Pierce 660 nm Protein Assay

Introduction

The Thermo Scientific™ Pierce™ 660 nm Protein Assay reagent is a ready-to-use formulation that offers rapid, accurate and reproducible colorimetric detection of minute amounts of protein in solution. Used in conjunction with the micro-volume capability of the Thermo Scientific™ NanoDrop™ spectrophotometers, the reagent provides an accurate and rapid means of protein quantitation with minimal consumption of sample. The ability of NanoDrop spectrophotometers to measure as little as 2 µL of protein samples allows significantly scaled-down reaction volumes, thereby using only a fraction of sample and reagent commonly needed for conventional cuvette-based instruments.

Note: All specifications and protocol instructions presented below are specific to the pedestal mode for NanoDrop One/One^C instruments. Please refer to the reagent manufacturer for additional guidance when utilizing the cuvette mode of the NanoDrop One^C.

Dynamic Range

The assay has a linear range of 50-2000 µg/mL using a 1:15 sample to reagent ratio. The sensitivity of the assay may be increased by using a 1:7.5 sample to reagent ratio yielding a linear range of 25-1000 µg/mL.

Supplies

Equipment:

- NanoDrop One/One^C Spectrophotometer
- 0.5-2 µL pipettor (and low retention tips) and 10-1000 µL pipettors

Materials:

- Low lint laboratory wipes
- 0.5 mL microcentrifuge tubes or 0.2 mL mini-centrifuge strip tubes and caps

Reagents:

- Pierce 660 nm Reagent, Pierce Product # 22660
- Pierce pre-diluted BSA standards Pierce Product # 23208(OPTIONAL) or other protein standard
- PR-1 Reconditioning kit Part # Chem-PR1-KIT

Assay Recommendations

- Measure 2 µL sample aliquots.
- Re-condition pedestals with PR-1 upon assay completion.

Sample Preparation

1. Equilibrate all reagents, unknowns and protein standards to room temperature. Mix thoroughly but gently to avoid introducing micro bubbles.

2. Prepare a zero reference (0 mg/mL protein).

   - **For a 1:15 sample to working reagent ratio**: Add 10 µL of the assay buffer to 150 µL of the Pierce 660 reagent.

   - **For a 1:7.5 sample to working reagent ratio**: Add 20 µL of the assay buffer to 150 µl of the Pierce 660 reagent.

   Note: The zero reference solution is used as the 'blank'. This is unlike the other colorimetric assays run on NanoDrop instruments where water is used for the 'blank' measurement.

3. Prepare standards and samples.

   - **For a 1:15 sample to working reagent ratio**: Add 10 µL of each standard and sample to 150 µL of the Pierce 660 reagent.

   - **For a 1:7.5 sample to working reagent ratio**: 20 µL of each standard and sample to 150 µL of the Pierce 660 reagent.

4. Mix each standard and unknown sample thoroughly by gently pipetting up and down several times. If necessary, collect the solution at the bottom of the tube by a brief centrifugation.

5. Incubate at room temperature for 5 minutes.

Thermo Scientific NanoDrop Products Wilmington, Delaware USA Technical support: nanodrop@thermofisher.com
302-479-7707 Toll free (US & Canada): 877-724-7690 www.thermofisher.com/nanodrop

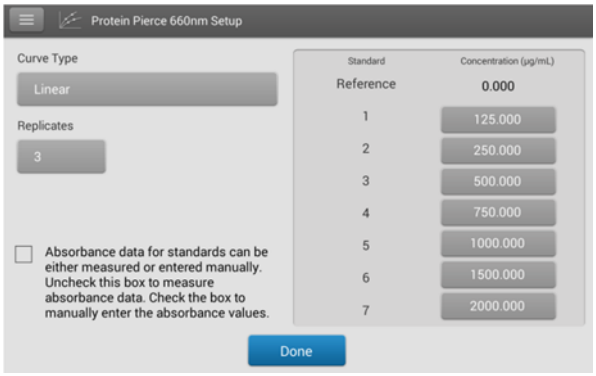
Protocol

1. Tap the **Protein** tab from the Home screen. Tap the **Protein Pierce 660 nm** application button.

2. On the left side of the screen, select the Curve Type and number of replicates to measure. We recommend selecting the **Linear** curve type and measuring 3 replicates of each standard.

   Optional: The user has the option to measure the absorbance of each standard or enter the manufacturer supplied standard absorbance values manually. In the bottom left pane, select the box to enter the manufacture supplied standard absorbance values manually. Deselect the box to measure the absorbance of each standard. See image below.

3. In the table on right side of the screen, enter the values for each standard concentration. The software allows for the reference and up to 7 additional standards. The zero reference and standards can be measured with up to 3 replicates. Tap **Done**.



