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 ul 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 the pedestal mode for NanoDrop 2000/2000c instruments. Please refer to the reagent manufacturer for additional guidance when utilizing the cuvette mode of the NanoDrop 2000c.

Dynamic Range

The assay has a linear range of 50-2000 ug/ml using a sample to reagent ratio of 1:15. 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 ug/ml.

Supplies

Equipment:

- NanoDrop 2000/2000c Spectrophotometer
- 0.5-2 ul pipettor (low retention tips)

Materials:

- Low lint laboratory wipes
- 0.5 ml Eppendorf 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)

Assay Recommendations

- Use 2 ul aliquots for all measurements
- Mix all solutions thoroughly but gently to avoid micro bubbles.

Sample Preparation

1. Equilibrate all reagents, unknowns and protein standards to room temperature.

2. Mix each stock standard solution and unknown sample gently prior to use.

3. Prepare a zero reference (0 mg/ml protein) by adding 10 of the assay buffer to 150 ul of the Pierce 660 reagent.

Note: Whether using a predefined standard curve or generating a new curve, 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.

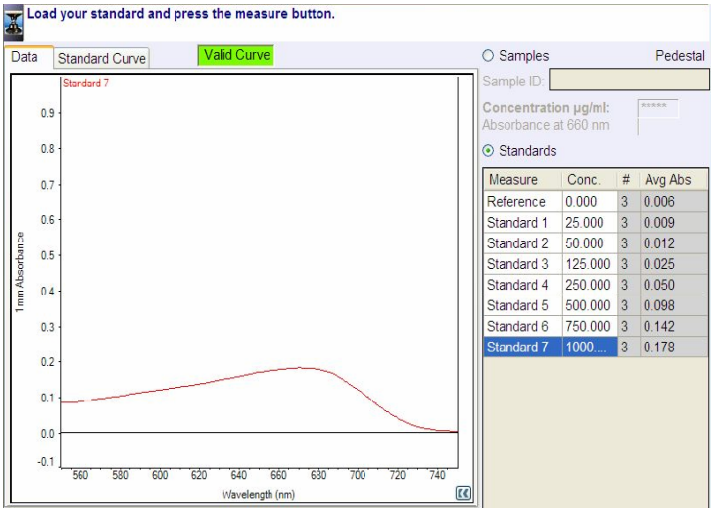
4. Transfer 10 ul of each sample or standard to 150 ul of the Pierce 660 reagent.

5. Mix each standard and unknown sample thoroughly by gently pipetting up and down several times.

6. Collect the solution at the bottom of the tube by a brief centrifugation.

7. Incubate at room temperature for 5 minutes.

Example spectrum of a Pierce 660 nm Protein Assay sample.



Protocol

1. Select the **Protein Pierce 660 nm** application from the Home page. 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.

4. 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 **Overlay spectra** to display multiple spectra at a time.

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.

   – Pedestal Option: Pipette 2 µL 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.

7. Under the Standards tab, highlight a standard and load as described for the blank above. Click **Measure**. Measure all standards prior to measuring samples.

8. 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. Click **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.

   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.

Standard Curve Data

BSA (µg/mL)	A660 (n=3)	St dev
125	0.016	0.001
250	0.030	0.001
500	0.058	0.001
750	0.087	0.001
1000	0.108	0.002
1500	0.187	0.001
2000	0.209	0.003

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

BSA (µg/mL)	A660 (n=3)	St dev
25	0.009	0.001
50	0.012	0.001
125	0.026	0.001
250	0.050	0.002
500	0.097	0.001
750	0.143	0.002
1000	0.178	0.002

Table 2. Typical absorbance values for a 1:7.5 sample to reagent ratio assay using the Pierce 660 nm Protein Assay.