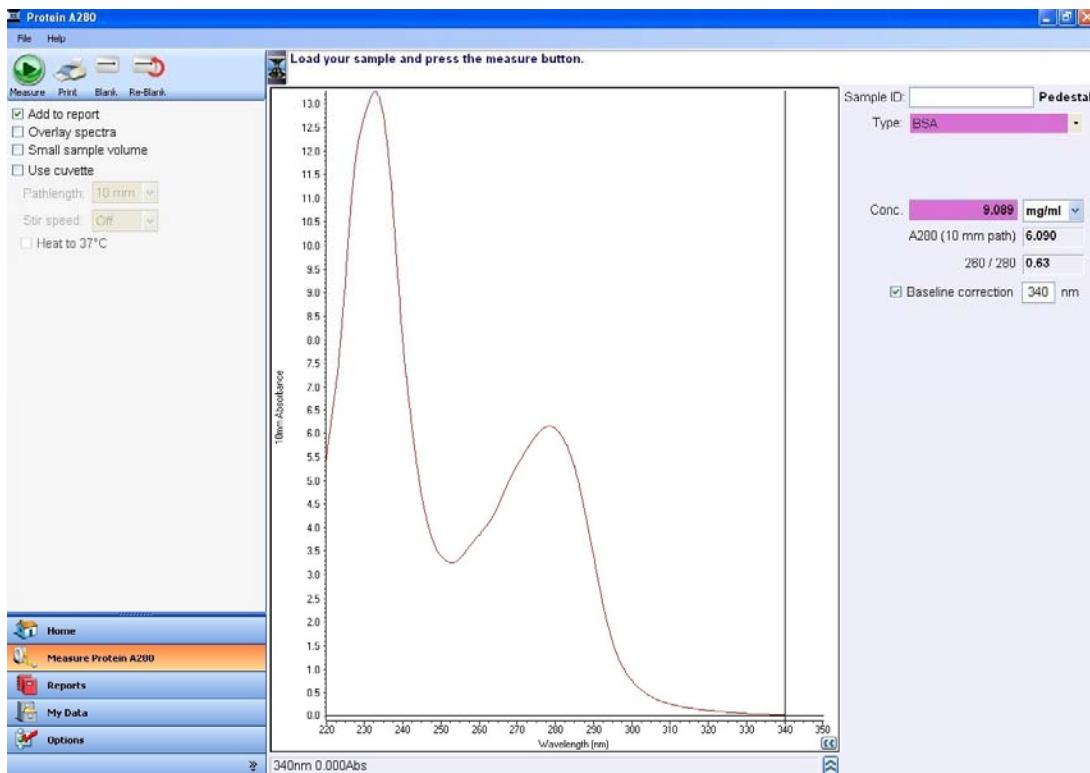
When using the cuvette option, it is essential that sufficient sample volume be used to ensure that the light path is passing through a representative portion of the sample. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

### Pedestal Reconditioning

Solutions and reagents containing surfactants may "un-condition" the measurement pedestal surfaces so that the liquid column does not form. If this occurs, use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. Additional information about the PR-1 kit may be found on our website.

## Unique Screen Features

The right pane displays features specific to the Protein A280 application. Task bars in the left pane not described below are described in “[Software Overview](#).”



The spectral display shows data for the current sample normalized to a 10 mm path for all measurements including measurements made with any cuvette pathlength (2000c only).

The following features are to the right of the spectral display:

- Sample ID** - field into which a sample ID is entered. The appropriate sample ID should be entered prior to each measurement.
- Type** - six sample types (options) are available for purified protein analysis and concentration measurements. The options can be viewed by selecting the drop down list adjacent to the **Type** box. The sample type (color-keyed) is selected by highlighting the preferred option in the drop down list. A description of each sample type is provided below. Default is 1 Abs = 1 mg/mL

1 Abs = 1 mg / mL	A general reference setting based on a 0.1% (1 mg/mL) protein solution producing an Absorbance at 280 nm of 1.0 A (where the pathlength is 10 mm or 1 cm).
BSA	Bovine Serum Albumin reference. Unknown (sample) protein concentrations are calculated using the mass extinction coefficient of 6.7 at 280 nm for a 1% (10 mg/mL) BSA solution.
IgG	IgG reference. Unknown (sample) protein concentrations are calculated using the mass extinction coefficient of 13.7 at 280 nm for a 1% (10 mg/mL) IgG solution.
Lysozyme	Lysozyme reference. Unknown (sample) protein concentrations are calculated using the mass extinction coefficient of 26.4 at 280 nm for a 1% (10 mg/mL) Lysozyme solution.
Type: Other protein (E & MW)	User-entered values for molar extinction coefficient (M-1 cm-1) and molecular weight (MW) in kilo Daltons (kDa) for their respective protein reference.
e / 1000 M.W. (kDa)	0.00 0.00
Type: Other protein (E 1%)	User-entered mass extinction coefficient (L gm-1cm-1) for a 10 mg/mL (1%) solution of the respective reference protein.
Ext. Coeff, E 1% L/gm-cm	0.00

- **Ext. Coeff, E1% L/gm-cm** - appears when **Other Protein (E1%)** is selected. The appropriate extinction coefficient should be entered prior to making a measurement.
- **$\epsilon/1000$  and M.W. (kDa)** - appears when **Other protein (E & MW)** is selected. The appropriate  $\epsilon/1000$  and M.W. (kDa) should be entered prior to making a measurement.
- **Conc.** - concentration based on absorbance at 280 nm and the selected extinction coefficient. Concentration units are chosen from the adjacent drop down list. The default selection is mg/mL.
- **A280 (10 mm path)** - absorbance at 280 nm for the protein sample being measured. The displayed absorbance value is normalized to a 10 mm path.
- **260/280**- ratio of the absorbance at 260 and 280 nm.
- **Baseline correction:** if selected, the default wavelength for the bichromatic normalization is 340 nm. The user can manually enter a different wavelength for the bichromatic normalization of the absorbance data. In either case, the baseline is automatically set to the absorbance value of the sample at the selected wavelength. All wavelength data will be referenced off of this value. If the baseline correction is not utilized, the spectra may be offset from the baseline and the calculated protein concentration may be higher than the true value.

### Making Protein A280 Measurements

1. Select the **Protein A280** application from the main menu. If the wavelength verification window appears, ensure the arm is down and click **OK**.
2. Select the type of sample to be measured from the **Type** drop-down list. The default setting is 1 Abs = 1 mg/mL.
3. Choose the concentration units from the drop-down list adjacent to the color coded box. Default is mg/mL.
4. A default wavelength of 340 nm is automatically used for a bichromatic normalization. Select an alternative reference wavelength or choose not to have the spectrum normalized by deselecting the **Baseline correction** box.
  - Select the file drop-down option **Use current settings as default** as a convenient way to limit set-up time for each new workbook.
5. Select **Add to report** to automatically include all measurements in the current report. The default setting is for all samples to be added to reports. The **Add to report** checkbox must be selected prior to a measurement to save the sample data to a workbook.
6. Select **Overlay spectra** to display multiple spectra at a time.
7. Establish a blank using the appropriate buffer. The blank solution generally is the buffer that the molecule of interest is suspended or dissolved in. This solution should be the same pH and of a similar ionic strength as the sample solution.
  - Pedestal Option: Pipette 2  $\mu$ L of the appropriate blanking solution onto the bottom pedestal, lower the arm and click the **Blank** button.
  - Cuvette Option (Model 2000c only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

**Note:** The arm must be down for all measurements, including those made with cuvettes. It is recommended that cuvettes be removed from the instrument prior to making a pedestal measurement to ensure that the pedestal arm can move to the proper starting position.

8. Enter a sample ID in the appropriate field, load the first sample as described for the blank above and click **Measure**.

**Note:** A fresh aliquot of sample should be used for each measurement.

**After the measurement:**

- Simply wipe the upper and lower pedestals using a dry laboratory wipe and the instrument is ready to measure the next sample.
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples.

## Proteins & Labels

### Overview

The Proteins & Labels application can be used to determine protein concentration (A280 nm) as well as fluorescent dye concentration (protein array conjugates). It can also be used to measure the purity of metalloproteins (such as hemoglobin) using wavelength ratios.

### Measurement Concentration Range

The NanoDrop 2000/2000c can accurately measure fluorescent-dye and protein concentrations up to 100 pmol/ $\mu$ L (Cy3) and 20 mg/ml (BSA) respectively without dilution. Refer to "[Measurement Ranges](#)" for additional information.

### Sample Volume Requirements

Although sample size is not critical, it is essential that a liquid column is formed when using the pedestal option so that the gap between the upper and lower measurement pedestals is bridged with sample.

The dominant factor determining the surface tension of a droplet is the hydrogen bonding of the lattice of water molecules in solution. Generally, all additives including protein, buffer salts and detergent-like molecules may reduce the surface tension by interfering with the hydrogen bonding between water molecules. Although 1  $\mu$ L volumes are usually sufficient for most sample measurements, increasing the sample size to 2  $\mu$ L for protein measurements will ensure proper column formation for samples with reduced surface tension.

### Pedestal Reconditioning

Solutions and reagents containing surfactants may "un-condition" the measurement pedestal surfaces so that the liquid column does not form. If this occurs, use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. Additional information about PR-1 may be found on our [website](#).

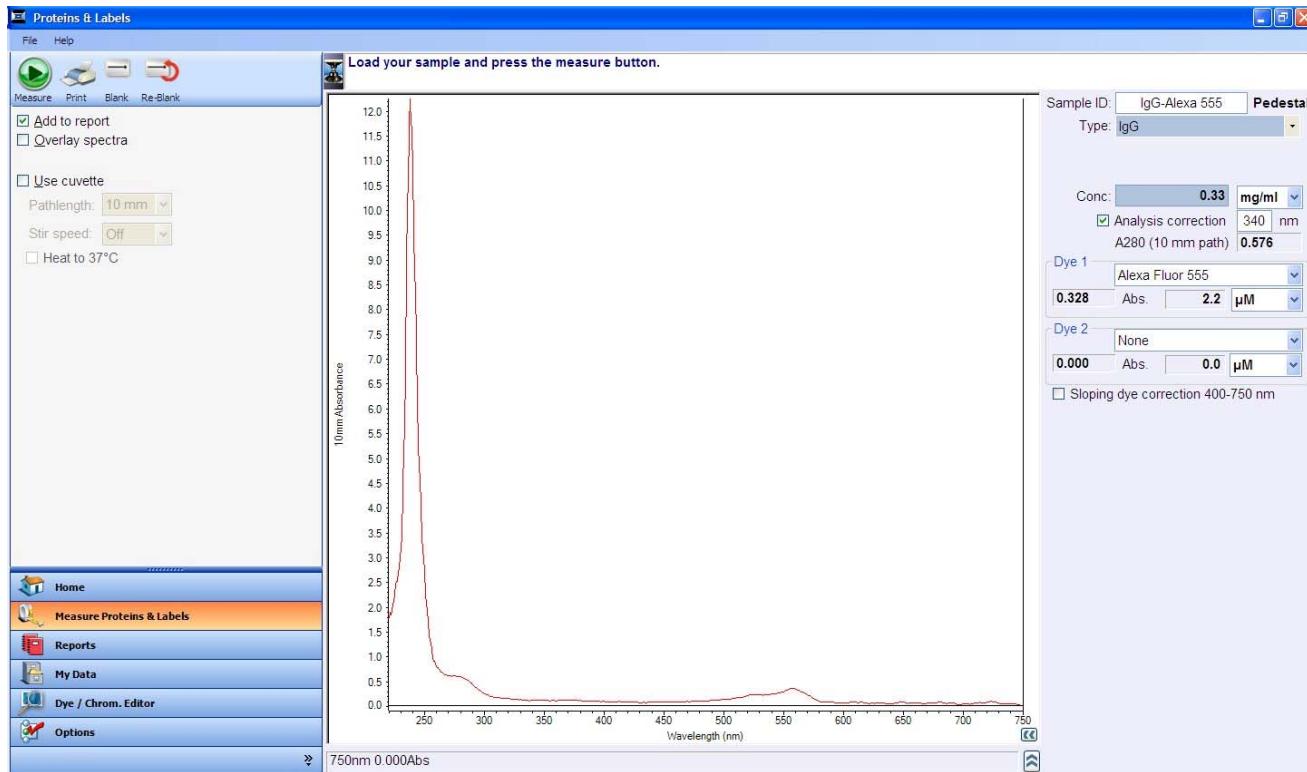
### Dye/Chromophore Editor

The NanoDrop 2000/2000c software allows the user to either select from a list of pre-defined dyes or enter new dyes using the Dye Chromophore Editor. To enter a new dye, select the **Show** column box (the last row). This will activate the fields for manual entry of the appropriate information. Refer to the dye manufacturer for the appropriate correction factors for user entered dyes. The 280 nm corrections will be automatically utilized for protein sample concentration calculations when the respective dye is selected. Once entered, the information is saved.

To delete a user defined dye, highlight the row of interest by clicking in the grey box to the left of the (+) icon and either use the keyboard Delete key or right-click to bring up the delete option. Pre-defined dyes, identified by the lock icon adjacent to the dye entry in the editor, may not be edited or deleted. The default settings are Cy3 for Dye 1 and Cy5 for Dye 2.

## Unique Screen Features

The right pane displays features specific to the Protein & Labels application. Task bars in the left pane not described below are described in "[Software Overview](#)".



The spectral display shows data for the current sample normalized to a 10 mm path for all measurements including measurements made with any cuvette pathlength (model 2000c only).

The following features are to the right of the spectral display:

- **Sample ID** - field into which sample ID is entered. The appropriate sample ID should be entered prior to each measurement.
- **Type** - six sample types (options) are available for purified protein analysis and concentration measurement. All of the options can be viewed by selecting the drop down menu adjacent to the box. The sample type (color-keyed) is selected by using the mouse to highlight the preferred option. A complete description of each sample type is provided within the Protein A280 application. The default setting is 1 Abs = 1 mg/mL.
- **Ext. Coeff, E1% L/gm-cm** - appears only when the Other Protein (E1%) sample type is selected. The appropriate extinction coefficient should be entered prior to making a measurement.
- **ε/1000 and M.W. (kDa)** - appears when **Other protein (E & MW)** is selected. The appropriate ε/1000 and M.W. (kDa) should be entered prior to making a measurement.
- **Conc** - based on absorbance at 280 nm and the selected extinction coefficient. Concentration units may be selected from the adjacent drop list. Default is mg/mL.
- **Dye 1 (or 2)** - user selected dye(s). The defaults are Cy3 for Dye 1 and Cy5 for Dye 2. Abs. is the normalized absorbance of the respective dye at the 1 mm pathlength.
  - **Abs-** the normalized absorbance of the respective dye at the 1 mm pathlength.

- **uM** - is the concentration based upon the respective dye's extinction coefficient. Concentration units may be selected from the adjacent drop-down list box.
- **A280** - displays the absorbance at 280 nm for the protein sample measured. The displayed absorbance value is normalized to a 10 mm path.

**Note:** The displayed A280 value is the difference in absorbance of the sample at 280 nm compared to the absorbance of the sample at 750 nm. The A280 value used to calculate the protein concentration takes into account any appropriate dye correction factors, the absorbance correction due to the selected analysis correction nm as well as the 750 nm baseline. Therefore, the displayed A280 value may not be the value used to calculate the protein concentration.

- **Analysis correction** -the absorbance at the specified correction wavelength is subtracted from the absorbance at the analysis wavelength before calculating the concentration. This only affects the reported protein concentration.
- **Sloping Dye Correction** - when selected the software will subtract the value of a sloping baseline from 400 to 750 nm from the absorbance at the Dye wavelength. This only affects the reported absorbance of the dye peak(s) and dye concentration(s).

## Making Proteins & Labels Measurements

1. Select the **Proteins & Labels** application from the main menu. If the wavelength verification window appears, ensure the arm is down and click **OK**.
2. Select the type of sample to be measured from the **Type** drop-down list. The default setting is 1 Abs = 1 mg/mL.
3. Choose the concentration units from the drop-down list adjacent to the color coded concentration box. The default units are mg/mL.
4. Use the drop-down lists in the Dye 1 or Dye 2 box to select the appropriate dye (or dyes). The default for Dye 1 is Cy3 and the default for Dye 2 is Cy5. If the protein has been labeled with only one dye choose **None** as the dye type for Dye 2.
5. A default wavelength of 340 nm is automatically used for a bichromatic normalization for the protein component of the sample. Select an alternative reference wavelength or choose not to have the spectrum normalized by deselecting **Analysis correction**.
  - Select the file drop-down option **Use current settings as default** as a convenient way to limit set-up time for each new workbook.
6. Select **Add to report** to automatically include all measurements in the current report. The default setting is for all samples to be added to reports. The **Add to report** checkbox must be selected prior to a measurement to save the sample data to a workbook.
7. Select **Overlay spectra** to display multiple spectra at a time.
8. Select **Sloping dye correction 400-750 nm** if desired.
9. Establish a blank using the appropriate buffer. The reference or blank solution generally is the buffer that the molecule of interest is suspended or dissolved in. This solution should be the same pH and of a similar ionic strength as the sample solution.
  - Pedestal Option: Pipette 2  $\mu$ L of dH<sub>2</sub>O onto the bottom pedestal, lower the arm and click the **Blank** button.

- Cuvette Option (Model 2000c only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

**Note:** The arm must be down for all measurements, including those made with cuvettes. It is recommended that cuvettes be removed from the instrument prior to making a pedestal measurement to ensure that the pedestal arm can move to the proper starting position.

10. Enter a sample ID in the appropriate field, load the first sample as described for the blank above and click **Measure**.

**Note:** A fresh aliquot of sample should be used for each measurement.

#### After the measurement:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe and the instrument is ready to measure the next sample.
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples.

## Protein BCA

### Overview

The BCA (Bicinchoninic Acid) assay is a colorimetric method for determining the total protein concentration in unpurified protein samples. It is often used for dilute protein solutions and/or proteins in the presence of components that have significant UV (280 nm) absorbance. Unlike the Protein A280 application, the Protein BCA application requires a standard curve be generated before sample protein concentrations can be measured.

The method uses bicinchoninic acid (BCA) as the detection reagent for Cu+1, which is formed when Cu+2 is reduced by protein in an alkaline environment. A purple-colored 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 normalized at 750 nm. Pre-formulated reagents of BCA and CuSO<sub>4</sub>, are available in kit form from Thermo Fisher Scientific.

### BCA Assay Measurement Ranges

Commercial BCA Protein kit manufacturers typically outline procedures for different protein concentration ranges:

- A regular assay uses a 20:1 reagent / sample volume ratio to detect a concentration range between ~0.20 mg/mL to 8.0 mg/mL (BSA). When setting up an assay for pedestal measurements, a minimum sample volume of 4 µL in 80 µL of BCA reagent is recommended.
- A mini assay uses a 1:1 reagent / sample volume ratio to detect a concentration range between 0.01 mg/mL to 0.20 mg/mL (BSA). To prepare sufficient volume of these 1:1 mixtures for pedestal measurements, a minimum sample volume of 10 µL and 10 µL of BCA reagent (in a PCR tube) is recommended.

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.

**Note:** If running the assay at 60°C, doubling the volumes may afford greater insurance against skewed results from evaporation / condensation within the sealed reaction tube.

Protein standards (BSA) for generating a standard curve may also be provided by the manufacturer for the BCA assay. Since the NanoDrop 2000/2000c pedestal option 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.

### Sample Volume Requirements

Although sample size is not critical, it is essential that a liquid column is formed when using the pedestal option so that the gap between the upper and lower measurement pedestals is bridged with sample.

The dominant factor determining the surface tension of a droplet is the hydrogen bonding of the lattice of water molecules in solution. Generally, all additives including protein, buffer salts and detergent-like molecules may reduce the surface tension by interfering with the hydrogen bonding between water molecules. Although 1 µL volumes are usually sufficient for most sample measurements, increasing the sample size to 2 µL for protein measurements will ensure proper column formation for samples with reduced surface tension.

