A Modulus™ Single Tube Multimode Reader Method for

Coomassie Plus – The Better Bradford™ Assay Kit

1. INTRODUCTION

The Modulus™ Single Tube Multimode Reader by Turner BioSystems in combination with the Pierce's Coomassie Plus™ Assay kit provides a convenient procedure for quantifying protein. When the Coomassie dye binds protein the absorption maximum shifts from 465 nm to 595 nm. The Absorbance Module detects as little as 25 µg of BSA in 1.5 mL of Coomassie Plus Reagent.

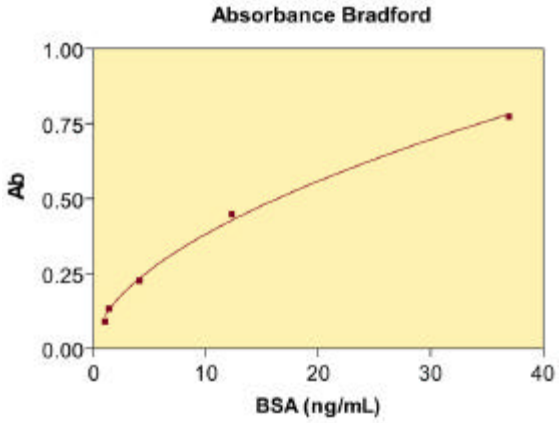

Figure 1. *Bradford Assay was performed on the Modulus using the Absorbance Module, the 600 nm filter, and the Coomassie Plus reagent. Each point represents the average of replicate samples after background subtraction (n=2).*

2. MATERIALS

- Modulus™ Single Tube Multimode Reader (P/N 9200-000 or 9200-002)
- Absorbance Module (P/N 9200-050)
- 600 nm filter paddle (P/N 9200-052)
- 10 x 10 mm disposable methacrylate cuvettes (7000-959)
- Coomassie Plus – The Better Bradford Assay kit (Pierce catalog #'s 23225 and 23227)

3. PREPARATION

NOTE: Store assay reagent at 4°C. Unopened vials of bovine serum albumin (BSA) standard may be stored at room temperature.

3.1 BSA Standard Curve

Prepare a serial dilution of BSA that covers the range for your samples. For example, create a two-fold dilution series from 1000 µg/mL to 125 µg/mL. Make sure to include a blank solution (diluent only) in your standard curve preparation.

NOTE: Prepare the dilution series in the same diluent as the samples for best results.

3.2 Coomassie Plus Reagent

Determine the total volume of reagent required. Each sample and standard requires 1.5 mL of reagent.

3.2.1 Gently invert the bottle of Coomassie Plus Reagent solution to mix the solution before removing the amount necessary for the assay.

3.2.2 Equilibrate the reagent to room temperature before use.

NOTE: Coomassie Plus Reagent may form dye-dye and dye-protein aggregates when left undisturbed. Gentle mixing dissolves the aggregates. For best results, mix the reagent before dispensing and again before measuring absorbance.

3.3 Samples

3.3.1 For each standard or sample, pipette 50 µL into an individual 10 x 10 mm methacrylate cuvette.

3.3.2 Add 1.5 mL of the Coomassie Plus reagent to each cuvette.

3.3.3 Incubate the samples and standards for 10 minutes at room temperature.

3.4 Instrument Setup

3.4.1 Power OFF the Modulus. Install the Absorbance Module into the sample compartment according to *Operating Manual*.

3.4.2 Insert the 600 nm filter paddle in the Absorbance Module.
3.4.3 Turn ON the Modulus and use the touchscreen to choose the Absorbance operation mode.

3.4.4 Touch “Calibrate” and use the black cuvette to set the Modulus to calibrate the zero (dark) reading.

3.4.5 Use a cuvette containing 2 mL of ultrapure water to calibrate the baseline (100% transmittance) reading.

3.4.6 Touch “OK” to accept the calibrations and return to the “Home” screen.

4. SAMPLE ANALYSIS

4.1 Insert the sample or standard into the Absorbance Module and touch “Measure Absorbance” to commence measurement.

4.2 Record the results in Absorbance units (Ab).

4.3 Use a standard curve to determine the protein concentration of each unknown sample. A four-parameter (quadratic) or best-fit curve provides the best accuracy.

5. ABOUT PIERCE

Coomassie-Plus – The Better Bradford is a registered trademark of Pierce Biotechnology, Inc.

Orders for Pierce products may be placed by:

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6. ABOUT TURNER BIOSYSTEMS

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