Protocol

NanoDrop Ultra Spectrophotometers and Fluorometers

Pierce 660 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 Ultra instruments. Please refer to the reagent manufacturer for additional guidance when utilizing the cuvette mode of the Thermo Scientific™ NanoDrop™ Ultra^{C} Spectrophotometer or Thermo Scientific™ NanoDrop™ Ultra^{C} FL Spectrophotometer and Fluorometer.

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 Ultra/Ultra^{C}/Ultra FL/Ultra^{C} FL Spectrophotometer
- 0.5–2 µL pipettor (low retention tips)
- 10–1,000 µL pipettor (low retention tips)

Materials:

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

Recommended Reagents:

- Pierce 660 nm Reagent, #22660
- Pierce Pre-Diluted BSA standards, #23208 (or other commercially prepared protein standard)
- NanoDrop PR-1 Reconditioning kit, #CHEM-PR1-KIT

Assay Recommendations

- Measure 2 µL sample aliquots.
- It is recommended that a new standard curve be generated for each assay.
- 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 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.

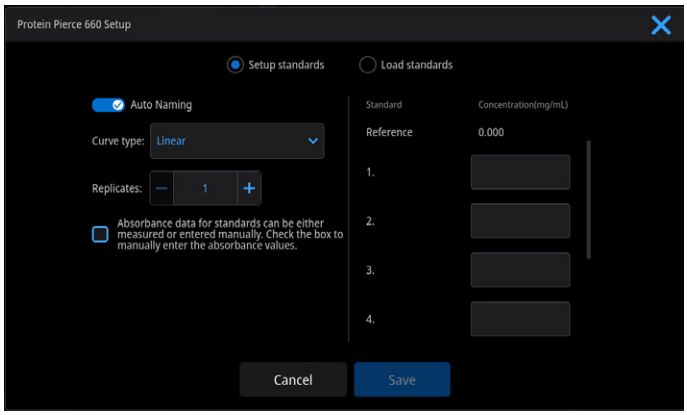
   Note: The zero reference solution is used as the 'blank.' This is unlike the other colorimetric assays run on NanoDrop instruments where water is used for the 'blank' measurement.
3. Prepare standards and samples.
   - **For a 1:15 sample to working reagent ratio:** Add 10 µL of each standard and sample 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.

Protocol

1. From the home screen, select the **Proteins** tab, then select **Protein Pierce 660**.
2. Within the left panel of the screen, enable or disable Auto Naming (depending on your preference) using the provided toggle; select the Curve Type from the drop-down menu; and indicate the number of Replicates to measure using the -/+ buttons. We recommend selecting the **Linear** curve type and measuring 3 replicates of each standard.

   Note: The curve type cannot be changed after the assay is in progress.

   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 manufacturer-supplied standard absorbance values manually. Deselect the box to measure the absorbance of each standard. See image below.
3. Enter the values for each standard concentration in the table within the right pane. 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.



   Note: The minimum requirement for standard curve generation is 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.
4. After all standard concentrations have been added and settings have been updated, select **Save**.

   Note: Use the **Load standards** option at the top to load a previously run standard curve by selecting the curve and selecting **Save**. Only standard curves that were previously generated on the instrument will appear.
5. If using a NanoDrop Ultra^{C} or NanoDrop Ultra^{C} FL model, ensure that **Pedestal** is selected as the measurement pathway at the top of the screen.

6. 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.
7. Pipette 2 µL of the blanking solution onto the lower pedestal, lower the arm, then select **Blank**.

   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.
8. Lift arm and clean both pedestals with new laboratory wipe.
9. Follow the direction at the top of the screen to measure the reference and standards. After each measurement, wipe the upper and lower pedestals using a dry laboratory wipe.

   Note: The arm must be down for all measurements.
10. After all standard measurements have been made, a pop-up box will indicate Standards Completed.

    – Select **Load More Standards** to reopen the Protein Pierce 660 Setup window. Enter any additional standards and then select **Save**. You will then be prompted to measure the additional standards.

    – Select **Remeasure Standards** to view the measurement screen.

      From the measurement screen, open the Sample Details box for the standard you would like to remeasure. This can be done by pressing and holding the row of the desired standard; select **Remeasure**. Confirm by selecting **Yes** and you will then be prompted to remeasure the standard.

    – Select **Measure Samples** to continue with sample measurement.

      Note: Once a sample is measured, the standards can no longer be remeasured.
11. After selecting **Measure Samples**, enter a sample ID at the top of the screen. Load 2 µL of sample onto the lower pedestal. Select **Measure**.

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

Cleaning the instrument after a measurement

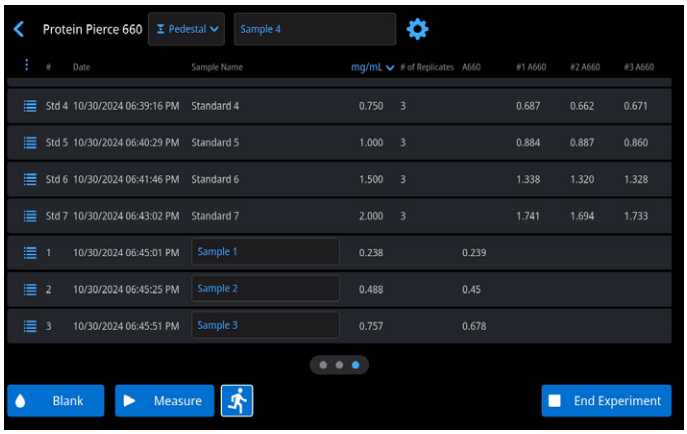
– Simply wipe the upper and lower pedestals using a dry laboratory wipe and the instrument is ready to measure the next sample.

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)	St dev	%CV
0	0.076	0.006	NA
125	0.141	0.010	7.0
250	0.229	0.004	1.7
500	0.433	0.009	2.0
750	0.673	0.013	1.9
1000	0.877	0.015	1.7
1500	1.329	0.009	0.7
2000	1.723	0.025	1.5

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

For additional information regarding the Pierce 660nm assay and reagents, please refer to the manufacturer's product literature supplied with the Pierce Protein Assay.