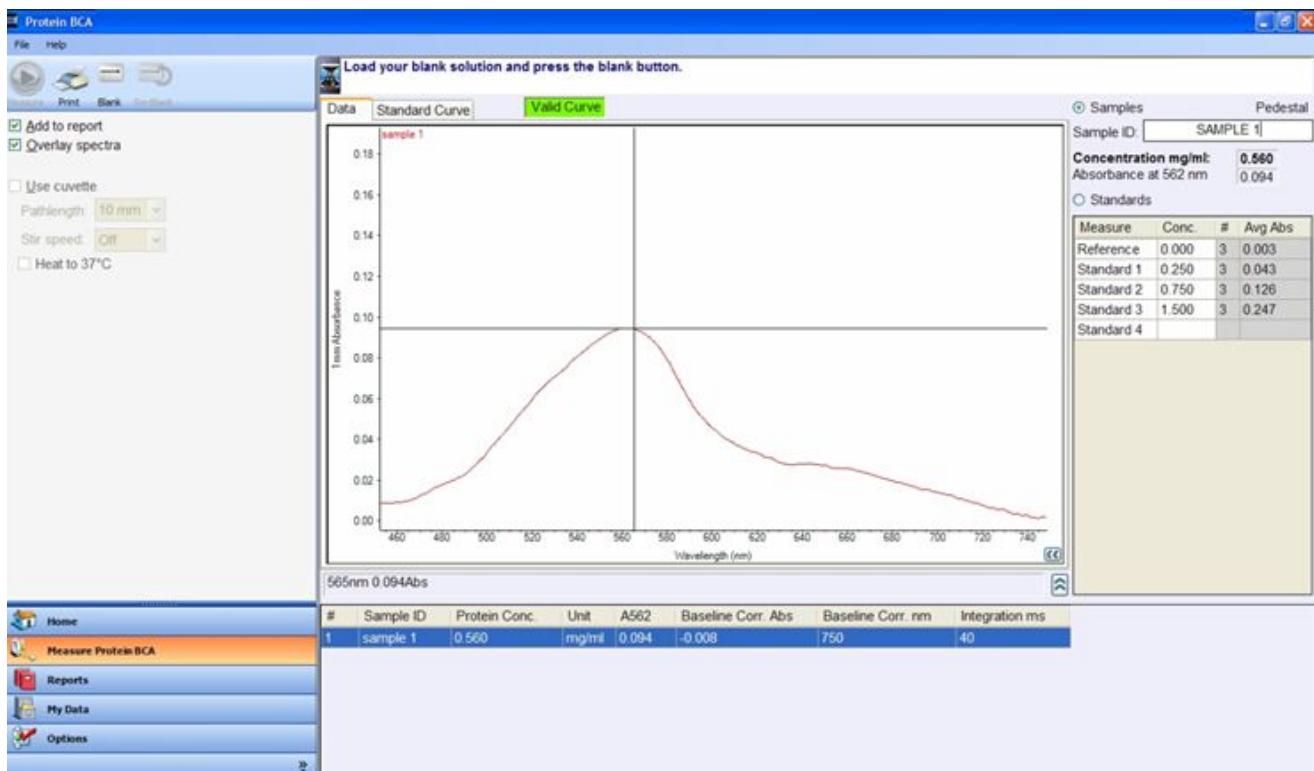
When using the cuvette option, it is essential that sufficient sample volume be used to ensure that the light path is passing through a representative portion of the sample. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for recommended volumes.

## Pedestal Reconditioning

Solutions and reagents containing surfactants may “un-condition” the measurement pedestal surfaces so that the liquid column does not form. If this occurs, use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. Additional information about PR-1 may be found on our [website](#).

## Unique Screen Features

The right pane displays features specific to the BCA Protein application. Task bars in the left pane not described below are described in [“Software Overview.”](#)



The spectral display shows data for the current sample normalized to a 1 mm path if measured using the pedestal mode or the pathlength selected if using the cuvette option (model 2000c only). The pathlength information is indicated on the Y axis.

- **Data tab** - displays current measurement data.
- **Standard Curve tab** - displays the plot of the standard curve, the  $R^2$  value and the type of curve being displayed. Curve types include interpolation, linear and polynomials. The default selection is “linear.”
- **Valid / Invalid Curve** - indicates whether the minimum number of standards has been measured. Once the criteria have been met, the standard curve status will change from Invalid Curve (red) to Valid Curve (green).

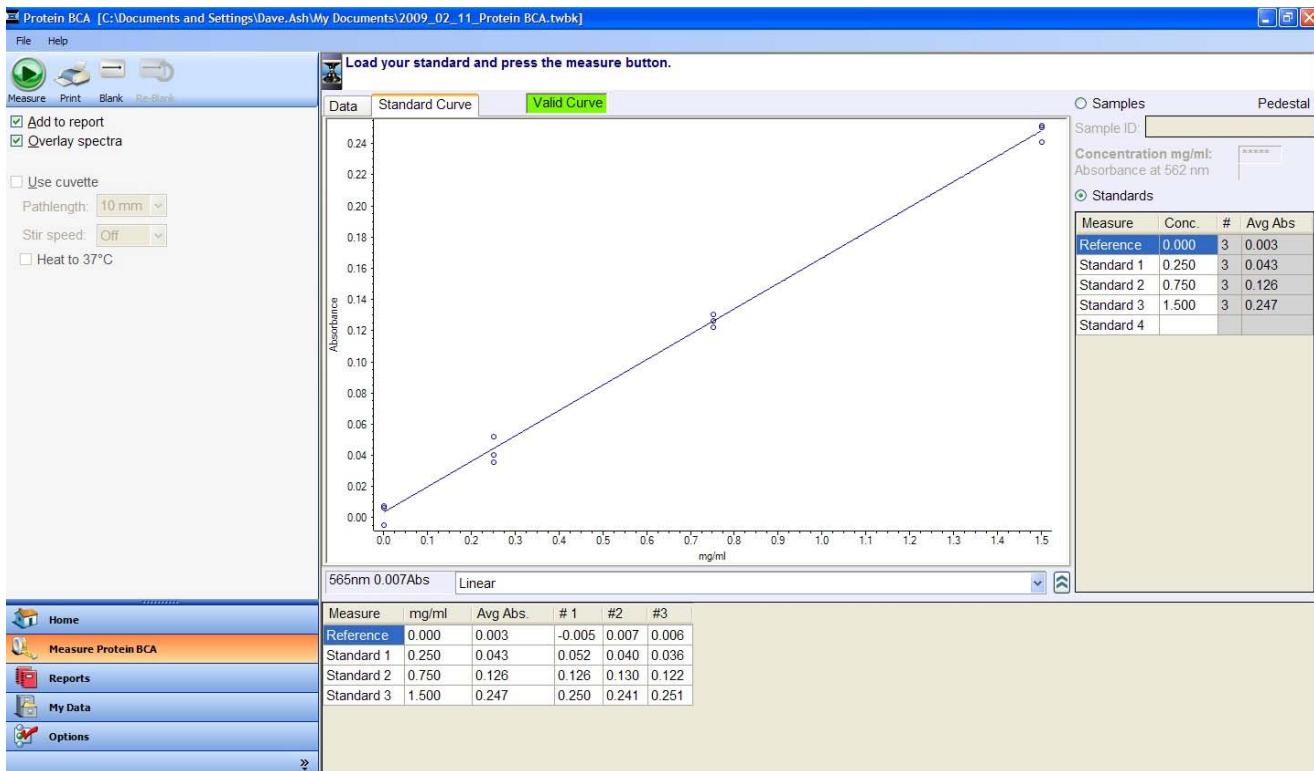
**Note:** The status is only an indicator that the minimum number of required points has been established for the curve fit type selected. The Standard Curve Status indicator does not validate the integrity of the curve. Additional standards may be required to cover expected assay concentration range.

- **Samples radio button** - Once the criterion has been met for a valid curve, the Sample ID field will become active when selected. The sample ID should be entered prior to making the measurement.

- **Standards radio button** - enables entry of standard names and concentrations into the standards table when selected.
- **Concentration mg/mL** - concentration of current sample in mg/mL.
- **Absorbance at 562 nm** - the Cu-BCA complex's absorbance at 562 nm.

## BCA Standard Curves

A standard curve is required for BCA assays.



Example Standard Curve: 20:1 reagent/sample volume

- A standard curve can be generated using a minimum of two points which may include two standards or a reference (BCA reagent/assay buffer only – no protein) and one standard.
- The multi-point curve capability allows for multiple replicates for up to seven standards. There is no set order in which standards must be run.
- Additional standards may only be added before unknown samples are measured. Once the first sample is measured, no additional standards or replicates may be added.
- A standard may be deleted at any time before a sample has been measured. Right-click the results table to the far right to delete a standard or clear the entire standards table. Right-click a standard within the replicates table under the graph display to delete individual standard replicates.
- Deleted standards cannot be added back or re-measured once the first sample is measured.
- In order to establish a new standard curve, a new workbook must be created. Colorimetric applications will offer several options regarding the use of previous standard curves when appending sample data to previous workbooks. It is recommended that the user follow the protein assay manufacturer's guidelines for generating standard curves before appending new data to a workbook.

**Note:** If selecting a previously saved workbook, all concentration calculations for newly measured samples will be based upon the standard absorbance values saved in the workbook. Each workbook will archive only one standard curve.

## Making BCA Assay Measurements

- Refer to the manufacturers' guidelines and recommendations for sample preparation.
- The Zero Reference standard is a solution of the same buffer/dye reagent composition as the other standards and samples but without any protein added.
- Prepare both standards and unknowns in the same manner. Use a diluent of the same pH and ionic strength for all blanks, standards and unknown samples.
- Standards diluted from a stock must cover the expected range of the unknown samples. Sample protein concentrations are not extrapolated beyond measured standard concentrations.

## Procedure

1. Select the **Protein BCA** application from the main menu. If the wavelength verification window appears, ensure the arm is down and click **OK**.
2. Enter the values for each standard concentration in the right pane table. The software allows for the reference and up to 7 additional standards. The Reference and/or standards can be measured in replicates.

**Note:** The minimum requirement for standard curve generation is the measurement of two standards or the measurement of the zero reference and at least one standard. It is recommended that additional standards be included as necessary to cover the expected assay concentration range.

3. Select **Add to report** to automatically include all measurements in the current report. The default setting is for all samples to be added to reports. The **Add to report** checkbox must be selected prior to a measurement to save the sample data to a workbook.
  - Select the file drop-down option **Use current settings as default** as a convenient way to limit set-up time for each new workbook.
4. Select **Overlay spectra** to display multiple spectra at a time.
5. Establish a blank using the appropriate buffer. The blank for colorimetric assays is generally dH<sub>2</sub>O.
  - Pedestal Option: Pipette 2 µL of dH<sub>2</sub>O onto the bottom pedestal, lower the arm and click **Blank**.
  - Cuvette Option (Model 2000c only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

**Note:** The arm must be down for all measurements, including those made with cuvettes. It is recommended that cuvettes be removed from the instrument prior to making a pedestal measurement to ensure that the pedestal arm can move to the proper starting position.

6. Under the Standards tab, highlight a standard and load as described for the blank above. Click **Measure** to initiate the measurement. Be sure to measure all standards prior to measuring samples.
7. After all of the Standards have been measured, click on the **Samples** radio button. Enter a sample ID. Load 2 µL of sample when using the pedestal or insert the cuvette. Click **Measure** to initiate the measurement. It is not necessary to blank the instrument between the standard and the unknown sample measurements.

**Note:** A fresh aliquot of sample should be used for each measurement.

**After the measurement:**

- Simply wipe the upper and lower pedestals using a dry laboratory wipe and the instrument is ready to measure the next sample.
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples.

## Protein Lowry

### Overview

The Lowry assay is an alternative method for determining protein concentration based on the widely used and cited Lowry procedure for protein quantitation. Like the other colorimetric assays, the Lowry Assay requires a standard curve be generated before sample proteins can be measured.

The Lowry procedure involves reaction of protein with cupric sulfate in alkaline solution, resulting in formation of tetradeinate copper-protein complexes. The Folin-Ciocalteu Reagent is effectively reduced in proportion to the chelated copper-complexes resulting in a water-soluble blue product that is measured at 650 nm and normalized at 405 nm. Pre-formulated reagents, utilized in the assay, are available in kit form from numerous manufacturers.

### Lowry Assay Measurement Range

To accurately prepare standards, it is recommended that a 20 µL minimum sample volume and 100 µL of Lowry reagent be used for each reaction. On the NanoDrop 2000/2000c the Lowry assay can be used to measure samples from ~0.20 mg/mL up to 4 mg/mL.

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 (BSA) for generating a standard curve may also be provided by the manufacturer for the Lowry assay. Since the NanoDrop 2000/2000c pedestal option 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.

### Sample Volume Requirements

Although sample size is not critical, it is essential that a liquid column is formed when using the pedestal option so that the gap between the upper and lower measurement pedestals is bridged with sample.

The dominant factor determining the surface tension of a droplet is the hydrogen bonding of the lattice of water molecules in solution. Generally, all additives including protein, buffer salts and detergent-like molecules may reduce the surface tension by interfering with the hydrogen bonding between water molecules. Although 1 µL volumes are usually sufficient for most sample measurements, increasing the sample size to 2 µL for protein measurements will ensure proper column formation for samples with reduced surface tension.

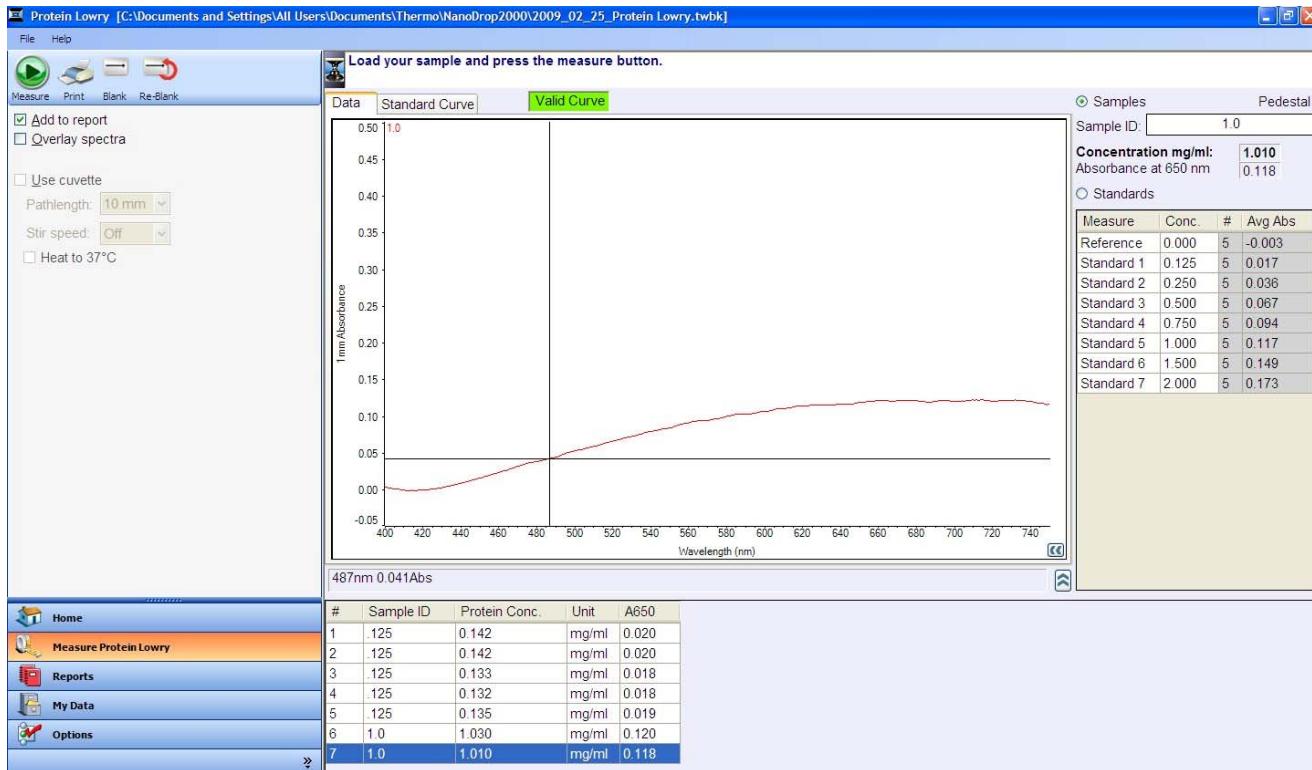
When using the cuvette option, it is essential that sufficient sample volume be used to ensure that the light path is passing through a representative portion of the sample. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for recommended volumes.

### Pedestal Reconditioning

Solutions and reagents containing surfactants may “un-condition” the measurement pedestal surfaces so that the liquid column does not form. If this occurs, use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. Additional information about PR-1 may be found on our [website](#).

## Unique Screen Features

The right pane displays features specific to the Protein Lowry application. Task bars in the left pane not described below are described in “[Software Overview](#).”



The spectral display shows data for the current sample normalized to a 1 mm path if measured using the pedestal mode or the pathlength selected if using the cuvette option (model 2000c only). The pathlength information is indicated on the Y axis.

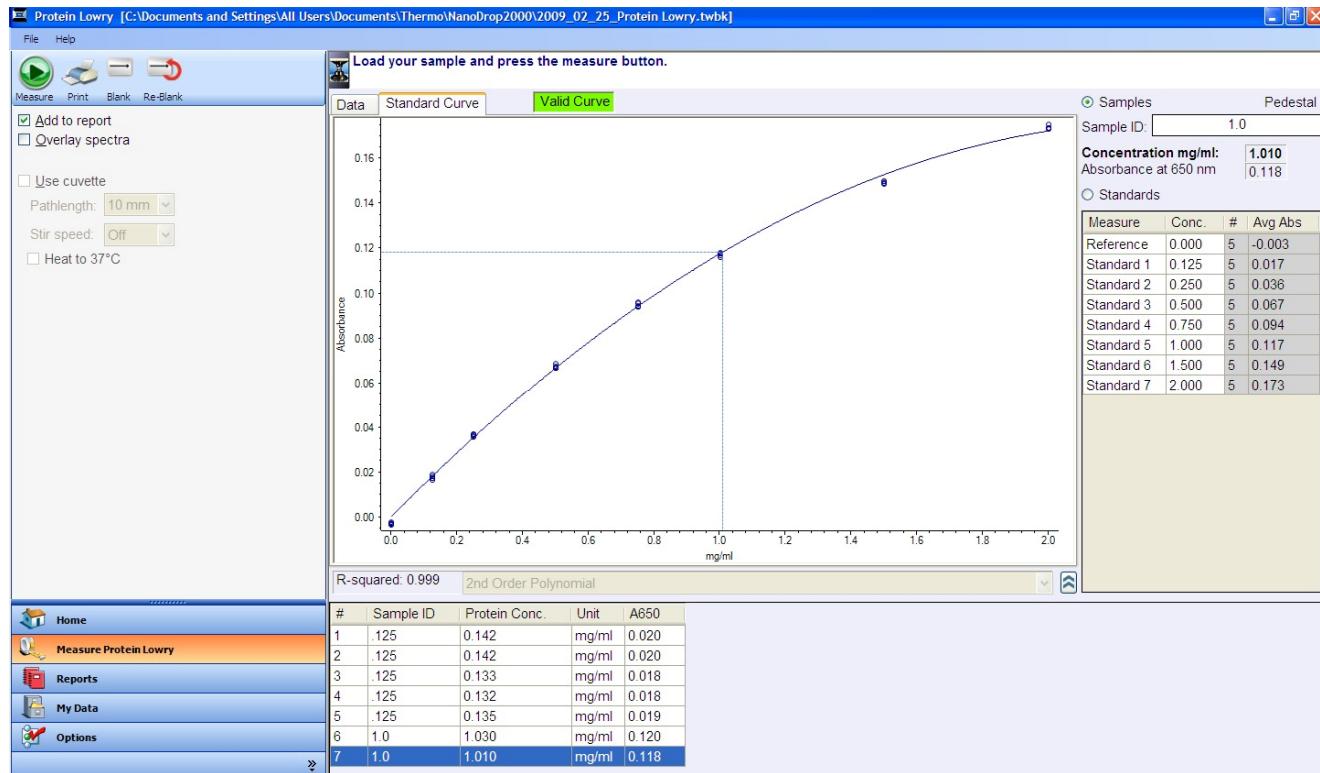
- **Data tab** - displays current measurement data
- **Standard Curve tab** - displays the plot of the standard curve, the  $R^2$  value and the type of curve being displayed. Curve types include interpolation, linear and polynomials. The default selection is “linear.”
- **Valid / Invalid Curve** - indicates whether the minimum number of standards has been measured. Once the criteria have been met, the standard curve status will change from Invalid Curve (red) to Valid Curve (green).