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

4. Establish a blank using the appropriate buffer. It is advisable to use the dye reagent and protein buffer ("0" reference) without any protein added as both the blank and zero reference sample for this assay.
   - Pedestal Option: Pipette 2 µl of blank solution onto the bottom pedestal, lower the arm and tap **Blank**.
   - Cuvette Option: (Model NanoDrop One^{C} 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: 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. A message at the top of the screen will prompt you to, "**Clean both pedestals and Load - Reference 0.000**," if you are using the pedestal mode. If you are using the cuvette mode, a message will prompt you to, "**Insert Cuvette and Load - Reference 0.000**." Load the reference sample in the manner described for loading the blank.

6. After each measurement, wipe the upper and lower pedestals using a clean, dry laboratory wipe.

7. Once the reference and at least one standard have been measured, the message **Invalid Curve** in red at the top right of the screen will switch to **Valid Curve** in blue.

8. After all standard measurements have been made a pop-up box will indicate **Standards Complete**. Select **Load more standards** to enter and measure additional standards or **Run samples** if standard measurements are complete.

9. After selecting **Run samples**, enter a sample ID at the top of the screen. Load 2 µL of sample when using the pedestal. Tap **Measure**.

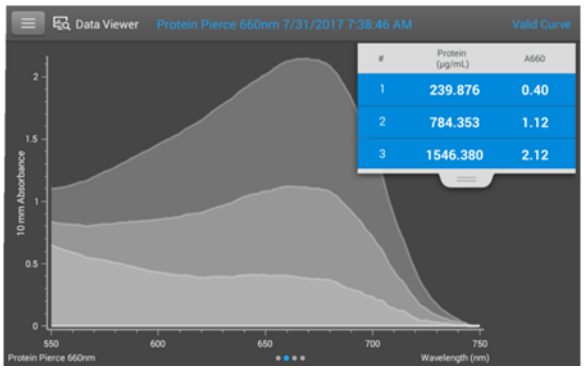
10. It is not necessary to blank the instrument between the standard and the unknown sample measurements.

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

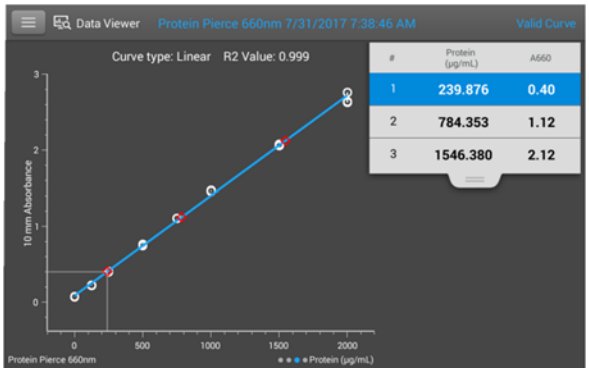
11. After completing all Standard and Sample measurements, it is good practice to re-condition the pedestals using PR-1.

After the measurements:

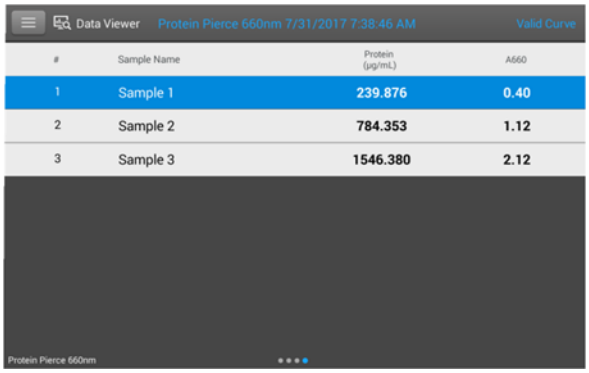
1. Select each sample to display multiple spectra at a time.



2. Swipe the screen to the left to view the curve.



3. Swipe the screen again to the left to view your measurement results.



Standard Curve Data

BSA (µg/mL)	A660 (n=3)	Std. dev.	%CV
0	0.072	0.003	NA
125	0.223	0.004	1.6
250	0.403	0.002	0.5
500	0.757	0.013	1.7
750	1.107	0.002	0.2
1000	1.467	0.006	0.4
1500	2.065	0.012	0.6
2000	2.676	0.074	2.8

Table 1. Typical absorbance values for a 1:15 sample to reagent ratio assay using the Pierce 660 nm Protein Assay.

For additional information regarding the Pierce 660nm assay and reagents: www.thermoscientific.com/pierce

Thermo Scientific NanoDrop Products
302-479-7707
Wilmington, Delaware USA
Toll free (US & Canada): 877-724-7690
Technical support: nanodrop@thermofisher.com
www.thermofisher.com/nanodrop