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 microvolume 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.

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 8000 Spectrophotometer
- Low volume 8-channel pipettor for loading samples onto measurement pedestals (low retention tips)

Materials:

- Low lint laboratory wipes
- 0.5 ml Eppendorf tubes (for stock reagent)
- 0.2 ml mini-centrifuge strip tubes and caps or 96 well PCR plate (for standards and sample reactions)

Recommended Reagents:

- Pierce 660 nm Reagent, Pierce product number 22660
- Pierce pre-diluted BSA standards, Pierce product number 23208 (optional) or other protein standard
- PR-1 Reconditioning Kit, part number CHEM-PR1-KIT

Assay recommendations

- Measure 2 µL standard and sample aliquots.
- Making triplicate measurements for both standards and samples is good practice. Use fresh aliquots for all replicate measurements.
- Use an 8-channel pipettor to simultaneously load all 8 measurement positions. **Note: The use of a single channel pipettor to load multiple positions may result in erroneous results**.

Sample ID entry

The NanoDrop 8000 spectrophotometer offers several options for entering sample IDs. When making only a few measurements, it is easy to either type in sample names prior to measurement or use the Manual Plate Setup. When measuring several samples, the user may load a list of predefined sample IDs. The lists may be created in Excel or Notepad but must be saved as a .txt file. It is recommended that a list file be generated prior to starting the assay if many samples are to be measured.

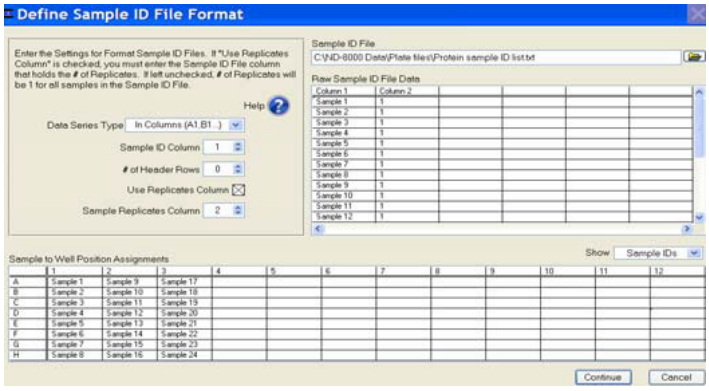

Figure 1. The NanoDrop 8000 software allows the use of predefined sample ID lists

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Pierce 660 nm Protein Assay sample preparation

1. Equilibrate all reagents and samples to room temperature, then mix each thoroughly but gently to avoid micro bubbles.

2. Prepare a zero reference (0 mg/ml protein) by adding 10 of the assay buffer to 150 µL 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.**

3. Transfer 10 µL of each sample or standard to 150 µL of the Pierce 660 reagent. Mix each standard and unknown sample thoroughly by gently pipetting up and down several times.

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

5. Incubate at room temperature for 5 minutes.

Protocol

1. Clean pedestals by pipetting 2 µL of dH_2O onto each all 8 lower pedestals, and then lowering the arm so that the water makes contact with both the upper and lower measurement surfaces. Raise the arm and wipe all pedestals with a dry laboratory wipe.

2. Launch the NanoDrop 8000 software and open the **Pierce 660 nm** module.

3. Initialize the instrument by loading 2 µL of dH_2O to all 8 lower pedestals, lower the arm and click **OK**. When initialization is complete, use a dry laboratory wipe to wipe the water from all measurement surfaces.

4. From the **Standards Choose Source** window select the standard curve source. It is recommended that new standard absorbance values be measured each time the assay is run. Manually enter standard concentration values or enter a concentration series using a previously stored standard curve. If using a preloaded standard curve, continue to step 7.

Measurements Table — Double Click on any row to change the concentration or delete replicates.

	Standard	mg/ml	Ave Abs.	Abs. 1	Abs. 2	Abs. 3	Abs. 4	Abs. 5
Active	A - Reference	0.000						
Active	B - Standard 1	125.0						
Active	C - Standard 2	250.0						
Active	D - Standard 3	500.0						
Active	E - Standard 4	750.0						
Active	F - Standard 5	1000						
Active	G - Standard 6	1500						
Active	H - Standard 7	2000						

Figure 2. The NanoDrop 8000 software allows the use of a predefined standard curve or the user may build a new curve

5. Use an 8-channel pipettor to transfer 2 µL of dH_20 onto each of the 8 lower pedestals. Lower the arm and click **Blank**. When the measurement is complete wipe the pedestals with a lab wipe.

6. Gently mix the standards, then use a 8-channel pipettor to simultaneously load the reference (reagent and buffer, no protein) and standards to generate a new standard curve. Use fresh 2 µL aliquots to measure additional replicates.

7. Select the sample ID loading mode when prompted. Refer to page 1 for additional details.

8. Gently mix the samples and use an 8-channel pipettor to simultaneously load multiple pedestal positions. Use fresh 2 µL aliquots for each replicate.

9. After completing all the measurements, recondition the pedestals with PR-1.

Performance data

BSA (µg/mL)	A660 (n=5)	St Dev	%CV
0	0	NA	NA
125	.016	.001	6.25
250	.030	.001	3.33
500	.062	.001	1.61
750	.088	.002	2.27
1000	.110	.002	1.81
1500	.165	.001	.606
2000	.196	.002	1.02

Table 1. Typical performance data for the 15:1 reagent to sample ratio protein assay using a BSA Standard Curve

Typical Pierce 660 nm spectrum

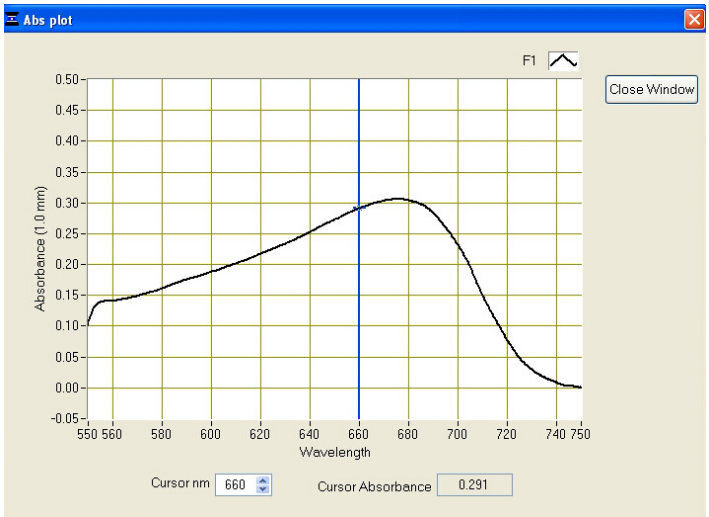

Figure 3. Example spectrum of Protein 660 nm reagent protein sample

Find out more at **thermofisher.com/nd8000**

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