**Note:** The status is only an indicator that the minimum number of required points has been established for the curve fit type selected. The Standard Curve Status indicator does not validate the integrity of the curve. Additional standards may be required to cover expected assay concentration range.

- **Samples radio button** - Once the criterion has been met for a valid curve, the Sample ID field will become active when selected. The sample ID should be entered prior to making the measurement.
- **Standards radio button** - enables entry of standard names and concentrations into the standards table when selected.
- **Concentration mg/mL** - concentration of current sample in mg/mL.
- **Absorbance at 650 nm** - the tetradeinate copper-protein complex's absorbance at 650 nm.

## Lowry Standard Curves

A standard curve is required for Lowry assays.



Example Standard Curve: 5:1 reagent/sample volume.

- A standard curve can be generated using a minimum of two points which may include two standards or a reference (Lowry reagent/assay buffer only – no protein) and one standard.
- The multi-point curve capability allows for multiple replicates for up to seven standards. There is no set order in which standards must be run.
- Additional standards may only be added before unknown samples are measured. Once the first sample is measured, no additional standards or replicates may be added.
- A standard may be deleted at any time before a sample has been measured. Right-click the results table to the far right to delete a standard or clear the entire standards table. Right-click a standard within the replicates table under the graph display to delete individual standard replicates.
- Deleted standards cannot be added back or re-measured once the first sample is measured.
- In order to establish a new standard curve, a new workbook must be created. Colorimetric applications will offer several options regarding the use of previous standard curves when appending sample data to previous workbooks. It is recommended that the user follow the protein assay manufacturer's guidelines for generating standard curves before appending new data to a workbook.

**Note:** If selecting a previously saved workbook, all concentration calculations for newly measured samples will be based upon the standard absorbance values saved in the workbook. Each workbook will archive only one standard curve.

## Making Lowry Assay Measurements

- Refer to the manufacturers' guidelines and recommendations for sample preparation.
- The Zero Reference standard is a solution of the same buffer/dye reagent composition as the other standards and samples but without any protein added.
- Prepare both standards and unknowns in the same manner. Use a diluent of the same pH and ionic strength for all blanks, standards and unknown samples.
- Standards diluted from a stock must cover the expected range of the unknown samples. Sample protein concentrations are not extrapolated beyond measured standard concentrations.

### Procedure:

1. Select the **Protein Lowry** application from the main menu. If the wavelength verification window appears, ensure the arm is down and click **OK**.
2. Enter the values for each standard concentration in the right pane table. The software allows for the reference and up to 7 additional standards. The Reference and/or standards can be measured in replicates.

**Note:** The minimum requirement for standard curve generation is the measurement of two standards or the measurement of the zero reference and at least one standard. It is recommended that additional standards be included as necessary to cover the expected assay concentration range.

3. Select **Add to report** to automatically include all measurements in the current report. The default setting is for all samples to be added to reports. The **Add to report** checkbox must be selected prior to a measurement to save the sample data to a workbook.
  - Select the file drop-down option **Use current settings as default** as a convenient way to limit set-up time for each new workbook.
4. Select **Overlay spectra** to display multiple spectra at a time.
5. Establish a blank using the appropriate buffer. The blank for colorimetric assays is generally dH<sub>2</sub>O.
  - Pedestal Option: Pipette 2 µL of dH<sub>2</sub>O onto the bottom pedestal, lower the arm and click **Blank**.
  - Cuvette Option (Model 2000c only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.
6. Under the Standards tab, highlight a standard and load as described for the blank above. Click **Measure** to initiate the measurement. Be sure to measure all standards prior to measuring samples.
7. After all of the Standards have been measured, click on the **Samples** radio button. Enter a sample ID. Load 2 µL of sample when using the pedestal or insert the cuvette. Click **Measure** to initiate the measurement. It is not necessary to blank the instrument between the standard and the unknown sample measurements.

**Note:** A fresh aliquot of sample should be used for each measurement.

**After the measurement:**

- Simply wipe the upper and lower pedestals using a dry laboratory wipe and the instrument is ready to measure the next sample.
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples.

## Protein Bradford

### Overview

The Bradford Assay is a commonly used method for determining protein concentration. It is often used for more dilute protein solutions where lower detection sensitivity is needed and/or in the presence of components that also have significant UV (280 nm) absorbance. Like the other colorimetric assays, the Bradford assay requires a standard curve be generated before sample proteins can be measured.

The Bradford procedure uses the protein-induced absorbance shift of Coomassie Blue dye to 595 nm as a measure of protein concentration. The bound protein-dye complex is measured at 595 nm and normalized at 750 nm. A single stabilized reagent mixture containing Coomassie Blue dye, alcohol, and surfactant in kit form is available from numerous manufacturers.

### Bradford Assay Measurement Ranges

Commercial Bradford protein kit manufacturers typically outline procedures for different protein concentration ranges:

- A regular assay uses a 50:1 reagent / sample volume ratio to detect a concentration range between ~0.1 mg/mL to 8.0 mg/mL (BSA). The best linearity is in the 0.01- 1 mg/mL range. When setting up an assay for pedestal measurements, a minimum sample volume of 4  $\mu$ L in 200  $\mu$ L of Bradford reagent is recommended.
- A mini assay uses a 1:1 reagent / sample volume ratio to detect a concentration range between 15  $\mu$ g/mL – 125  $\mu$ g/mL (BSA). To prepare sufficient volume of these 1:1 mixtures for pedestal measurements, a minimum sample volume of 10  $\mu$ L and 10  $\mu$ L of BCA reagent (in a PCR tube) is recommended.

Follow the assay kit manufacturer's recommendations for all standards and samples (unknowns), ensuring they are subjected to the same timing and temperature throughout the assay.

Protein standards (BSA) for generating a standard curve may also be provided by the manufacturer for the Bradford assay.

**Note:** Since the NanoDrop 2000/2000c pedestal option 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.

Coomassie dye-dye and Coomassie dye-protein aggregates are frequently encountered in Coomassie dye-based protein assays. Particulates often form with increasing development time, which can cause significant fluctuations in Absorbance readings. It is also important to note the total analyte (protein-dye) signal at 595 nm is limited to ~ 0.150 A as a result of the 1.0 mm pathlength of the pedestal, the Bradford (Coomassie dye) reagent concentration, and the acidic pH. Making measurements in triplicate of standards and samples (unknowns) is good practice, particularly with the limited assay signal obtained with the Bradford Assay. Using the cuvette option of the NanoDrop 2000 c will result in a larger signal.

### Sample Volume Requirements

Although sample size is not critical, it is essential that a liquid column is formed when using the pedestal option so that the gap between the upper and lower measurement pedestals is bridged with sample.

The dominant factor determining the surface tension of a droplet is the hydrogen bonding of the lattice of water molecules in solution. Generally, all additives including protein, buffer salts and detergent-like molecules may reduce the surface tension by interfering with the hydrogen bonding between water molecules. Although 1  $\mu$ L volumes are usually sufficient for most sample measurements, increasing the sample size to 2  $\mu$ L for protein measurements will ensure proper column formation for samples with reduced surface tension.

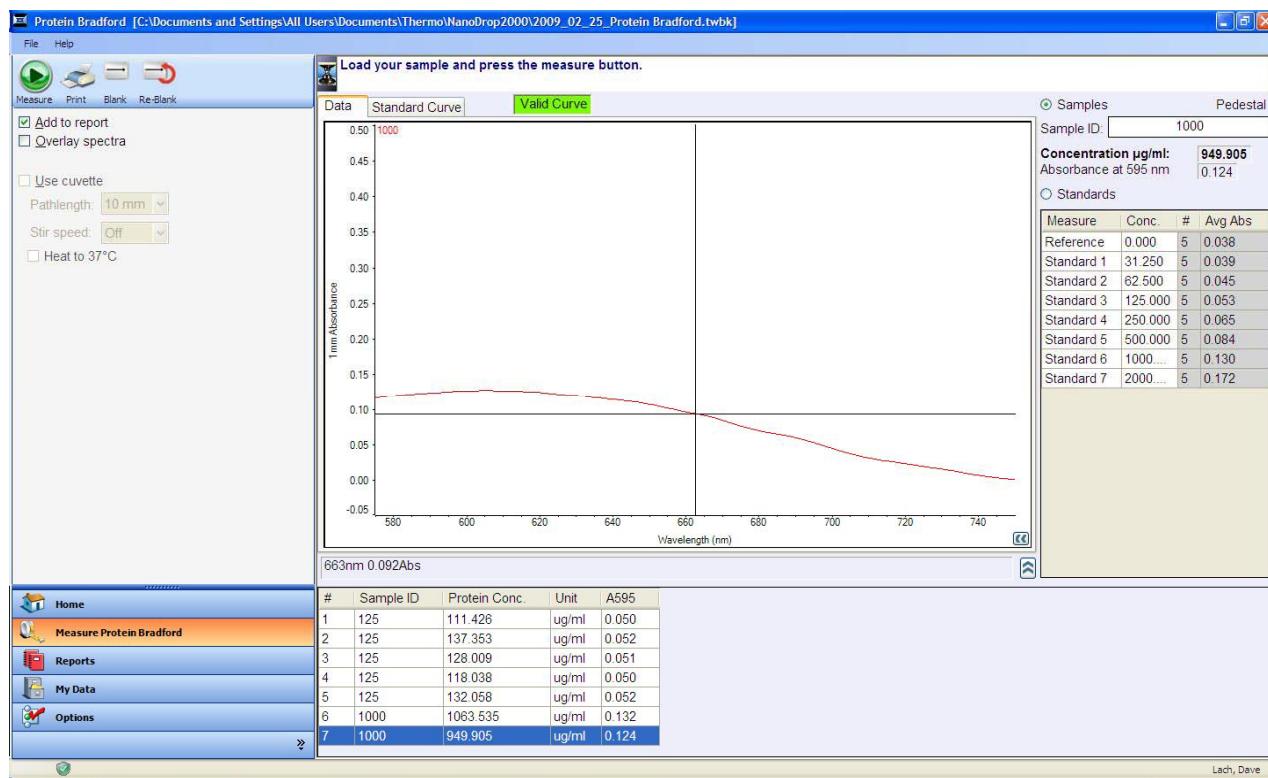
When using the cuvette option, it is essential that sufficient sample volume be used to ensure that the light path is passing through a representative portion of the sample. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for recommended volumes.

## Pedestal Reconditioning

Solutions and reagents containing surfactants may “un-condition” the measurement pedestal surfaces so that the liquid column does not form. If this occurs, use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. Additional information about PR-1 may be found on our [website](#).

## Unique Screen Features

The right pane displays features specific to the Protein Bradford application. Task bars in the left pane not described below are described in “[Software Overview](#).”



The spectral display shows data for the current sample normalized to a 1 mm path if measured using the pedestal mode or the pathlength selected if using the cuvette option (model 2000c only). The pathlength information is indicated on the Y axis.

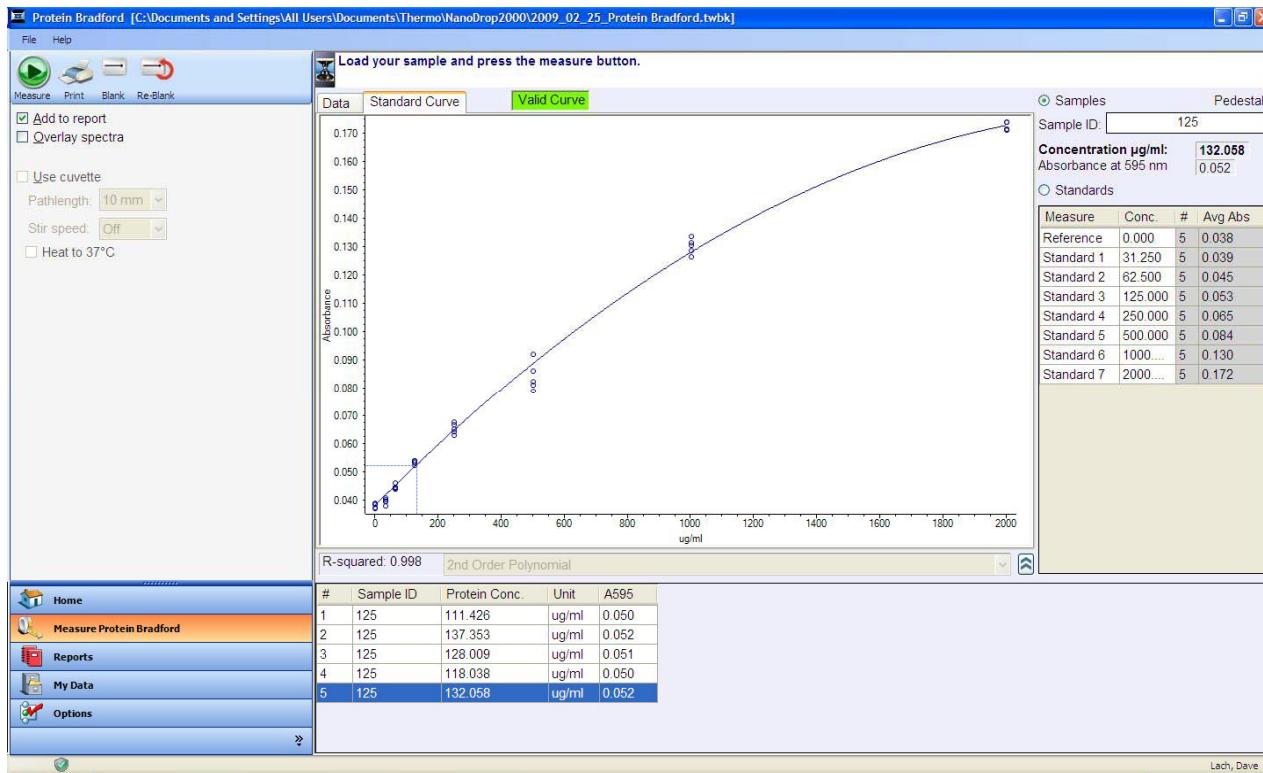
- **Data tab** - displays current measurement data
- **Standard Curve tab** - displays the plot of the standard curve, the  $R^2$  value and the type of curve being displayed. Curve types include interpolation, linear and polynomials. The default selection is “linear.”
- **Valid / Invalid Curve** –indicates whether the minimum number of standards has been measured. Once the criteria have been met, the standard curve status will change from Invalid Curve (red) to Valid Curve (green).

**Note:** The status is only an indicator that the minimum number of required points has been established for the curve fit type selected. The Standard Curve Status indicator does not validate the integrity of the curve. Additional standards may be required to cover expected assay concentration range.

- **Samples radio button** – Once the criteria have been met for a valid curve, the Sample ID field will become active when selected. The sample ID should be entered prior to making the measurement. The sample ID should be entered prior to making the measurement.
- **Standards radio button** – enables entry of standard names and concentrations into the standards table when selected.
- **Concentration ug/ml** - concentration of current sample in ug/ml.
- **Absorbance at 595 nm** – absorbance of the Coomassie dye complex at 595 nm.

## Bradford Standard Curves

A standard curve is required for Bradford assays.



Example Standard Curve: 50:1 reagent/sample volume.

- A standard curve can be generated using a minimum of two points which may include two standards or a reference (Bradford reagent/assay buffer only – no protein) and one standard.
- The multi-point curve capability allows for multiple replicates for up to seven standards. There is no set order in which standards must be run.
- Additional standards may only be added before unknown samples are measured. Once the first sample is measured, no additional standards or replicates may be added.
- A standard may be deleted at any time before a sample has been measured. Right-click the results table to the far right to delete a standard or clear the entire standards table. Right-click a standard within the replicates table under the graph display to delete individual standard replicates.
- Deleted standards cannot be added back or re-measured once the first sample is measured.

- In order to establish a new standard curve, a new workbook must be created. Colorimetric applications will offer several options regarding the use of previous standard curves when appending sample data to previous workbooks. It is recommended that the user follow the protein assay manufacturer's guidelines for generating standard curves before appending new data to a workbook.

**Note:** If selecting a previously saved workbook, all concentration calculations for newly measured samples will be based upon the standard absorbance values saved in the workbook. Each workbook will archive only one standard curve.

## Making Bradford Assay Measurements

- Refer to the manufacturers' guidelines and recommendations for sample preparation.
- The Zero Reference standard is a solution of the same buffer/dye reagent composition as the other standards and samples but without any protein added.
- Prepare both standards and unknowns in the same manner. Use a diluent of the same pH and ionic strength for all blanks, standards and unknown samples.
- Standards diluted from a stock must cover the expected range of the unknown samples. Sample protein concentrations are not extrapolated beyond measured standard concentrations.

## Procedure

- Select the **Protein Bradford** application from the main menu. If the wavelength verification window appears, ensure the arm is down and click **OK**.
- Enter the values for each standard concentration in the right pane table. The software allows for the reference and up to 7 additional standards. The Reference and/or standards can be measured in replicates.

**Note:** The minimum requirement for standard curve generation is the measurement of two standards or the measurement of the zero reference and at least one standard. It is recommended that additional standards be included as necessary to cover the expected assay concentration range.

- Select **Add to report** to automatically include all measurements in the current report. The default setting is for all samples to be added to reports. The **Add to report** checkbox must be selected prior to a measurement to save the sample data to a workbook.
  - Select the file drop-down option **Use current settings as default** as a convenient way to limit set-up time for each new workbook.
- Select **Overlay spectra** to display multiple spectra at a time.
- Establish a blank using the appropriate buffer. The blank for colorimetric assays is generally dH<sub>2</sub>O.
  - Pedestal Option: Pipette 2 µL of dH<sub>2</sub>O onto the bottom pedestal, lower the arm and click **Blank**.
  - Cuvette Option (Model 2000c only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

**Note:** The arm must be down for all measurements, including those made with cuvettes. It is recommended that cuvettes be removed from the instrument prior to making a pedestal measurement to ensure that the pedestal arm can move to the proper starting position.
- Under the Standards tab, highlight a standard and load as described for the blank above. Click **Measure** to initiate the measurement. Be sure to measure all standards prior to measuring samples.

7. After all of the Standards have been measured, click on the **Samples** radio button. Enter a sample ID. Load 2  $\mu\text{L}$  of sample when using the pedestal or insert the cuvette. Click **Measure** to initiate the measurement. It is not necessary to blank the instrument between the standard and the unknown sample measurements.

**Note:** A fresh aliquot of sample should be used for each measurement.

#### After the measurement:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe and the instrument is ready to measure the next sample.
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples.

## Protein Pierce 660 nm

### Overview

The Thermo Scientific Protein Pierce 660 nm assay reagent is a ready-to-use formulation that offers rapid, accurate and reproducible colorimetric detection of minute amounts of protein in solution. The reagent is ideal for measuring total protein concentration in samples containing reducing agents and/or detergents.

### Protein Pierce 660 nm Reagent and Protocols

This protocol uses a 15:1 reagent/sample volume ratio. When setting up an assay for pedestal measurements, a minimum sample volume of 4  $\mu\text{L}$  in 60  $\mu\text{L}$  of reagent is recommended.

Follow the assay kit manufacturer's recommendations for all standards and samples (unknowns), ensuring they are subjected to the same timing and temperature throughout the assay.

Protein standards (BSA) for generating a standard curve may also be provided by the manufacturer for the Pierce 660 nm assay.

### Pierce 660 nm Assay Measurement Range

When using a dilution of 4  $\mu\text{L}$  of sample in 60  $\mu\text{L}$  of reagent, the Protein Pierce 660 nm assay concentration range of detection is 50  $\mu\text{g}/\text{ml}$  to 125  $\mu\text{g}/\text{ml}$ . Refer to "[Measurement Ranges](#)" for additional information.

### Sample Volume Requirements

Although sample size is not critical, it is essential that a liquid column is formed when using the pedestal option so that the gap between the upper and lower measurement pedestals is bridged with sample.

