Thermo SCIENTIFIC

PROTOCOL NanoDrop 2000/2000c

Bradford Protein Assay

Introduction

Use of the coomassie G-250 dye in a colorimetric reagent for the detection and quantitation of total protein was first described by Dr. Marion Bradford in 1976. Protein binds to the coomassie dye in the acidic environment of the reagent. This results in a spectral shift from the reddish/brown form of the dye (absorbance maximum at 465 nm) to the blue form of the dye (absorbance maximum at 610 nm). The difference between the two forms of the dye is greatest at 595 nm, the optimal wavelength to measure the blue color from the coomassie dye-protein complex. In conjunction with the micro-volume capability of the Thermo Scientific NanoDrop Spectrophotometers, the assay provides an accurate means of protein quantitation with minimal consumption of sample.

Note: All specifications and protocol instructions presented below are specific to 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 Micro assay has a linear range of 15–100 µg/ml using a 1:1 sample to reagent ratio. The Standard assay has a higher range of 100-1000 µg/ml may be obtained using a 1:30 sample to reagent ratio.

Supplies

Equipment:
- NanoDrop 2000/2000c Spectrophotometer
- 0.5-2 µl pipettor (low retention tips)

Materials:
- Low lint laboratory wipes
- 0.5 ml Eppendorf tubes or 0.2 ml mini-centrifuge strip tubes and caps

Recommended Reagents:
- Coomassie Plus reagent, Pierce Product # 23236,23238
- 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 ul sample aliquots
- It is recommended that a new standard curve be generated for each assay
- Making measurements in triplicate of standards and samples is good practice, particularity with the limited assay signal obtained with the Bradford Assay.
- Re-condition pedestals with PR-1 upon assay completion

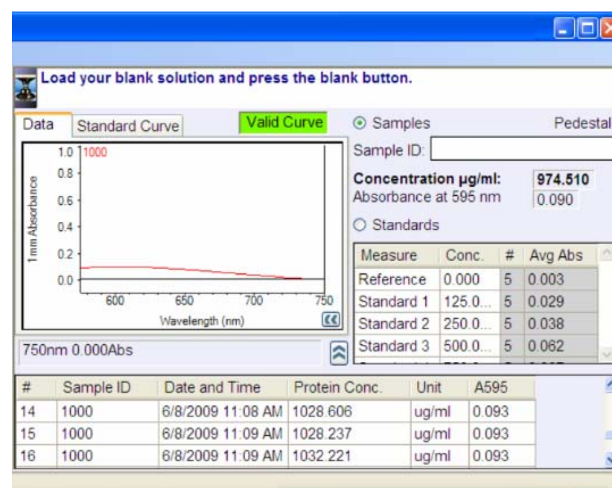
Sample Preparation

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

2. Micro assay (1:1 sample to reagent ratio): add 10 µl of working reagent to each of the standards and sample tubes. Standard assay (1:30 sample to reagent ratio): add 300 ul of working reagent to each of the standard and sample tubes.

3. Add 10 µl of each standard and sample to each of the reagent tubes. Mix well by gentle vortexing. If necessary, collect the solution at the bottom of the tube by a brief centrifugation.

4. Follow reagent manufacturer's recommended incubation time.



Typical absorbance spectrum of a Bradford protein assay sample measurement.

Protocol

1. Select the **Protein Bradford** 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. In the left window pane 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 dH_2O. It is advisable to use the dye reagent and protein buffer ("0" reference) without any protein added as the 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**.

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

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.

After the measurement:

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

– Cuvette Option: Remove the cuvette, rinse thoroughly and dry between samples.

Standard Curve Data

BSA (µg/mL)	A595 (n=5)	St dev	%CV
0	.035	.001	NA
125	.054	.001	1.6
250	.069	.003	4.9
500	.099	.005	4.5
750	.130	.001	1.1
1000	.151	.002	1.6
1500	.172	.001	.8
2000	.188	.002	1.2

Typical absorbance values for a Standard assay using 1:30 sample to reagent ratio assay using the Pierce Coomassie Plus reagent.

For additional information regarding the Bradford assay and reagents, please refer to the Pierce Website (http://www.piercenet.com/).