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 of 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 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 which may be obtained using a 1:30 sample to reagent ratio.

Supplies, Materials & Reagents

- NanoDrop One/One^{c} spectrophotometer
- 0.5 - 2 µL pipettor (and low retention tips) and 10 - 1000 µL pipettors.
- Low lint laboratory wipes
- 0.5mL microcentrifuge tubes or 0.2 mL mini-centrifuge strip tubes and caps
- 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 µL sample aliquots
- Making standard and sample measurements in triplicate 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. Mix thoroughly but gently to avoid introducing micro bubbles.

2. Add the appropriate reagent volume to each microcentrifuge tube or mini-centrifuge strip well.
   - **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 µL 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. 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.

5. Follow reagent manufacturer's recommended incubation time.

Protocol

1. Select the **Proteins Tab** from the New Experiment Screen. Select the **Protein Bradford** application.

2. On the left side of the screen, select the Curve Type and number of replicates to measure using the drop down menus. The Pierce protocol recommends using a 2^{nd} order polynomial. Please note, the curve type cannot be changed after the assay is in progress.

3. Enter the values for each standard concentration in the table on the right. 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. Select **DONE**.

   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.

4. Establish a blank using dH_2O.

   - **Pedestal Option**: Pipette 2 µL of blank solution onto the bottom pedestal, lower the arm and select **Blank**.

   - **Cuvette Option (Model NanoDrop One^c only)**: Select the cuvette option in the NanoDrop One^c software. 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. After loading the cuvette with the appropriate volume, select **Blank**.

     Note: The arm must be down for all measurements, except 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.

5. 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.

6. After the last standard is measured, you can choose to load more standards or run samples using the window that appears in the lower right corner of the screen. To proceed with sample measurements, select the **Run Samples** radio button, and select **DONE**.

7. Enter a sample ID in the sample ID field located in the upper left corner of the screen. Load 2 µL of sample when using the pedestal. Select **Measure**.
8. After completing all standard and sample measurements, it is good practice to re-condition the pedestals using PR-1.

9. 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.

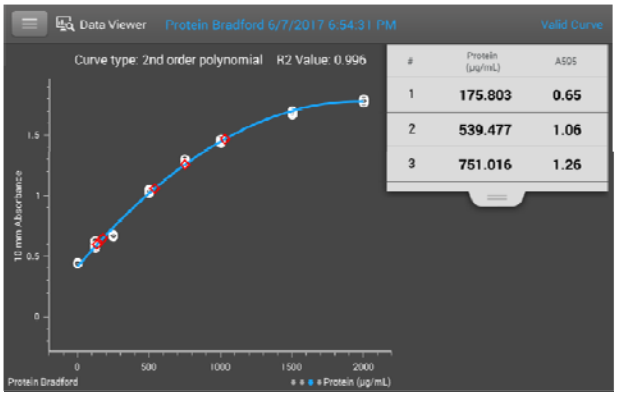
10. Cleaning the instrument after a measurement:

    - **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, and clean according to the manufacturer's recommendations.

Standard Curve Data

BSA (µg/mL)	A595 (n=3)	St dev	%CV
0	0.44	0.003	NA
125	0.593	0.028	4.7
250	0.666	0.007	1.1
500	1.037	0.013	1.2
750	1.289	0.018	1.4
1000	1.445	0.015	1.0
1500	1.683	0.017	1.0
2000	1.783	0.012	0.7



Typical absorbance values and standard curve 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.thermoscientific.com/Pierce).

Find out more at thermofisher.com/nanodrop

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