The dominant factor determining the surface tension of a droplet is the hydrogen bonding of the lattice of water molecules in solution. Generally, all additives including protein, buffer salts and detergent-like molecules may reduce the surface tension by interfering with the hydrogen bonding between water molecules. Although 1  $\mu\text{L}$  volumes are usually sufficient for most sample measurements, increasing the sample size to 2  $\mu\text{L}$  for protein measurements will ensure proper column formation for samples with reduced surface tension.

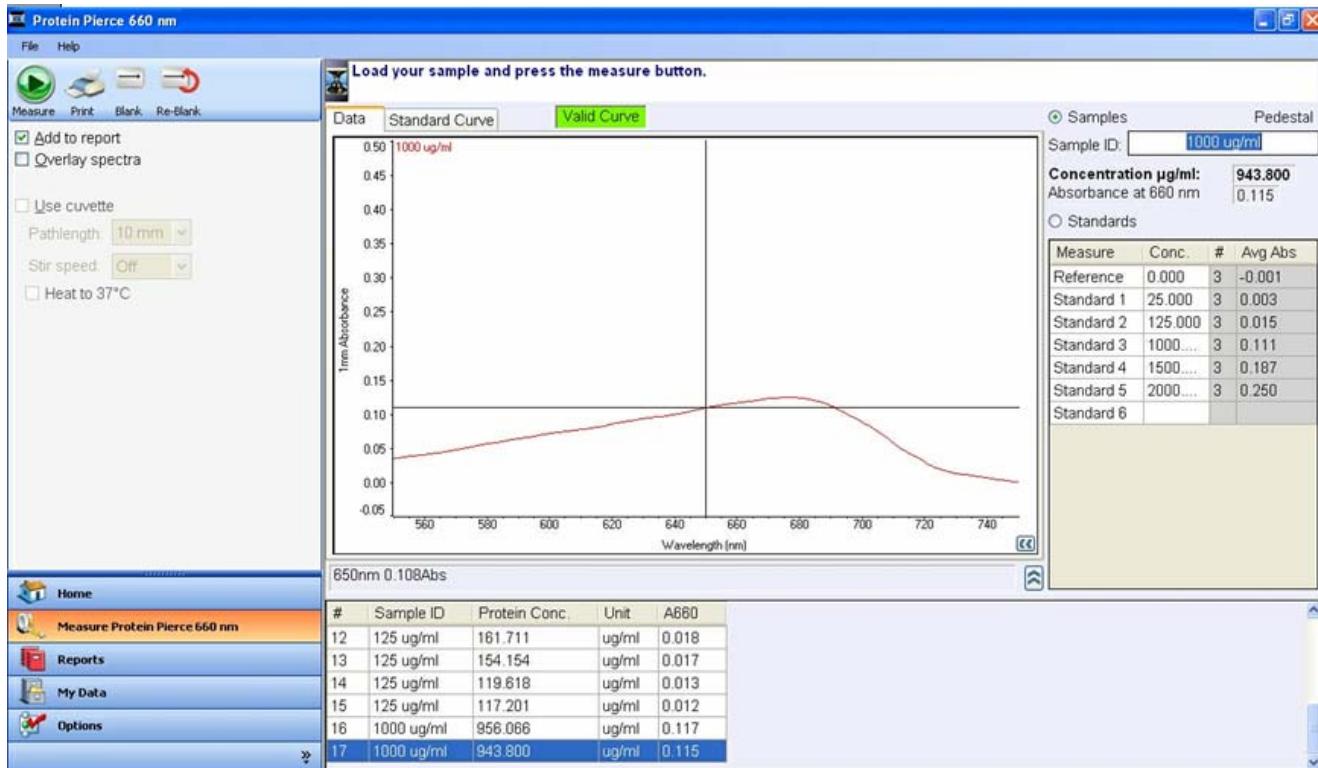
When using the cuvette option, it is essential that sufficient sample volume be used to ensure that the light path is passing through a representative portion of the sample. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for recommended volumes.

### Pedestal Reconditioning

Solutions and reagents containing surfactants may "un-condition" the measurement pedestal surfaces so that the liquid column does not form. If this occurs, use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. Additional information about PR-1 may be found on our [website](#).

## Unique Screen Features

The right pane displays features specific to the Protein Pierce 660 nm application. Task bars in the left pane not described below are described in "[Software Overview](#)".



The spectral display shows data for the current sample normalized to a 1 mm path if measured using the pedestal mode or the pathlength selected if using the cuvette option (model 2000c only). The pathlength information is indicated on the Y axis.

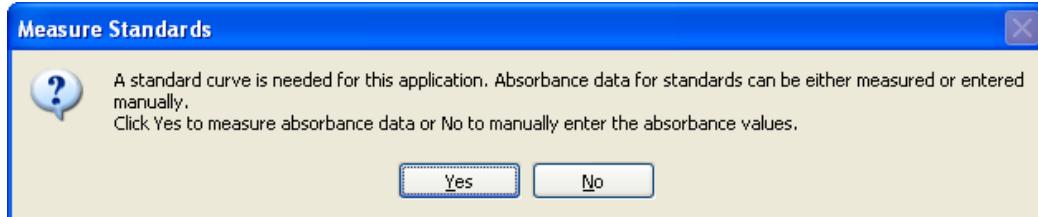
- **Data tab** - displays current measurement data.
- **Standard Curve tab** - displays the plot of the standard curve, the  $R^2$  value and the type of curve being displayed. Curve types include interpolation, linear and polynomials. The default selection is "linear."
- **Valid / Invalid Curve** –indicates whether the minimum number of standards has been measured. Once the criterion has been met, the standard curve status will change from Invalid Curve (red) to Valid Curve (green).

**Note:** The status is only an indicator that the minimum number of required points has been established for the curve fit type selected. The Standard Curve Status indicator does not validate the integrity of the curve. Additional standards may be required to cover expected assay concentration range.

- **Samples radio button** – Once the criterion has been met for a valid curve, the Sample ID field will become active when selected. The sample ID should be entered prior to making the measurement. The sample ID should be entered prior to making the measurement.
- **Standards radio button** – enables entry of standard names and concentrations into the standards table when selected.
- **Concentration  $\mu\text{g}/\text{ml}$**  - concentration of current sample in  $\mu\text{g}/\text{ml}$ .
- **Absorbance at 660 nm** – absorbance of the Coomassie dye complex at 660 nm for the 1 mm pathlength.

## Protein Pierce 660 nm Assay Workbooks

When the Protein Pierce 660 nm application is selected from the main menu, the following message will appear:

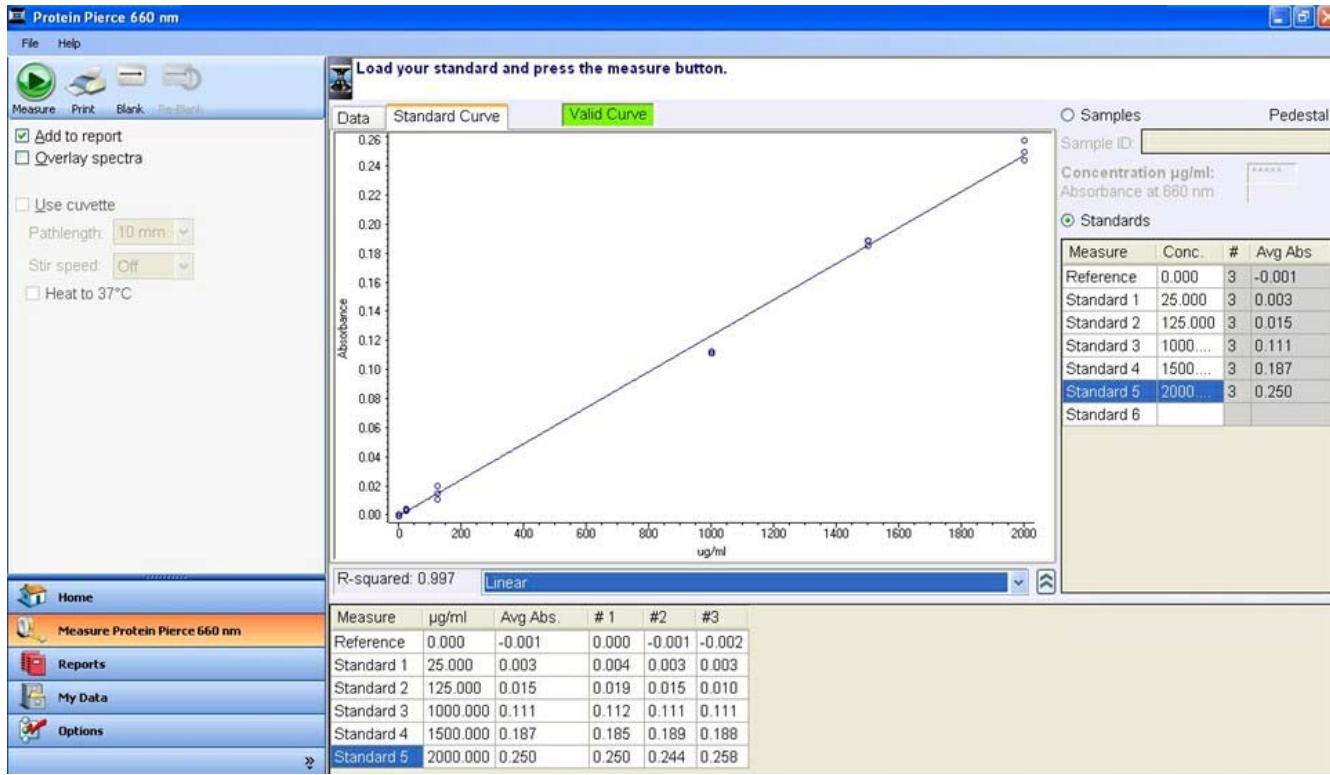


Although standard curve absorbance values may be entered manually rather than measured, it is recommended that the user follow the manufacturer's suggestions for generating new standard curves for each assay.

In order to establish a new standard curve, a new workbook must be created. If a previously saved workbook is used, all concentration calculations for the newly measured samples will be based upon the saved curve archived in the workbook.

**Note:** Each workbook will archive only one standard curve.

## Standard Curves



Example Standard Curve: 15:1 reagent/sample volume

A standard curve is required by the software every time the Protein Pierce 660 nm assay is run. Although curves can be saved and reloaded in the NanoDrop 2000/200c software, it is recommended that the user follow manufacturers' guidelines regarding the use of saved curves when running this assay.

A blank must be measured before the standard curve may be generated. It is advisable to use the dye reagent without any protein added as both the blank and the zero reference sample.

**Note:** This is unlike the other colorimetric assays on the NanoDrop 2000/2000c where it is recommended that water be used as the blank.

- A standard curve can be generated using a minimum of two points which may include two standards or a reference (Protein Pierce reagent/assay buffer only – no protein) and one standard.
- The multi-point curve capability allows for multiple replicates for up to seven standards. There is no set order in which standards must be run.
- Additional standards may only be added before unknown samples are measured. Once the first sample is measured, no additional standards or replicates may be added.
- A standard may be deleted at any time before a sample has been measured. Right-click the results table to the far right to delete a standard or clear the entire standards table. Right-click a standard within the replicates table under the graph display to delete individual standard replicates.
- Deleted standards cannot be added back or re-measured once the first sample is measured.
- In order to establish a new standard curve, a new workbook must be created. Colorimetric applications will offer several options regarding the use of previous standard curves when appending sample data to previous workbooks. It is recommended that the user follow the protein assay manufacturer's guidelines for generating standard curves before appending new data to a workbook.

**Note:** If selecting a previously saved workbook, all concentration calculations for newly measured samples will be based upon the standard absorbance values saved in the workbook. Each workbook will archive only one standard curve.

## Making Protein Pierce 660 nm Assay Measurements

### Sample and Standards Preparation

- Refer to the manufacturers' guidelines and recommendations for sample preparation.
- The Zero Reference standard is a solution of the same buffer/dye reagent composition as the other standards and samples but without any protein added.
- Prepare both standards and unknowns in the same manner. Be sure to use a diluent of the same pH and ionic strength for all blanks, standards and unknown samples.
- Standards diluted from a stock must cover the expected range of the unknown samples. Pre-diluted standard sets may be available from some manufacturers.

### Procedure

1. Select the **Protein Pierce 660 nm** application from the main menu. If the wavelength verification window appears, ensure the arm is down and click **OK**.
2. Enter the values for each standard concentration in the right pane table. The software allows for the reference and up to 7 additional standards. The Reference and/or standards can be measured in replicates.

**Note:** The minimum requirement for standard curve generation is the measurement of two standards or the measurement of the zero reference and at least one standard. It is recommended that additional standards be included as necessary to cover the expected assay concentration range.

3. Select **Add to report** to automatically include all measurements in the current report. The default setting is for all samples to be added to reports. The **Add to report** checkbox must be selected prior to a measurement to save the sample data to a workbook.

- Select the file drop-down option **Use current settings as default** as a convenient way to limit set-up time for each new workbook.
4. Select **Overlay spectra** to display multiple spectra at a time.
  5. Establish a blank using the appropriate buffer. It is advisable to use the dye reagent and protein buffer without any protein added as both the blank and zero reference sample.
    - Pedestal Option: Pipette 2  $\mu$ L of blank solution onto the bottom pedestal, lower the arm and click **Blank**.
    - Cuvette Option (Model 2000c only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.
- Note:** The arm must be down for all measurements, including those made with cuvettes. It is recommended that cuvettes be removed from the instrument prior to making a pedestal measurement to ensure that the pedestal arm can move to the proper starting position.
6. Under the Standards tab, highlight a standard and load as described for the blank above. Click **Measure** to initiate the measurement. Be sure to measure all standards prior to measuring samples.
  7. After all of the Standards have been measured, click on the **Samples** radio button. Enter a sample ID. Load 2  $\mu$ L of sample when using the pedestal or insert the cuvette. Click **Measure** to initiate the measurement. It is not necessary to blank the instrument between the standard and the unknown sample measurements.

**Note:** A fresh aliquot of sample should be used for each measurement.

#### After the measurement:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe and the instrument is ready to measure the next sample.
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples.

## Cell Cultures

### Overview

Using an absorbance spectrophotometer to monitor light scattered by non-absorbing suspended cells is common practice in life science laboratories. Such applications, more than any other, accentuate the differences amongst the optical systems of the numerous spectrophotometer designs.

The most distinct difference between the NanoDrop 2000/2000c pedestal “absorbance” values for microbial cell cultures and those made with the cuvettes will be attributable to the shorter pathlength (1 mm vs. 1 cm.) Measurements made using the pedestal mode will be approximately 10 fold lower than those made using a 10 mm pathlength cuvette. Values between the NanoDrop 2000c and classical cuvette based spectrophotometers may not be exactly the same as readings are dependent on both the optics of a specific spectrophotometer as well as the cell type in suspension.

The Cell Cultures application displays the sample spectrum from 250 nm to 700 nm.

### Sample Size Requirements

Although sample size is not critical, it is essential that a liquid column is formed when using the pedestal option so that the gap between the upper and lower measurement pedestals is bridged with sample. For cell cultures, it is also important to ensure that the cells have not settled and that a representative aliquot has been taken of the cell culture. It is recommended that 2 µL volumes be used when making cell culture measurements.

When using the cuvette option, it is essential that sufficient sample volume be used to ensure that the light path is passing through a representative portion of the sample. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for recommended volumes.

### Sample Homogeneity

Ensure that the cell suspension is homogeneous before removing an aliquot for its “absorbance” measurement. Vigorous mixing may be required before measuring aliquots of concentrated samples on the measurement pedestals. Use the stirring capability when making microbial cell culture cuvette measurements.

### Measurement Range

Due to its shorter pathlengths, the NanoDrop 2000/2000c can directly monitor concentrated cell suspensions. Since the entire spectrum is displayed, dilute samples exhibiting low absorbance at 600 nm may be monitored at lower wavelengths, for example 400 nm. Alternatively, use the cuvette mode available in the 2000c model to monitor dilute microbial cell cultures.

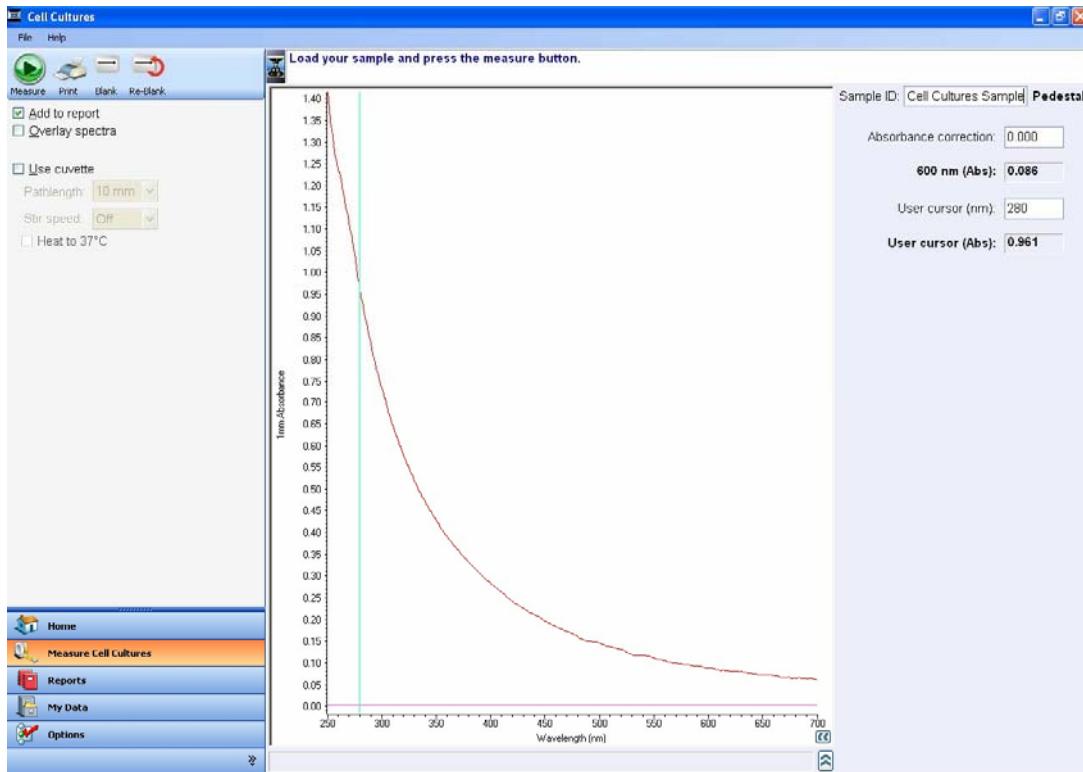
### Decontamination of Measurement Pedestals

If decontamination is necessary, a sanitizing solution, such as a 0.5% solution of sodium hypochlorite (1:10 dilution of common commercial bleach solutions – freshly prepared), can be used to ensure that no biologically active material is present on the measurement pedestals.

**Note:** Do not use a squirt bottle to apply the diluted bleach. Always dampen a laboratory wipe with the bleach then clean the top and bottom pedestal surfaces as well as the outside of the instrument. Remove the bleach solution with a lab wipe dampened with water. Do not squirt any cleaning solution into the cuvette holder assembly as the liquid may drip into the instrument.

## Unique Screen Features

The right pane displays features specific to the Cell Cultures application. Task bars in the left pane not described below are described in “[Software Overview](#).”



The spectral display shows data for the current sample normalized to a 1 mm path if measured using the pedestal mode or the selected pathlength if using the cuvette option (model 2000c only). The pathlength information is indicated on the Y axis.

- **Absorbance correction** - user defined baseline cursor value. The baseline may be moved by selecting the cursor and vertically dragging it to a different position or by entering a specific value.
- **600 nm (Abs)** – 600 nm absorbance value, as measured from the user defined baseline.
- **User cursor (nm)** - user defined cursor position. The cursor may be moved by selecting it and horizontally dragging to a different position or by entering the desired wavelength.
- **User cursor (Abs)** - absorbance value at the user defined wavelength, as measured from the user defined baseline.

## Making Cell Cultures Measurements

1. Select the **Cell Cultures** application from the main menu. If the wavelength verification window appears, ensure the arm is down and click **OK**.
2. Select **Add to report** to automatically include all measurements in the current report. The default setting is for all samples to be added to reports. The **Add to report** checkbox must be selected prior to a measurement to save the sample data to a workbook.
3. Select **Overlay spectra** to display multiple spectra at a time.

4. Establish a blank using the appropriate buffer. The reference or blank solution generally is the buffer that the molecule of interest is suspended or dissolved in. This solution should be the same pH and of a similar ionic strength as the sample solution.
  - Pedestal Option: Pipette 2  $\mu$ L of the appropriate blanking solution onto the bottom pedestal and click **Blank**.
  - Cuvette Option (Model 2000c only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

**Note:** The arm must be down for all measurements, including those made with cuvettes. It is recommended that cuvettes be removed from the instrument prior to making a pedestal measurement to ensure that the pedestal arm can move to the proper starting position

5. Enter a Sample ID in the appropriate field, load the first sample as described for the blank above and click **Measure**.

**Note:** It is very important to ensure that cells have not settled prior to taking an aliquot for measurement. A fresh aliquot of sample should be used for each measurement.

#### After the measurement:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe and the instrument is ready to measure the next sample.
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples.

## Application FAQs

**Q: Does the NanoDrop 2000/2000c produce results for a continuous spectrum or just selected wavelengths?**

**A:** Continuous spectrum from 190-840 nm. The range will vary for specific applications.

**Q: What are the sample size requirements for the NanoDrop 2000/2000c pedestal?**

**A:** As little as 1  $\mu$ L is usually sufficient for most applications. Generally all additives including protein, DNA, RNA, buffer salts, and detergent like molecules reduce the surface tension by interfering with the hydrogen bonding between water molecules. Although 1  $\mu$ L volumes are usually sufficient for most sample measurements, increasing the sample size to 2  $\mu$ L will ensure proper column formation for samples with reduced surface tension properties.

**Q: When is a 0.5  $\mu$ L volume sufficient?**

**A:** The **small sample volume** option is available in the Nucleic Acid or Protein A280 applications for samples that have 10 mm equivalent absorbance values of 3.0 or higher ( $>150 \text{ ng}/\mu\text{L}$  dsDNA or  $>4.5 \text{ mg}/\text{mL}$  BSA).

**Q: What are the sample size requirements when using a cuvette with the NanoDrop 2000c?**

**A:** It is essential to ensure that the sample volume in the cuvette is adequate to allow the light to pass through a representative portion of the sample when making a cuvette measurement. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for recommended volumes.

**Q: What sort of accuracy should I expect with the NanoDrop 2000/2000c?**

**A:** Typically within 2%.

**Q: What sort of reproducibility should I expect with the NanoDrop 2000/2000c?**

**A:** Typically  $\pm 0.1\text{mg}/\text{mL}$  for samples  $< 10 \text{ mg}/\text{mL}$  and  $\pm 2\%$  for samples  $> 10 \text{ mg}/\text{mL}$ .

**Q: What biomolecules are routinely analyzed using the NanoDrop 2000/2000c?**

**A:** The NanoDrop 2000/2000c is routinely used to determine the concentrations of dsDNA, RNA, ssDNA, fluorescent dyes incorporated into micro array and proteomic probes, microbial cell cultures as well as virtually any aqueous analyte that exhibits absorbance in the 190 to 840 nm range.

**Q: Do nucleic acids require purification prior to measurement on the NanoDrop 2000/2000c?**

**A:** Yes. Absorbance measurements are not specific for a particular nucleic acid and will be affected by the presence of nucleotides and other molecular contaminants which absorb at 260 nm.

**Q: I am seeing abnormal 260/230 ratios. What is wrong?**

**A:** Some buffers and reagents contain fluorophores with their own unique peaks that may carry through during the purification process. To check, measure a sample of your buffer against a water blank.

**Q: Where can I find my data?**

**A:** The user is prompted to designate a workbook (.twbk) prior to making measurements. This workbook may be stored at a location of the user's preference. All measurements are also automatically archived at the following location:

XP: C:\Documents and Settings\All Users\Shared Documents\Thermo\NanoDrop2000\Autosave

Vista: C:\Users\Public\Public Documents\Thermo\Autosave\ (Software Application)

**Q: Can I quantify proteins using the NanoDrop 2000/2000c?**

**A:** Yes. Options include the Protein A280 application for purified proteins and the Proteins & Labels application for antibodies and other proteins incorporating fluorescent labels. In addition, colorimetric assays such as BCA, Bradford, Lowry, and Protein Pierce 660 nm can be run using the NanoDrop 2000/2000c.

**Q: What is the dynamic (concentration) range for proteins that can be measured on the NanoDrop 2000/2000c?**

**A:** BSA concentrations between 0.1 mg/mL to 400 mg/mL can be measured using the Protein A280 application.

**Q: I am using a colorimetric method (e.g. Bradford, BCA etc.,) to determine the protein concentration. Can I measure the sample using the A280 method on the NanoDrop instrument?**

**A:** The Protein A280 application is most applicable to purified proteins. Colorimetric assays such as BCA, Pierce 660 nm, Bradford, and Lowry are generally used for uncharacterized protein solutions and cell lysates. If you are currently using a colorimetric assay to measure proteins, it is recommended that you use one of the pre-programmed colorimetric methods available on the NanoDrop 2000/2000c.

**Q: Do I have to generate a new standard curve each time I run a colorimetric assay on the NanoDrop 2000/2000c?**

**A:** No. You may choose to load a previously generated standard curve. However, it is recommended that you follow the colorimetric reagent manufacturer's recommendations.

**Q: Can I measure proteins that are not predefined into the NanoDrop 2000/2000c software?**

**A:** Yes, you can measure any protein that has absorbance at 280 nm with a known extinction coefficient and molecular weight.

## 4. Method Editor

The Method Editor application and the List of Custom Methods box on the main menu are used to create, edit, save and view custom methods. The Editor Options task bar will be displayed when the Method Editor application is selected and is used to set limits prior to utilizing the Method Editor.

### Editor Options

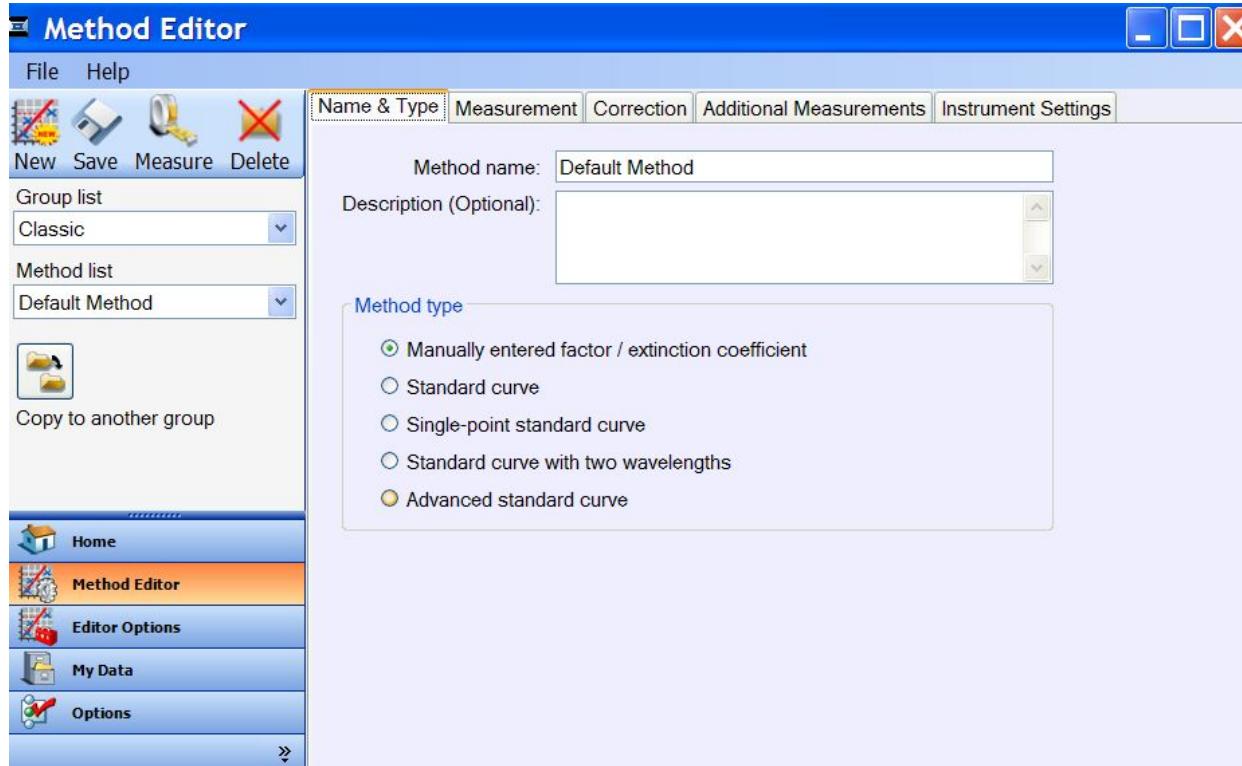
This feature includes 3 tabs to define the limits for settings available within the Method Editor:

- **Formula** - a list of predefined formulas which allows for entry and editing of user defined formulas. Formula selected within this tab will be available for use within the Method Editor. Predefined methods identified by the lock icon may not be edited or deleted.

**Note:** The Reset icon will delete all user-entered and saved formulas and revert to the default list.

- **Units** - the list of default and user-defined units that will be available for use within a custom method. Predefined units, identified by the lock icon, may not be edited or deleted.
- **Advanced** - a list of pre-defined standard curve equations for advanced custom method operations. Additional user-defined equations can be added. Predefined equations, identified by the lock icon, may not be edited or deleted.

### Method Editor Features



## Left Pane

When the **Method Editor** task bar is selected, the following icons will appear at the top of the left pane:

- **New** - creates a new custom method.
- **Save** - saves a new custom method. This icon will be grayed out until the minimum setting requirements for a new method have been entered. A new custom method may be saved to Groups other than Classic by using the **Copy to another group** icon located under the method list drop down box.
- **Measure** - opens a new custom method once the method is saved to at least one group. This icon will be grayed out until the minimum setting requirements for a new method have been entered.
- **Delete** - deletes the highlighted method in the method list box from the selected group.

Under the icons are two drop-down list boxes:

- **Group List** – selects the group to which the new methods is saved. The default group is Classic.
- **Method List** - displays the available methods associated with the selected group.

## Right Pane

- **Name & Type** - defines the custom method name and type. The type refers to the method of calculating the sample concentration and offers a choice of multiplying absorbance values by factors/extinction coefficients or utilizing standard curves.

Method types include:

- **Manually entered factor/extinction coefficient** - a molecular weight is required if the results units and the user defined factor/extinction coefficient units are not both weight based and molarity based. Click **Information** icons within the Method Editor for additional information.
- **Standard Curve** - uses a simple standard curve based on the absorbance of a set of standards at the specified analysis wavelength to determine a sample concentration.
- **Single Point Standard Curve** - uses the sample absorbance value divided by the average absorbance value of the single standard to determine the sample concentration as a ratio of the standard concentration.
- **Standard Curve with two wavelengths** - calculates the sample concentration by taking the average of values determined by each of two standard curves associated with the user defined wavelengths.
- **Advanced Standard Curve** - calculates the sample concentration as determined by a standard curve generated as a result of a user defined equation.
- **Measurement** - defines the analysis wavelength and the preferred result units. The measurement results or concentration will be calculated using the absorbance value at the analysis wavelength and by applying the selected method type (factor or standard curve). The default label for the result name is Concentration. If an alternate user-defined label is entered, it will be utilized in the user-defined workbook and autosave files.
- The spectral display shows data for the current sample normalized to a 1 mm pathlength if measured using the pedestal mode or the selected pathlength if using the cuvette option (model 2000c only). The pathlength is indicated on the Y axis.
- **Correction** - selects both an across the spectrum baseline correction and a choice of three corrections for the entered analysis wavelength. The analysis corrections of None, Single point, or Sloping baseline subtract from the absorbance of the analysis wavelength. The baseline correction offsets the spectrum such that the absorbance at the correction wavelength is subtracted from the entire spectrum.

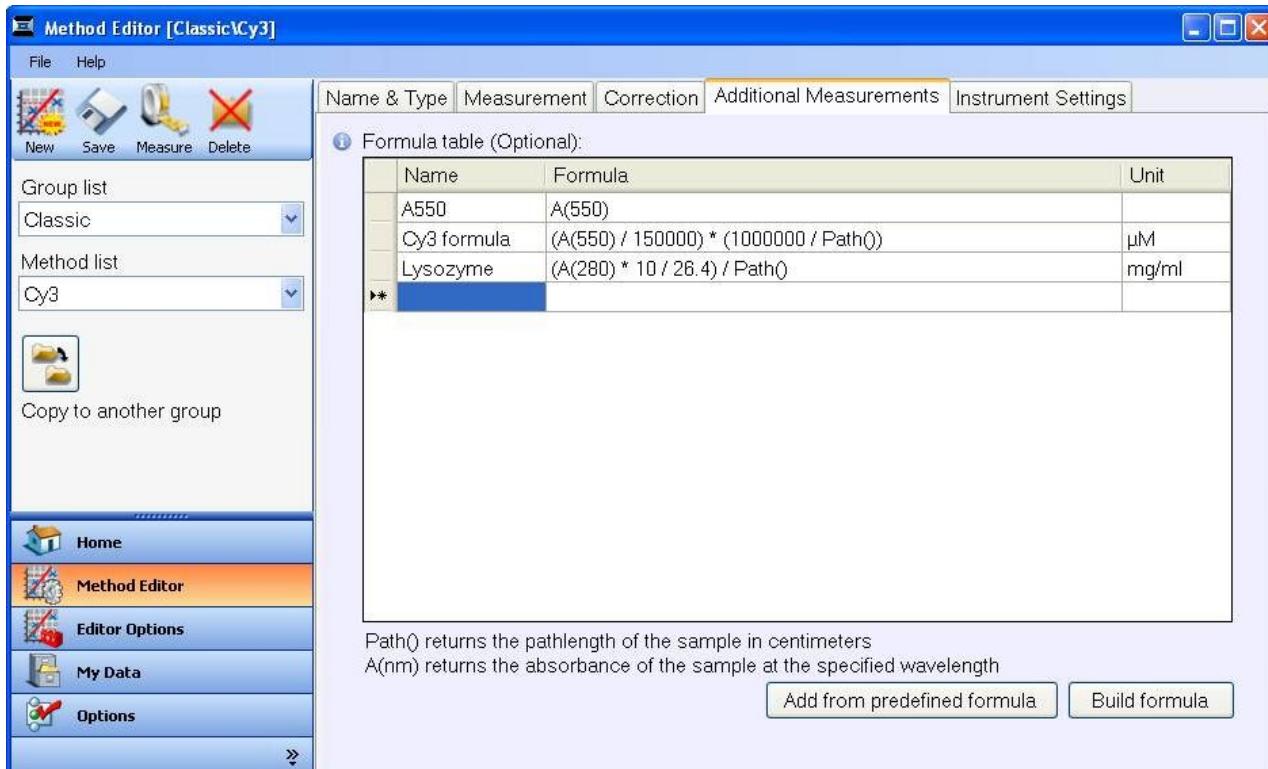
- **Additional Measurements** - adds user-selected or user-defined formulas for additional processing of data. Refer to “Creating New Formulas” below for additional details regarding how to build a custom formula.
- **Instrument settings** - selects the spectral display wavelength range for the method. The auto pathlength option automatically uses the optimal pedestal pathlength for high concentration samples. It is recommended that the auto pathlength feature be selected for most sample measurements.
  - When selected, the optimal shorter pedestal pathlength is used when the specified analysis wavelength has a 1 mm equivalent absorbance value >1.25. (For analysis nm between 190 to 219 nm, the change to the shorter pathlength occurs at 1 mm equivalent absorbance values of >1.0.)
  - When the auto pathlength is not selected, only the 1 mm pathlength is used. Concentrated samples may result in jagged peaks indicating detector saturation.
- **Standard** - an additional tab that appears when a standard curve option is selected as the method type. This tab is used to define the initial standard names and concentration values that appear in the custom method and is used to define the curve fit used in the method. Curve fit choices include: linear, linear through zero, interpolation, 2<sup>nd</sup> and 3<sup>rd</sup> order polynomials.
  - The Standard tab is also used to define, save or recall a series of standard concentration values. Although standard concentration values for a method can be entered within this page, the values may also be entered or modified when the method is opened to make measurements. Absorbance data associated with a set of standards is not entered or recalled using this feature.
  - A standard curve can be generated using a minimum of two points which may include two standards or a reference (assay buffer only – no protein) and one standard.
  - The multi-point curve capability allows for multiple replicates for up to seven standards. There is no set order in which standards must be run.
  - Additional standards may only be added before unknown samples are measured. Once the first sample is measured, no additional standards or replicates may be added.
  - A standard may be deleted at any time before a sample has been measured. Right-click the results table to the far right to delete a standard or clear the entire standards table. Right-click a standard within the replicates table under the graph display to delete individual standard replicates.
  - Deleted standards cannot be added back or re-measured once the first sample is measured.
  - In order to establish a new standard curve, a new workbook must be created.

### **Creating New Formulas**

The Additional Measurements tab is used to define measurements that are included in the report in addition to the principle concentration setup on the Measurement tab of a method.

New measurement values are defined by entering the desired value name, the formula used to calculate the value, and the unit of measure associated with the value. A formula can be keyed-in manually, selected from a list of predefined formulas, or constructed from a set of equation templates using the “Build formula” button.

Each formula consists of a mathematical expression written in a form similar to that used in a computer language or command script language. They are composed of constant values, arithmetic operations, a set of mathematical functions, and functions that extract values from the sample data collected.



Example: Lysozyme       $(A(280) * 10 / 26.4) / \text{Path}()$       mg/ml

The above example will result in a "Lysozyme" column to be added to the report. The value will be the absorbance at 280 nm multiplied by an adjustment factor of 10 divided by the constant 26.4 divided by the pathlength used in centimeters.

**Note:** The 10 fold factor is used to adjust the E1% extinction coefficient value to a 0.1% value. This adjustment allows the protein concentration to be reported in units of mg/ml.

Pedestal measurements will use the value 0.1 for the pathlength whereas cuvette measurements will apply the user-selected pathlength.

All equation functions are case sensitive. Spaces are not allowed between a function name and "(" For example- A(280) is correct, but A (280) is incorrect.

Min(x, y)	Minimum of x and y
Max(x, y)	Maximum of x and y
abs(x)	Absolute value of x
Ceiling(x)	Round the fractional value of x up to an integer
Floor(x)	Round the fractional value of x down to an integer
Round(x)	Round decimal x to the nearest integer
Sqrt(x)	Square root of x
Area(x, y)	Area under the curve from wavelength x to y
Path()	Represents the pathlength in cm used to calculate concentration
A(x)	Absorbance at wavelength x
R(x)	Reflectance at wavelength x
T(x)	Transmittance at wavelength x
C(x)	Concentration of specified component x
Ln(x)	Natural logarithm of x
exp(x)	Returns e raised to the power of x where e is 2.718
Log(x) or log10(x)	Log base 10 of x
Pow(x, y)	Take x to the y power

## Sharing of Custom Methods

Custom methods may be shared by simply pasting a copy of the desired method into the appropriate folder. The method folder is located at:

XP C:\Documents and Settings\All Users\Shared Documents\Thermo\NanoDrop2000\Custom Methods  
Vista C:\Users\Public\Documents\Thermo\NanoDrop2000\Custom Methods\Classic

## Making Custom Method Measurements

1. Select the custom method from the main menu custom method list box. Alternatively, choose **Measure** from within the method editor.
2. Select **Add to Report** to automatically include all measurements in the current report. The default setting is for all samples to be added to reports. The **Add to report** checkbox must be selected prior to a measurement to save the sample data to a workbook.
3. Select **Overlay spectra** to display multiple spectra at a time.
4. If running a method that utilizes a standard curve, enter the values for each standard concentration in the right pane table. The software allows for the reference and up to 7 additional standards. The Reference and/or standards can be measured in replicates.

**Note:** The minimum requirement for standard curve generation is the measurement of two standards or the measurement of the zero reference and at least one standard. It is recommended that additional standards be included as necessary to cover the expected assay concentration range. Standards values be modified prior to measurement.

5. Establish a blank using the appropriate buffer. The reference or blank solution generally is the buffer that the molecule of interest is suspended or dissolved in. This solution should be the same pH and of a similar ionic strength as the sample solution.
  - **Pedestal Option:** Pipette 2  $\mu$ L of the appropriate blanking buffer onto the bottom pedestal, lower the arm and click **Blank**.
  - **Cuvette Option** (Model 2000c only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

**Note:** The arm must be down for all measurements, including those made with cuvettes. It is recommended that cuvettes be removed from the instrument prior to making a pedestal measurement to ensure that the pedestal arm can move to the proper starting position.

6. For method utilizing standards click on the **Samples** radio button after all standards have been measured. Enter a sample ID. Load 1-2  $\mu$ L of sample when using the pedestal or insert the cuvette.
7. Click **Measure** to initiate the measurement. It is not necessary to blank the instrument between the standard and the unknown sample measurements.

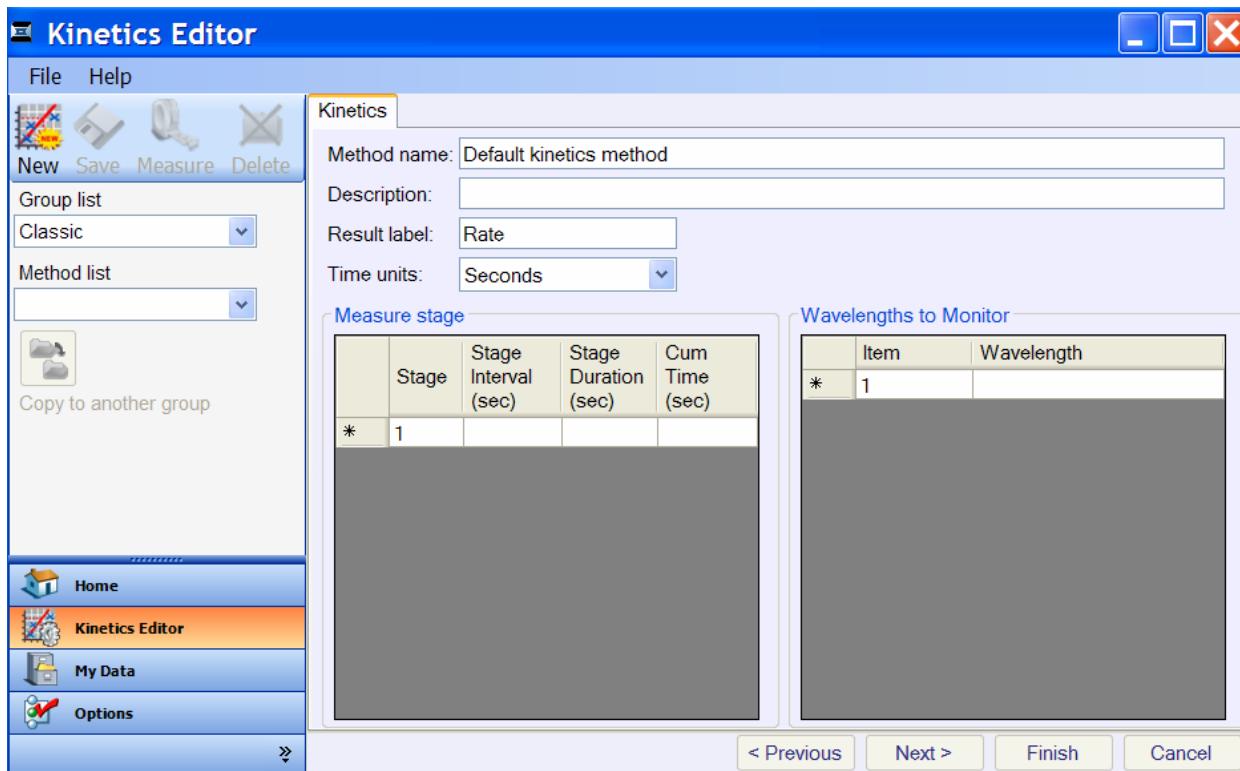
**Note:** A fresh aliquot of sample should be used for each measurement.

### After the measurement:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe and the instrument is ready to measure the next sample.
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples.

## 5. Kinetics

The NanoDrop 2000c can be used to make time-based kinetic measurements. The Kinetics Editor is used to create, edit, save and view custom kinetic methods. Measurements can also be made without saving a method. The software does not support kinetics measurements using the pedestal mode.



### Screen Features

#### Left Pane

When the **Kinetics Editor** is selected, the following icons will be displayed at the top of the left pane:

- **New** - creates a new custom method.
- **Save** - saves the current method definition. When creating a new method the icon will be greyed until the minimum setting requirements for a new method have been met.

**Note:** A new Kinetics method may be saved to groups other than Classic by using the **Copy to another method** icon located under the **Method List** drop down box.

- **Measure** - starts a kinetics method measurement. The kinetics method does not need to be saved prior to running. This icon will be grayed out until the minimum setting requirements for a new method have been entered.
- **Delete** - deletes a kinetics method. This icon will be grayed out until the minimum setting requirements for a new method have been entered.

#### Under the icons are two drop-down list boxes:

- **Group List** - defines a group of methods. The default is **Classic**.
- **Method List** - loads the selected method for editing.

## Right Pane

When **Kinetics Editor** is selected, clicking on the icon at the top left will start a new method. A two-step wizard style interface will appear at the bottom of the right pane. This wizard style interface includes **Previous**, **Next**, **Finish** and **Cancel** buttons on the bottom right of the page.

The right pane features include:

- **Kinetics Tab** - displays the current method name, description, result label and time units. The default setting for time units is seconds.

Two screen panels are used to define a method:

- **Measure Stage** – defines measurement stage intervals and the duration of each stage. To add the first stage, enter a value in the highlighted **Stage Interval** field. Next, click on and enter a value in the highlighted **Stage Duration** field. Click **Enter** and cumulative time will auto-populate. The next row will now be available for additional stage entries. The minimum stage interval allowed is 2.0 seconds.
- **Wavelengths to Monitor** - monitors multiple wavelengths during the reaction. To add the first wavelength, highlight the empty field and enter a value. The next row will become available for additional wavelength selections.
  - A cursor (colored vertical line) will be positioned at each of the monitored wavelengths. These cursors may be moved and the associated changes will be reflected in the rate display.
  - Additional wavelengths may be selected post measurement by positioning the black crosshair cursor at the wavelength of interest and clicking Add.
  - Wavelengths may be removed by selecting the associated vertical-line cursor and Remove.
  - A trace for each specified wavelength will be added to the top panel once the measurement has been initiated.
- **Instrument settings** - selects the spectral display wavelength range: UV, Visible, UV-Vis or custom.

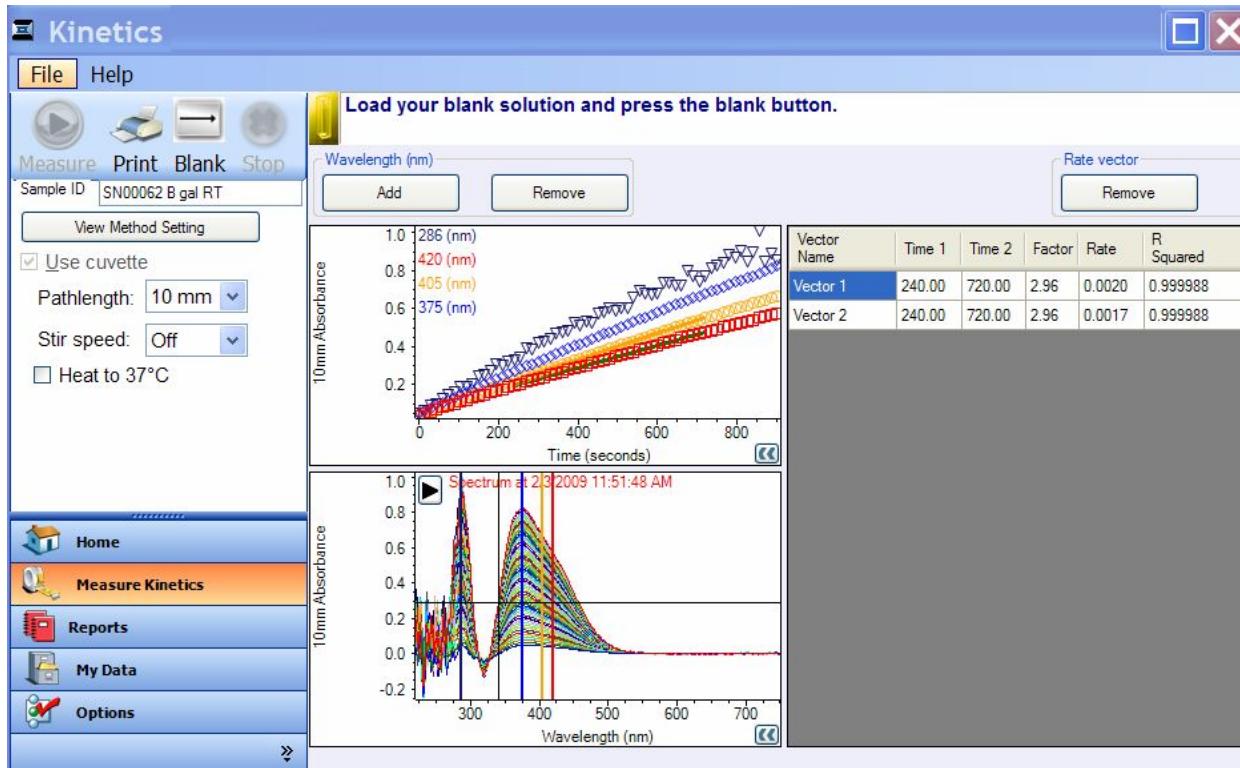
A Kinetics method can either be saved or immediately run without saving. To save a Kinetics method, click **Save** on the upper left side of the left pane.

To start the kinetics measurement, click on **Measure** on the upper left side of the left pane.

**Note:** When a measurement is initiated without first saving the method, the method settings will not be available for review or re-use. The method would have to be recreated for another sample to be run under the same conditions. Only one sample may be saved in each kinetic workbook. Kinetic data is not saved in autosave files.

A previously saved Kinetics method can be edited by highlighting the method name within the method list box on the "Home" screen and clicking **Kinetics Editor**. A previously saved Kinetics method can run by double-clicking on the method name within the method list box. Alternatively a button can be added to the "Home" screen for the method using the Option page.

## Data Acquisition Page



There are two graphical displays in the right pane of the Kinetics screen:

- The Wavelength table to the right of the display shows the absorbance values at each stage of the measurement for all of the wavelengths being monitored.
- The spectrum plot at the bottom of the display shows a trace for each measurement taken in each stage. The data is displayed with absorbance as the vertical axis and wavelength as the horizontal axis. Each spectrum in the display is color-coded. The time and date each spectrum was collected is shown in the upper-left corner of the spectrum plot area using the same color as the associated spectrum. The colored vertical-line crosshairs correspond to the wavelengths the method was initially set to monitor. They can be repositioned, or new ones added, to analyze other wavelengths of interest.
- The top Rate display allows rate vectors to be calculated for selected portions of associated data curves. A data curve is the series of absorbance values at the wavelength selected in the Spectrum plot area at each stage of the measurement. The data is shown as absorbance versus time where absorbance is the vertical axis and time is the horizontal axis. Right-click on a data series to copy the data to a clipboard.

## Calculating a Rate

1. If the wavelength of interest is not indicated by a cursor in the bottom display add the wavelength by positioning the vertical crosshair to the desired wavelength and choosing **Add**.
  - Up to 6 individual moveable cursors may be monitored and displayed.
2. Position the mouse over the initial time point of interest in the top wavelength display. Click and drag the mouse to draw a line to the point at the end time of interest. The vector color will correspond to the primary color associated with that wavelength cursor in the bottom panel although the associated wavelength ID in the top display will be colored red for the active wavelength.

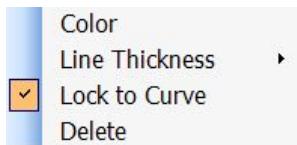
**Note:** Rate vectors are by default locked to the curve and will display the best fit for the two time points selected. Highlight the vector and right-click to deselect the **Lock to curve** feature to manually move the vector.

The data for the rate vector is shown in a table to the right of the Rate display. Fields include:

- **Vector Name** - name of the rate vector.
- **Time 1** - beginning time of the rate vector.
- **Time 2** - end time of the rate vector.
- **Factor** – user-entered rate scaling factor.
- **Rate** - calculated rate or slope between selected time points.
- **R Squared** –an indication of how well the rate vector fits the data points.

#### Working with Rate Vectors:

- To remove a particular rate vector, highlight the line and choose **Remove** in the rate vector box at the top right of the pane.
- To move a rate vector, drag it to the desired location on the data curve.
- To change the start or end times associated with a vector- click either end and resize the vector to reflect the desired time points. Alternatively, highlight the time in the rate table and overtype with the specific time point of interest.
- To change the attributes of a rate vector, right-click it to open the following menu:



- **Color** - changes the color of a rate vector.
- **Line Thickness** - changes the thickness of a rate vector.
- **Lock to Curve** - determines whether a curve fit is applied to a rate vector. De-select this option to manually move the vector.
- **Delete** - removes a rate vector.

#### Making Kinetic Measurements

1. Select a kinetics method from the main menu custom method list box. Alternatively, choose **Measure** from within the Kinetics Editor application.
2. Select the cuvette pathlength. The default selection is 10 mm.
3. Select **Heat to 37°C** if appropriate. Allow the cuvette holder to reach temperature before proceeding. When the **Heat to 37°C** box is selected, the current cuvette holder temperature will be displayed in the bottom status bar.

- It may take between 1- 10 minutes for the cuvette holder to reach temperature.
  - The heater will stop heating when another, workbook, application or method is opened.
4. Establish a blank using the appropriate buffer. Insert a cuvette with the blank solution into the instrument, noting the direction of the light path indicated by the etched arrow and choose **Blank**.
  5. The software will prompt for a workbook name and storage location. It is recommended that the name of the same be included as part of the workbook name. Only one sample is stored in each workbook.
    - The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

**Note:** The arm must be down for all measurements, including those made with cuvettes.

6. Enter a sample ID in the sample ID field in the left pane, inset the cuvette with the sample and choose **Measure**.
7. Remove the cuvette, and follow the recommendations of each specific cuvette manufacturer for the cleaning and maintenance of cuvettes.

**Note:** The sample ID field is located in the left pane for kinetic methods.

As only one sample is associated with each kinetic measurement workbook, the sample ID is not a field that can be selected under the reports configuration tab. The sample ID and method information can be included in a printed report by selecting the **Methods Info** on the **Print** tab under the **Reports** task bar.

## 6. Warranty and Maintenance

### Warranty

All NanoDrop spectrophotometers and accessories manufactured by Thermo Fisher Scientific are under warranty against manufacturing defects in parts and labor for a period of one year. Preventive maintenance, as well as additional one, two, and three year warranty extensions are available. More information about the various plans may be found on our [website](#).

### Replacement Parts

All service and replacement of parts for instruments sold to the US and Canada is done at the NanoDrop products factory in Wilmington, Delaware.

### Safety

Operating the instrument with the cover off exposes the operator to the instrument's sharp metal edges and delicate fiber optics. Removal of the cover may also void the warranty.

The NanoDrop 2000/2000c is designed only for indoor use under the following conditions:

- Temperature: 15-35 °C
- Humidity: 35-65 %

**Note:** The NanoDrop 2000/2000c is supplied with a 12V power supply. Use only the power supply provided with the instrument. The unit also comes with a grounded power cord. Plug this cord into a properly grounded outlet. Use of the instrument in a manner not specified by the manufacturer may impair the protection provided by the supplied power cord and power supply.

The power supply can remain plugged into the NanoDrop 2000/2000c while the instrument is not in use. When the instrument is plugged in but not in use, the power consumption is ~5 W and the flash lamp is not energized. The instrument does not utilize a power switch. It is recommended that the instrument not be positioned in a way that makes it difficult to unplug the power supply from the unit or the wall.

### WEEE Compliance

This product is required to comply with the European Union's Waste Electrical & Electronic Equipment (WEEE) Directive 2002/96/EC. If compliance is required, the instrument is marked with the following symbol:

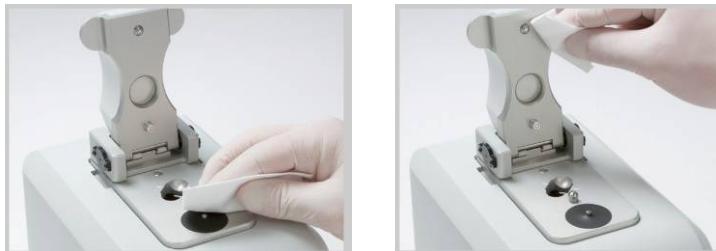


We have contracted with one or more recycling/disposal companies in each EU Member State, and this product should be disposed of or recycled through them. Further information regarding our compliance with these directives, the recyclers in your country, and information on our products which may assist the detection of substances subject to the RoHS Directive are available at [www.thermo.com/WEEERoHS](http://www.thermo.com/WEEERoHS).

## Maintenance of the Sample Retention System

### Cleaning

The primary maintenance requirement for the NanoDrop 2000/2000c is keeping the measurement pedestal surfaces clean. Upon completion of each sample measurement, wipe the sample from the upper and lower pedestals with a clean, lint-free lab wipe to prevent sample carryover and avoid residue buildup.



Although generally not necessary, 2 µL water aliquots can be used to clean the measurement surfaces after high concentration samples to ensure no residual sample is retained on either measurement surface.

1. Apply 3-5 µL of dH<sub>2</sub>O on to the bottom pedestal.
2. Lower the upper pedestal arm to form a liquid column; let it sit for approximately 2-3 minutes.
3. Wipe away the water from both the upper and lower pedestal with a clean lab wipe.

- **Between measurements**

Upon completion of each sample measurement, wipe the sample from both the upper and lower pedestals with a clean, dry, lint-free lab wipe, to prevent sample carryover and avoid residue buildup. It is not necessary to clean with dH<sub>2</sub>O between samples.

- **Between users**

A final cleaning of both measurement surfaces with dH<sub>2</sub>O is recommended before the first sample measurement and after the last sample measurement.

- **Additional cleaning**

When a more rigorous cleaning protocol is required (i.e. dried proteins) substitute 0.5M HCl for the dH<sub>2</sub>O in the procedure above. Follow with 3- 5 µL of dH<sub>2</sub>O.

- **Decontamination**

A sanitizing solution, such as a 0.5% solution of sodium hypochlorite (1:10 dilution of common commercial bleach solutions – freshly prepared), may be used to ensure that no biologically active material is present on the measurement pedestals. Follow with 3- 5 µL of dH<sub>2</sub>O.

The use of detergents or isopropyl alcohol is not recommended as they may un-condition the pedestal measurement surfaces. If a solution containing either is used, it is important to follow with 3- 5 µL of dH<sub>2</sub>O.

**Note:** Do not use a squirt or spray bottle to apply dH<sub>2</sub>O or any other liquid to the surface of the instrument.

A single page *Cleaning and Reconditioning Guide* can be accessed from the “Appendices” chapter of the *NanoDrop 2000 User Manual* PDF. It is recommended that this guide be printed and posted near the instrument as a reference.

The diaphragm around the pedestal is permanently affixed to the instrument. Do not attempt to remove the diaphragm for cleaning purposes. Avoid prolonged exposure of the diaphragm to HCl, alcohol, bleach, acetone or other solvents as the adhesive underneath may be affected. If the diaphragm comes loose- please contact [Technical Support](#). Outside of the US or Canada, contact your local NanoDrop products distributor.

## Pedestal Reconditioning

The Bradford reagent as well as other buffers containing surfactants may “un-condition” the measurement pedestal surfaces so that the liquid column does not form well with 1  $\mu\text{L}$  samples. Use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. This will “re-condition” the surfaces allowing the liquid sample column to form.

### Procedure

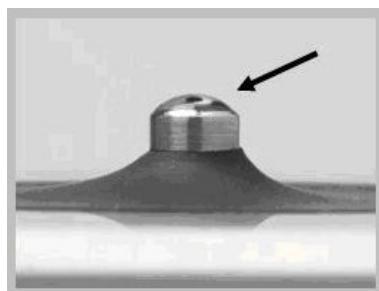
1. Open the vial containing PR-1 and use the applicator provided in the kit to remove a pin-head sized amount of the compound.
2. Apply a very thin, even layer of PR-1 to the flat surface of the upper and lower pedestals.
3. Wait 30 seconds for the PR-1 to dry.
4. Fold a clean, dry laboratory wipe into quarters and remove the PR-1 by rubbing the surface of the upper and lower pedestals until all compound residue is removed.



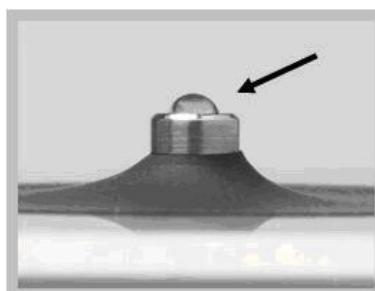
**Note:** The black appearance of the removed residue on the laboratory wipe is normal.

The reconditioning process is complete once the laboratory wipe no longer shows black residue. Use canned air to remove excess lint from the diaphragm.

To check the effectiveness of the reconditioning, pipette a 1  $\mu\text{L}$  aliquot of dH<sub>2</sub>O onto the lower measurement pedestal and visually verify that the water “beads” up. Additional information about the PR-1 kit may be found on our [website](#).



Droplet “flattens” out on un-conditioned pedestal



Droplet “Beads” up on properly conditioned pedestal

As an alternative to using the PR-1 Kit, the pedestals may be reconditioned as follows:

1. Fold a clean dry lab wipe over several times to increase its thickness.
2. Press the lab wipe firmly down on the lower pedestal and "buff/rub" aggressively at least 50 times. The lab wipe may rip during this process and may need to be refolded several times. The upper pedestal should also be buffed but care should be taken not to put too much force on the upper arm.
3. Use canned air to remove any excess lint from the diaphragm.

## Solvent Compatibility

The NanoDrop 2000/2000c Spectrophotometer pedestals are compatible with most solvents typically used in life science laboratories. These include: methanol, ethanol, n-propanol, isopropanol, butanol, acetone, ether, chloroform, carbon tetrachloride, DMSO, DMF, Acetonitrile, THF, toluene, hexane, benzene, sodium hydroxide, sodium hypochlorite (bleach), dilute HCl, dilute HNO<sub>3</sub>, dilute acetic acid.

All forms of Hydrofluoric Acid (HF) are incompatible, as the fluoride ion will dissolve the quartz fiber optic cable.

Do not allow alcohol, bleach, acetone or other solvents to remain on the diaphragm surrounding the pedestal for more than a minute as the adhesive keeping the seal in place may be adversely affected. If the seal comes loose, please contact [Technical Support](#). Contact your local distributor for service and Technical Support outside of the US or Canada.

## Decontamination of Measurement Pedestals

If decontamination is necessary, a sanitizing solution, such as a 0.5% solution of sodium hypochlorite (1:10 dilution of common commercial bleach solutions, freshly prepared), may be used to ensure that no biologically active material is present on the measurement pedestals.

**Note:** Do not use a squirt or spray bottle to apply the diluted bleach. Always dampen a laboratory wipe with the bleach to clean the top and bottom pedestal surfaces as well as the outside of the instrument. Remove the bleach solution with a lab wipe dampened with water.

## Maintenance of the Cuvette System

The cuvette holder assembly block may be cleaned of excess dust using canned air. If any liquid gets into the block, it is best to use a lab wipe or a lab cotton swab to absorb the liquid.

**Note:** Do not squirt any cleaning solution into the cuvette holder as the liquid may drip into the instrument.

Follow the recommendations of each specific cuvette manufacturer for the cleaning and maintenance of cuvettes. Cuvettes with scratches in the optical path should not be used. Ensure all optical surfaces are free of lint and fingerprints prior to insertion in the NanoDrop 2000c cuvette holder.

## Maintenance FAQs

**Q. How do I clean the pedestal?**

**A:** Add 2-3  $\mu\text{L}$  of water to the bottom pedestal surface and lower the arm to form a liquid column. Raise the arm and wipe both measurement surfaces with a dry, lint-free laboratory wipe. Do not use detergents or isopropanol as cleaning agents as their use may result in the pedestals becoming unconditioned. When the pedestal becomes unconditioned sample droplets will "flatten-out" instead of "beading up" when applied to the bottom pedestal.

**Q. How do I clean the cuvette assembly block?**

**A:** Use a dry lab swab or lab wipe to wick away any spills within or around the assembly block. Do not squirt any liquid into the block as the liquid will drip into the instrument.

**Q: How often do I need to check the calibration of the NanoDrop 2000/2000c?**

**A:** We recommend confirming that the instrument is working within specifications about every 6 months. The Thermo Scientific Calibration Check Fluid (CF-1) is required to run the calibration check procedure. CF-1 is available from Thermo Fisher Scientific or one of its authorized distributors.

**Q: How do I check the accuracy of the NanoDrop 2000/2000c when using a cuvette?**

**A:** Although a specific calibration check procedure is not available for the NanoDrop 2000c we suggest the use of Thermo Scientific SPECTRONIC™ 10%T and 50%T neutral density filters.

**Q: Is the flash lamp continuously on, or is it on only when performing a measurement?**

**A:** The lamp is only on during measurements.

**Q: How long before I need to replace the flashlamp?**

**A:** The lamp is rated to last for 3.5 million measurements before replacement should be required.

**Q: Does the NanoDrop 2000/2000c require a computer to operate?**

**A:** Yes, the NanoDrop 2000/2000c connects to a computer with a USB cable. The cable, operating software and a one year warranty are supplied with each instrument.

**Q: Are there solvent restrictions?**

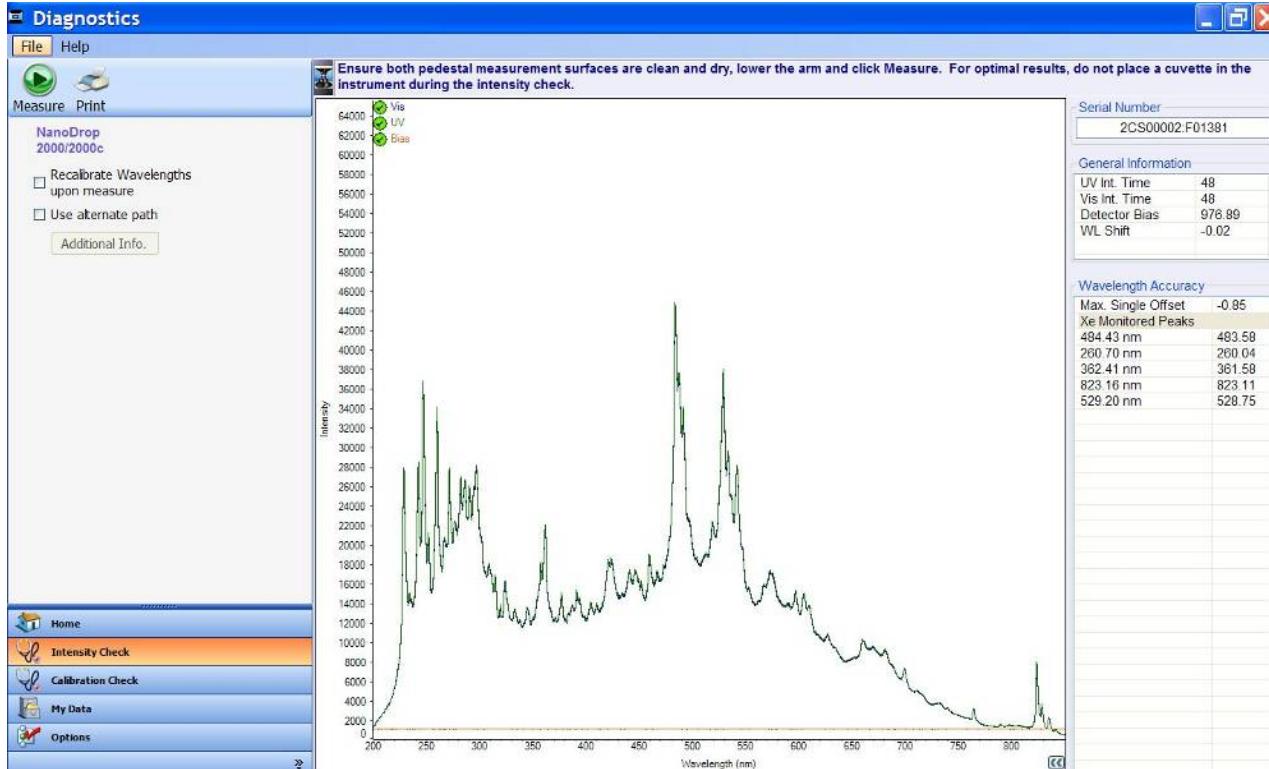
**A:** Do not use hydrofluoric acid on the pedestal as it can etch the quartz optical fiber. Most other laboratory solvents typically used in life science labs, including dilute acids, are compatible with the pedestal as long as they are immediately wiped away.

## 7. Diagnostics and Troubleshooting

The **Diagnostics** task bar accesses both the Intensity Check and the Calibration Check. The Intensity Check confirms that the internal spectrometer is functioning as expected and the Calibration Check confirms that the instrument is performing within the pedestal pathlength calibration specifications.

### Intensity Check

With the sampling arm down, click **Measure** to perform an Intensity Check.



If yellow triangles are displayed in the top left of the spectral display for either the Visible or UV indicator, please clean the pedestal as described below, and then repeat the Intensity Check.

A [Cleaning and Reconditioning Guide](#) can be accessed from the “Appendices” chapter of the *NanoDrop 2000 User Manual* PDF. It is recommended that this guide be printed and posted near the instrument as a reference.

1. Apply 3-5  $\mu\text{L}$  of  $\text{dH}_2\text{O}$  onto the bottom pedestal.
2. Lower the upper pedestal arm to form a liquid column; let it sit for approximately 2-3 minutes.
3. Wipe away the water from both the upper and lower pedestal with a clean, dry lab wipe.

If a yellow triangle is displayed in the top left of the spectral display for the Bias indicator, please ensure that the instrument is not located in a cold environment. Allow the unit to warm up to room temperature to repeat the Intensity check.

If problems persist, email the Intensity check results to [Technical Support](#). Outside of the US and Canada, please contact your local NanoDrop products distributor. The spectra are automatically saved as .JPG files at the following location:

Vista: C:\Users\Public\Public Documents\Thermo\Autosave\IntCheck

XP: C:\Documents and Settings\All Users\Documents\Thermo\NanoDrop2000\Autosave\IntCheck

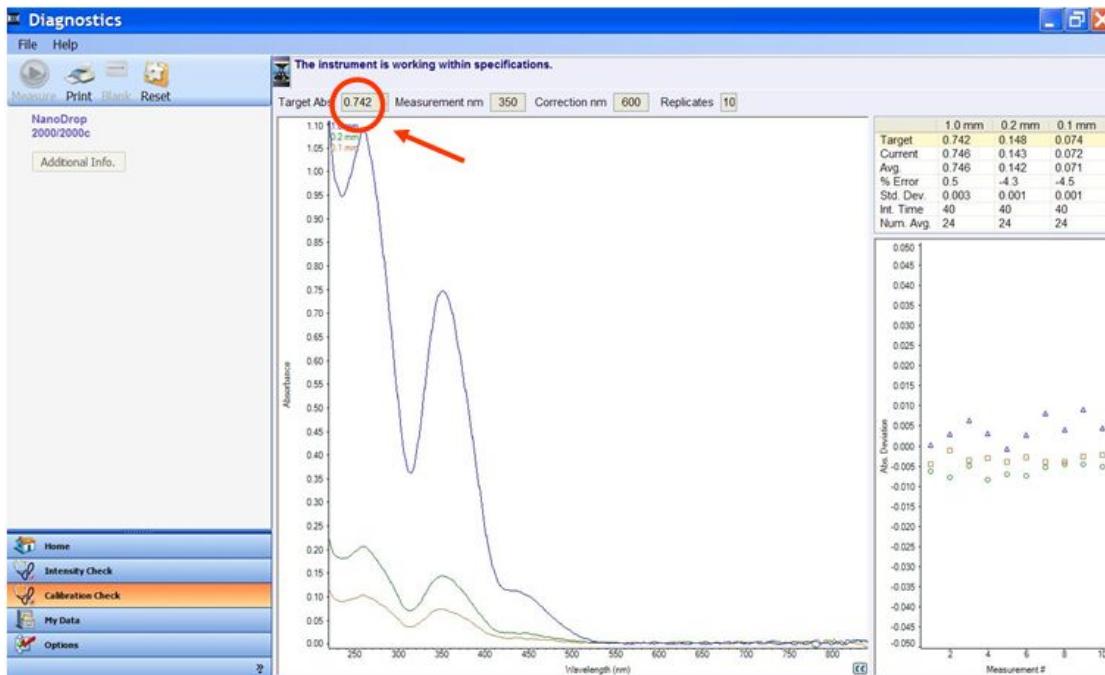
## Calibration Check

It is recommended that a pedestal calibration check be performed every six months to verify that the instrument is performing within specifications.

A vial of Thermo Scientific CF-1 calibration fluid is required to run the calibration check procedure. CF-1 is an aqueous potassium dichromate ( $K_2Cr_2O_7$ ) solution used to confirm the pathlength accuracy of NanoDrop 2000/2000c spectrophotometers. It is roughly ten times more concentrated than other commercially available  $K_2Cr_2O_7$  solutions used to confirm the calibration of standard spectrophotometers and is available through Thermo Fisher Scientific or your local distributor. Refer to the included material data safety sheet provided with the vial and take the appropriate laboratory precautions.

### Pedestal Calibration Check Procedure

1. Ensure the measurement pedestals are clean and that a 1  $\mu$ L water sample “beads” up on the lower pedestal.
2. Open the NanoDrop 2000/2000c software, and using the **Diagnostics** task bar, and choose **Calibration Check**.
3. Enter the actual Target Absorbance stated on the CF-1 vial in the appropriate field as shown in the image below.



4. Add 1  $\mu$ L of dH<sub>2</sub>O to the pedestal, lower the arm and click **Blank**.
5. Wipe the upper and lower pedestals using a dry laboratory wipe.
6. Vigorously shake the CF-1 vial to thoroughly mix the solution. Ensure that all of the solution has collected in the bottom portion of the ampoule before carefully breaking open the vial.

**Note:** The CF-1 calibration fluid is supplied in a single use vial. CF-1 must be used within one hour of opening the vial. Exposure to the environment or transferring of the fluid to another container may result in a significant change in concentration.

7. Follow the on-screen prompts in the Customer Guidance text box. Using individual 1 $\mu$ L aliquots of the CF-1 Calibration Fluid, measure 10 replicates. After the 10th measurement, the calibration check results will be displayed on-screen in the Customer Guidance text box.
8. If the instrument does not pass the calibration check using 1  $\mu$ L samples, immediately repeat the procedure again using 2  $\mu$ L samples.

If the procedure fails with 1  $\mu$ L volumes but passes with 2  $\mu$ L volumes, it is an indication that the pathlengths are within specifications but the pedestals may not be properly conditioned. Refer to the Pedestal Reconditioning procedure for more information.

If the instrument still does not pass the calibration check using 2  $\mu$ L samples recalibration is required. Contact Technical Support. Outside of the US and Canada, please contact your local NanoDrop products distributor.

A .JPG of the final results is automatically archived on the hard drive at the following location:

Vista: C:\Users\Public\Public Documents\Thermo\Autosave\CalibCheck

XP: C:\Documents and Settings\All Users\Documents\Thermo\NanoDrop2000\Autosave\CalibCheck

### Cuvette Calibration Check

Although a specific cuvette calibration check procedure is not available for the NanoDrop 2000c, we suggest the use of 10%T and 50%T neutral density filters such as those available from Thermo Scientific to monitor performance. The table below compliments the expected performance table found in the *SPECTRONIC™ Standards Operators Manual*.

<b>NanoDrop 2000c Expected performance using SPECTRONIC™ Standards Kit</b>	
White 50%T	$\pm 0.011A$
White 10%T	$\pm 0.044A$
Yellow 50%T	$\pm 0.011A$
Yellow 10%T	$\pm 0.044A$
0%T, full spectrum	2A*
Wavelength Accuracy	$\pm 1.5nm$
Stray Radiant Energy 220	2 A*
Stray Radiant Energy 340	2 A*
Stray Radiant Energy 400	2 A*

\*These light-blocking standards should achieve at least 2 A at the wavelength of interest.

## Hardware

### Connection Errors

If your instrument operates properly most of the time, but connection errors (e.g codes 1010, 1029 or 1039) appear intermittently, the instrument may not be receiving power, or recognizing the USB connection. Ensure that the USB and power cables are plugged into the back of the instrument, and that the instrument is receiving power.

If the error persists, contact Technical Support. Outside of the US and Canada, please contact your local NanoDrop products distributor.

### Power Restart

Many instrument issues can be addressed by a simple power restart. For error codes 1019, 1054, 1055 and 1056, follow the directions below:

1. Exit the software.
2. Disconnect the instrument power cord and USB cable.
3. Reconnect the instrument's power cord, then the USB cable.
4. Restart the software.

If the error persists, contact [Technical Support](#). Outside of the US and Canada, please contact your local NanoDrop products distributor.

## Low Signal Errors

Some error messages (e.g. 1009, 1022, 1023, and 1063) are triggered when little to no light reaches the detector during initialization or a measurement. Ensure the pedestal surfaces are clean and that there is not a cuvette in the cuvette assembly block.

Follow the pedestal cleaning steps as outlined below:

1. Apply 3-5  $\mu\text{L}$  of dH<sub>2</sub>O on to the bottom pedestal.
2. Lower the upper pedestal arm to form a liquid column; let it sit for approximately 2-3 minutes.
3. Wipe away the dH<sub>2</sub>O from both the upper and lower pedestals with a clean, dry lab wipe.

Typically dH<sub>2</sub>O is sufficient for removal of samples that have dried on the optical fiber pedestals. There are a few cases (i.e. dried proteins) that may require a more rigorous cleaning protocol. For these cases, we recommend that 0.5M HCl be substituted for the dH<sub>2</sub>O in step 1 above. Follow with 5  $\mu\text{L}$  of dH<sub>2</sub>O to remove any residual HCl.

**Note:** Do not use a squirt or spray bottle to apply de-ionized water or HCl.

If the troubleshooting steps outlined above do not fix the problem, perform an Intensity Check as previously described and send the results to [Technical Support](#).

## Liquid Column Breakage

**Measurement error**

 Error 1048: The ratio between the long path and short path absorbance is out of tolerance. Possible causes include: 1. The liquid column is not forming properly or broke during measurement. Clean and recondition the pedestals. If error persists, use a larger sample size (1.5 - 2  $\mu\text{L}$ ). 2. An air bubble in the sample. 3. The instrument is out of calibration. If error persists, contact Technical Support.

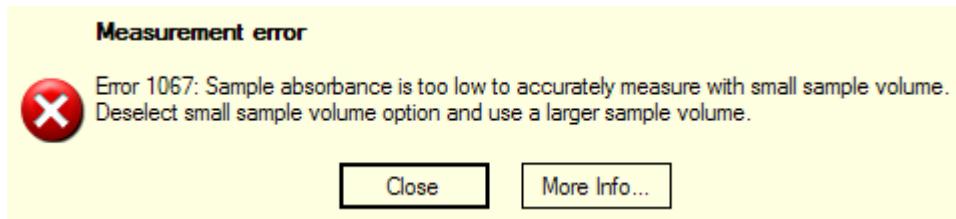
[Close](#)

This error occurs when a possible problem with the column is detected. The most common explanation is that the column is not forming properly due to the pedestal being “unconditioned.”

When a pedestal becomes unconditioned, sample droplets applied to the bottom pedestal will “flatten-out” and cover the entire pedestal surface rather than “bead up.” Buffers containing detergents and various other reagents may cause the pedestal surfaces to become unconditioned. We have noted that routine use of the Bradford reagent may result in difficulty forming columns with 1  $\mu\text{L}$  samples. Refer to the [Pedestal Reconditioning](#) procedure under “Warranty and Maintenance.”

If the warning persists and the user visually confirms that the liquid column is forming, perform a calibration check. If the instrument is out of calibration, contact [Technical Support](#). Outside of the US and Canada, please contact your local NanoDrop products distributor.

## Small Volume Sample Measurement



This error occurs when the **small sample volume** feature available in the Nucleic Acid and Protein A280 applications has been selected for use with a sample a 10 mm equivalent absorbance of less than 3.0. De-select the **small volume option**, and wipe away the aliquot. Use a larger sample volume aliquot and re-measure.

## Sample Related Issues

### Sample Carryover

Simple wiping of the upper and lower measurement pedestal with a dry laboratory wipe is highly effective in eliminating carryover for samples differing in concentration by as much as three orders of magnitude.

### Effect of Evaporation and Solvents

A fresh aliquot of sample should be used for each measurement. Evaporation of the sample during the measurement cycle usually has a minimal effect on absorbance readings and may result in a 1-2% increase in sample concentration. However, repeated measurements on the same sample aliquot will result in increasing concentrations and or column breakage. Highly volatile solvents, such as hexane, will likely evaporate before the measurement can be completed. Less volatile solvents such as DMSO can be used successfully.

The cuvette option (model 2000c only) may be useful for samples in volatile buffers or solvents.

### Sample Homogeneity

Sampling from non-homogeneous solutions, particularly when using small volumes, may cause significant measurement deviation in the data generated using all measurement technologies including spectrophotometer. Genomic DNA, lambda DNA and viscous solutions of highly concentrated nucleic acids are common examples that require careful attention to ensure homogeneity before sampling. Proteins are subject to denaturation, precipitation, and aggregation and therefore also require special handling to ensure homogeneity before sampling.

### Sample Measurement Accuracy and Reproducibility

Sample or aliquot non-homogeneity and/or liquid column breakage may result in erroneous or non-reproducible results. Follow the recommendations below to ensure accurate and reproducible results:

- **Ensure the pedestal surfaces are clean before opening the software.** A dirty sample pedestal (i.e. a pedestal with sample dried on to it) upon startup may cause erroneous absorbance readings (even negative values) and signal saturation. It is good practice to clean the pedestal surfaces with de-ionized water prior to opening the software.

**Note:** Do not use a squirt or spray bottle to apply de-ionized water.

- **Use a 1.5-2  $\mu$ L sample volume.** Unexpected results can occur when the liquid sample column is not completely formed during a measurement. During the measurement, visually confirm that the liquid column is formed. If necessary, use 1.5 to 2.0  $\mu$ L sample volume to ensure proper column formation. Also, proteins and solutions containing surfactants are known to “un-condition” the pedestal surfaces so that the liquid column does not form. If this occurs, use the Thermo Scientific Pedestal Reconditioning compound (PR-1) to recondition the pedestals. Refer to “[Pedestal Reconditioning](#)” for more details.
- **Heat DNA samples to 55 °C and gently vortex before measurement.** Due to the small volumes required by the NanoDrop 2000/2000c, it is important to ensure that the sample being measured is homogeneous. Field experience has shown that samples containing large molecules such as genomic or lambda DNA are particularly susceptible to non-homogeneity. Larger volumes used with cuvette-based measurements may minimize the effect of sample non-homogeneity.
- **Perform a Blanking Cycle.** This will confirm that the instrument is working as expected and that any sample carryover from previous measurements is not a concern. To run a blanking cycle, perform the following:
  1. Open the desired software application.
  2. Load an aliquot of the blank (the buffer, solvent, or carrier liquid used with your samples) onto the lower measurement pedestal and lower the sampling arm.
  3. Click **Blank** to initiate the measurement. When finished, wipe away the liquid with a dry lab wipe.
  4. Analyze a fresh aliquot of the blanking solution as though it were a sample. Load the buffer and click **Measure**. The result should be a spectrum that varies no more than 0.04 A (10mm absorbance equivalent)
  5. Wipe the blank from both pedestal surfaces with a laboratory wipe and repeat the process until the spectrum is within 0.04 A (10 mm path).
- **Confirm that blank and sample solvent are the same:** Some buffer components absorb in the UV range, therefore, it is critical to blank the instrument with the same solution that the sample is suspended in.
- **Confirm that the sample is not too dilute:** Analyzing samples at or near the detection limit will result in variable measurements. Refer to ‘Measurement Ranges’ for guidance on lower detection limits.
- **Confirm pedestal mode accuracy with CF-1.** CF-1 is a concentrated potassium dichromate calibration standard available from Thermo Fisher Scientific and its distributors. It is recommended that the calibration check procedure be performed every six months with a fresh vial of CF-1 to confirm that the instrument is performing within specifications.

## 260/280 Ratio

Some researchers may encounter a consistent 260/280 ratio change when switching from a standard cuvette spectrophotometer to the NanoDrop 2000/2000c. The three main causes for this are listed below:

- **Change in sample acidity**

Small changes in solution pH will cause the 260/280 to vary\*\*. Acidic solutions will under-represent the 260/280 ratio by 0.2-0.3, while a basic solution will over-represent the ratio by 0.2-0.3. If comparing the NanoDrop 2000/2000c to other spectrophotometers, it is important to ensure that the pH of an undiluted sample measured on the NanoDrop 2000/2000c is at the same pH as the diluted sample measured on the second spectrophotometer.

\*\* William W. Wilfinger, Karol Mackey, and Piotr Chomczynski, Effect of pH and Ionic Strength on the Spectrophotometric Assessment of Nucleic Acid Purity: BioTechniques 22:474-481 (March 1997)

- **Wavelength accuracy of the spectrophotometers**

Although the absorbance of a nucleic acid at 260nm is generally on a plateau, the absorbance curve at 280 nm is quite steeply sloped. A slight shift in wavelength accuracy will have a large effect on 260/280 ratios. For example, a  $\pm 1$  nm shift in wavelength accuracy will result in a  $\pm 0.2$  change in the 260/280 ratio. Since many spectrophotometers claim a 1 nm accuracy specification, it is possible to see as much as a 0.4 difference in the 260/280 ratio when measuring the same nucleic acid sample on two spectrophotometers that are both within wavelength accuracy specification.

- **Nucleotide mix in your sample**

The five nucleotides that comprise DNA and RNA exhibit widely varying 260/280 ratios\*\*. The following represent the 260/280 ratios estimated for each nucleotide if measured independently:

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

The resultant 260/280 ratio for the nucleic acid being studied will be approximately equal to the weighted average of the 260/280 ratios for the four nucleotides present. It is important to note that the generally accepted ratios of 1.8 and 2.0 for DNA and RNA are “rules of thumb.” The actual ratio will depend on the composition of the nucleic acid.

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

\*\* Leninger, A. L. Biochemistry, 2nd ed., Worth Publishers, New York, 1975

### Unusual Spectrum

A sample that is ragged or exhibits jagged “cuts” out of the spectrum but is an otherwise normal shape, may be the result of detector saturation. This may be caused by the software selecting too high of an integration time due to a dirty pedestal. Clean both the upper and lower pedestal measurement surfaces as previously described and restart the software.

If the problem persists contact [Technical Support](#). Outside of the US and Canada, please contact your local NanoDrop products distributor.

### Contact Technical Support

If after referring to the above troubleshooting tips you are unable to resolve your problem, please contact [Technical Support](#) for assistance. Outside of the US and Canada, please contact your local NanoDrop products distributor.

Thermo Fisher Scientific  
 3411 Silverside Road  
 Bancroft Building, Suite 100  
 Wilmington, DE 19810 U.S.A.  
 Telephone: (302) 479-7707  
 Fax: (302)792-7155  
 E-mail: [nanodrop@thermofisher.com](mailto:nanodrop@thermofisher.com)  
[www.nanodrop.com](http://www.nanodrop.com)

Please have the serial number of the instrument available. The number can be found on the bottom of the unit.

In addition, application screen captures and Intensity Check .JPGs are of great use in diagnosing problems. Email as attachments to Technical Support. Outside of the US and Canada, please contact your local NanoDrop products distributor.

## Data Archive Files

If you have questions about your data, please send the autosave file containing the data as an email attachment to Technical Support. Outside of the US and Canada, please contact your local NanoDrop products distributor.

All data is automatically archived as a workbook file (\*.twbk) to the following locations depending on the PC operating system:

Vista: C:\Users\Public\Public Documents\Thermo\Autosave\ (Software Module)

XP: C:\Documents and Settings\All Users\Shared Documents\Thermo\NanoDrop2000\Autosave\ (Software Module)

Alternatively, use the **My Data** task bar to locate the appropriate autosave data file. Open the file with the NanoDrop 2000/2000c software and use the file drop down **E-mail current workbook** option to send to Technical Support.

**Note:** Although all report fields may not be displayed when opening an autosave file, all fields are recoverable by Technical Support. Please do not send exported report files or user-defined workbooks as these files will not contain all the information needed for troubleshooting purposes.

## Troubleshooting FAQs

**Q: Why do I have negative absorbance values?**

**A:** A blank measurement was made either using a solution with more absorbance than the sample of interest or on a dirty pedestal. Clean the pedestal and make a new blank measurement with a fresh aliquot of the appropriate buffer.

**Q: Is simply wiping the pedestal surface enough to prevent carryover?**

**A:** The highly polished quartz and stainless steel surfaces of the sample retention system are resistant to sample adherence, making the use of dry laboratory wipes very effective in removing the sample. When using the cuvette option, it is best to follow the cleaning recommendations of the cuvette manufacturer.

**Q: How do I keep my sample from flattening out on the measurement pedestal?**

**A:** Many protein reagents and buffers contain surfactants that may interfere with the hydrophobic nature of the measurement pedestals, causing your samples to "flatten out." Use the NanoDrop PR-1 reconditioning compound as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. PR-1 kits are available through Thermo Fisher Scientific or your local distributor.

**Q: Can I make measurements on the pedestal if I leave a cuvette in the NanoDrop 2000c cuvette holder?**

**A:** It is highly recommended that cuvettes be removed from the instrument prior to making a pedestal measurement to ensure the pedestal arm moves to the proper starting position.

**Q: Is there a specific direction that the cuvette must be facing?**

**A:** Yes. The arrow etched into the assembly indicates the direction of the light path. Ensure the clear sides of the cuvette correspond to the direction of the arrow.

## 8. Appendices

### Quick Start Guide

The Quick Start Guide includes information about a recommended blanking procedure to ensure proper reference measurements. It may be useful to print and post this page near the instrument as a quick lab reference.

### Software Feature Overview

The Software Feature Overview is one page summary of key software features. It may be useful to print and post this page near the instrument as a quick lab reference.

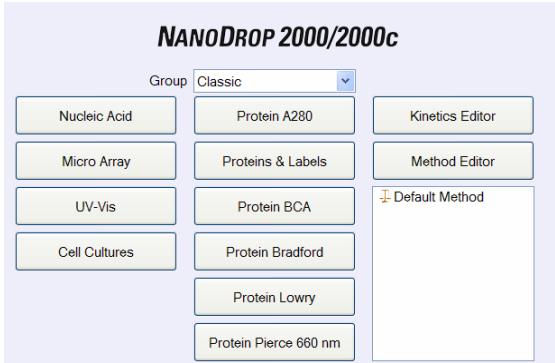
### Cleaning and Reconditioning Guide

The Cleaning and Reconditioning Guide is a one page document that provides recommendations for cleaning both the pedestal and the cuvette assembly holder. It may be useful to print and post this page near the instrument as a quick lab reference.

## Thermo Scientific NanoDrop 2000/2000c Spectrophotometers

**Quick Start**

1. Double click on the desktop NanoDrop™ 2000 software icon and select the application of interest. Follow the prompts for instrument initialization.
2. Ensure **Add to report** is selected in the left pane to automatically include all measurements in the saved report.
3. Establish a Blank using the appropriate buffer. Pipette 1-2 ul of the blanking buffer onto the bottom pedestal, lower the arm and click the **Blank** button. The blank solution is generally the same buffer that the molecule of interest is suspended or dissolved in.
  - For the NanoDrop 2000c model, select the **Use cuvette** box to make measurements with a cuvette.
  - Insert the cuvette noting the direction of the light path indicated by the etched arrow.
  - The arm must be down for all measurements-including measurements made with cuvettes.
  - The optical path is directed 8.5 mm above the bottom of the cuvette. Refer to the manufacturer for volume recommendations.
4. Wipe away the blank and enter the sample ID in the appropriate field. Pipette 1-2 ul of sample and hit **Measure**.
  - It is recommended that a fresh aliquot of sample be used for each measurement.



Pedestal measurement



Cuvette measurement

**After a measurement:**

- Wipe both measurement pedestals using a dry, lint-free laboratory wipe and the instrument is ready for the next sample.
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples.

**Blanking Cycle**

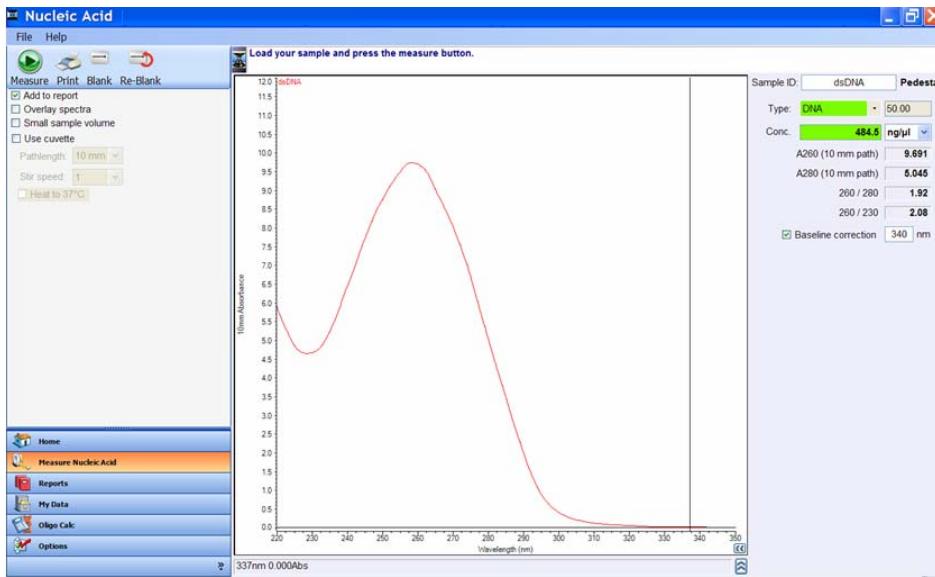
It is generally recommended that an aliquot of the blanking buffer be measured as if it were a sample. This will confirm that the instrument is working well and that any sample dried down from previous measurements is not a concern. To run a blanking cycle, perform the following:

1. Load an aliquot of the blank onto the lower measurement pedestal and lower the sampling arm into the down position.
2. Click on the **Blank** button to store the blank reference.
3. Analyze a fresh replicate of the blank as though it were a sample by selecting **Measure**. The result should be a spectrum that varies no more than 0.04 A (10 mm absorbance equivalent).
4. Wipe the blank from both measurement pedestal surfaces and repeat the process until the spectrum is within 0.04 A (10 mm path).

Although it is not necessary to blank between each sample, it is recommended that a new blank be taken every 30 minutes when measuring many samples.

For Technical Support contact us at 302-479-7707 or send an email to [nanodrop@thermofisher.com](mailto:nanodrop@thermofisher.com).

## Software Features



The NanoDrop 2000/2000c software interface is divided into a left pane and a right pane. Task bars and Action buttons are located in the left pane while the right pane acquisition pages display the sample spectra.

### Task Bars

**Home** - displays the main menu with the available selection of applications.

**Measure** (specific application)- active application screen.

**My Data** - manages data archiving and retrieval.

**Reports** - exportable user configurable report associated with current data set.

**Diagnostics** - accesses the Intensity and Calibration checks.

**Options** - includes tabs for account management and report printing options.

### Action Icons

**Measure** - initiates the measurement of a sample.

**Print Screen** - prints a copy of the spectrum and associated sample data to the default printer.

**Blank** - initiates the measurement of the buffer or carrier liquid in which the sample is suspended.

**Re-Blank** - establishes a new reference (blank) and recalculates the absorbance spectrum for the most recent sample and displays the revised spectrum on the screen.

### Optional Selections

**Add to Report** - used to indicate sample data that should be added to the current report.

**Overlay spectra** - selection of this feature displays multiple spectra at a time.

**Small sample volume** - 0.5 ul volume capability used for concentrated samples.

Nucleic acid and A280 applications only (examples  $\geq 150$  ng/ul dsDNA or  $\geq 4.5$  mg/ml BSA)

**Use cuvette** - select to enable cuvette measurements.

### Menu Bar

**File** - used to open and close workbooks.

**Help** - electronic Help files. May be accessed directly from many software screens.

Sample data is stored in workbooks at user-specified locations. Workbooks may be accessed through the **My Data** task bar in the left pane.

## Cleaning and Reconditioning

### Pedestal Cleaning

1. Apply 3-5 ul of dH<sub>2</sub>O on to the bottom pedestal. Never use a squirt bottle to apply de-ionized water or any other liquid to the surface of the instrument.
2. Lower the upper pedestal arm to form a liquid column; let it sit for approximately 2-3 minutes.
3. Wipe away the water from both the upper and lower pedestal with a dry, lint-free lab wipe.



- **Between measurements:** Wipe the sample from both the upper and lower pedestals with a clean, dry, lint-free lab wipe, to prevent sample carryover and avoid residue buildup.
- **Between users:** A final cleaning of both measurement surfaces with dH<sub>2</sub>O is recommended after the last sample measurement.
- **Additional cleaning:** Substitute 0.5M HCl for the dH<sub>2</sub>O in the procedure above when proteins have dried on the pedestal.
- **Decontamination:** Use a sanitizing solution, such as a 0.5% solution of sodium hypochlorite (1:10 dilution of common commercial bleach solution, freshly prepared), to decontaminate the measurement pedestals. Follow with 3- 5 ul of dH<sub>2</sub>O.

The use of detergents or isopropyl alcohol is **not** recommended as they may un-condition the pedestal measurement surfaces. If a solution containing either is used, it is important to follow with 3- 5 ul of dH<sub>2</sub>O.

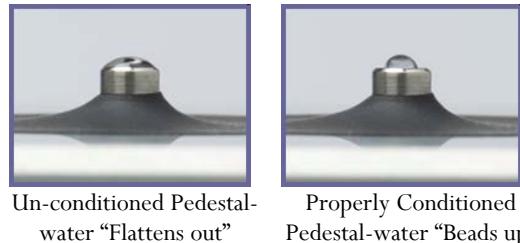
### Pedestal Reconditioning

Use the instrument pedestal reconditioning kit, PR-1, as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement.

1. Open the vial containing PR-1 and use the applicator provided in the kit to remove a pin-head sized amount of the compound.
2. Apply a very thin, even layer of PR-1 to the surface of the upper and lower pedestals and wait 30 seconds for the PR-1 to dry.
3. Fold a clean, dry laboratory wipe into quarters and remove the PR-1 by aggressively rubbing the surface of the upper and lower pedestals until all compound residue is removed. The appearance of a black residue on the laboratory wipe is normal.
4. Use canned air to remove excess lint from the diaphragm.

Test the effectiveness of the re-conditioning by pipetting a 1ul sample of dH<sub>2</sub>O (using a calibrated 2 ul pipettor) onto the lower measurement pedestal.

The figure on the left is a flat bead of water on an unconditioned pedestal. The figure on the right is a 1ul sample of dH<sub>2</sub>O on a properly conditioned pedestal.



### Cuvette Cleaning

- For routine, daily cleaning use a dry lab swab or lab wipe to wick away any spills within or around the cuvette holder assembly.
- The cuvette holder assembly may be cleaned of excess dust using canned air.
- DO NOT squirt any liquid into the block as the liquid will flow into the instrument. If liquid does get into the block, it is best to use a lab wipe or a lab cotton swab to absorb the liquid.
- Follow the recommendations of the cuvette manufacturer for the cleaning and maintenance of cuvettes.
- Cuvettes with scratches in the optical path should not be used. Ensure all optical surfaces are free of lint and fingerprints prior to insertion in the NanoDrop 2000c cuvette holder.

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