A GloMax® Multi Microplate Absorbance Method for

Coomassie (Bradford™) Assay Kit

INTRODUCTION

The GloMax® Multi Microplate reader used in conjunction with the Pierce's Coomassie (Bradford™) Assay Kit allows for rapid and accurate measurement of protein concentrations in small-volume microplates (200 µL per well). The Coomassie (Bradford™) Kit is a quick and easy to use modification of the well-known Coomassie-binding, colorometric method for total protein quantitation. When Coomassie dye binds protein in an acidic medium, an immediate shift in maximum absorption occurs from 465 nm to 595 nm with a concomitant change from brown to blue.

Performing the assay in a microplate is simple. Combine a small amount of protein sample with the assay reagent, mix well, incubate briefly, and measure the absorbance at 600 nm using the GloMax® Multi Microplate Absorbance Reader. Protein concentrations can be determined by use of a standard curve that is assayed alongside the unknowns. Since the color response with Coomassie (Bradford™) Kit is non-linear with increasing protein concentrations, it is important to run a standard curve with each assay.

Use of the GloMax® Multi Microplate Absorbance Reader in combination with Pierce's Coomassie (Bradford™) Assay Kit provides a convenient procedure for quantifying protein. The Absorbance Module is able to detect as little as 2.5 µg/mL of BSA when used with the Coomassie (Bradford™) Assay Kit..

MATERIALS REQUIRED

- GloMax® Multi Microplate Multimode Reader
- Absorbance Module
- Black/clear bottom 96-well microplates (Nalge Nunc, P/N 265301)
- Coomassie (Bradford™) Assay Kit (Pierce, 23200), containing:
  - Coomassie (Bradford™) Assay Reagent (950 mL of solution)
  - Albumin Standard Ampules (BSA), 2 mg/mL (10 x 1 mL ampules)

  Note: Handling, storage, and the use of reagents should be performed in accordance with the product information sheet supplied by Pierce.

EXPERIMENTAL PROTOCOL

Mixing and Equilibrating the Coomassie Reagent

Mix the Coomassie reagent solution immediately before use by gently inverting the bottle several times. Remove the amount of reagent needed and equilibrate it to room temperature (RT) prior to use.

Preparation of Diluted Albumin (BSA) Standards

Dilute stock of Albumin Standard (BSA) ampule according to the diluted albumin (BSA) standards described in Tables 1 and 2 using the same diluent as in the samples.

Standard Microplate Protocol (Upper Range)

1. Pipette 5 µL of each standard or unknown sample into the appropriate microplate wells.
2. Add 250 µL of Coomassie reagent to each well and mix with plate shaker for 30 seconds.

3. Remove plate from shaker. For maximum result consistency, incubate the microplate for 10 minutes at RT.

4. Measure the absorbance at 600 nm using the GloMax® Multi Microplate Multimode Reader.

5. Subtract the average value of the reagent blank from all other individual standard and unknown sample replicates. Use corrected data to generate a standard curve of absorbance verses protein concentration. Then use this standard curve to determine protein concentrations of unknown samples.

Table 1: High-Range Dilution

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	0	300 µL of stock	2000 µg/ml
B	125 µL	375 µL of stock	1500 µg/ml
C	325 µL	325 µL of stock	1000 µg/ml
D	175 µL	175 µL of vial B dilution	750 µg/ml
E	325 µL	325 µL of vial C dilution	500 µg/ml
F	325 µL	325 µL of vial E dilution	250 µg/ml
G	325 µL	325 µL of vial F dilution	125 µg/ml
H	400 µL	100 µL of vial G dilution	25 µg/ml
I	400 µL	0	0 µg/ml = Blank

Standard Microplate Protocol (Lower Range)

1. Pipette 150 µL of each standard or unknown sample into the appropriate microplate wells.

2. Add 150 µL of Coomassie reagent into each well and mix with plate shaker for 30 seconds.Remove plate from shaker. For maximum result consistency, incubate the microplate for 10 minutes at RT.

3. Remove plate from shaker. For maximum result consistency, incubate the microplate for 10 minutes at RT.

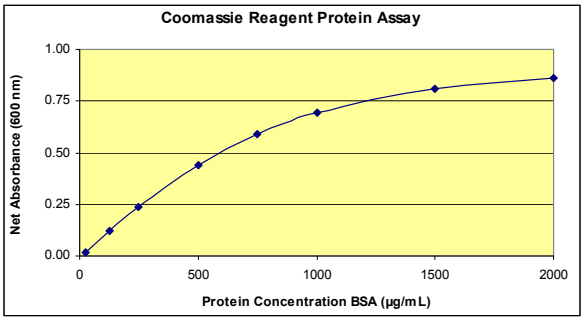
4. the absorbance at 600 nm using the GloMax® Multi Microplate Multimode Reader.

5. Subtract the average value of the reagent blank from all other individual standard and unknown sample replicates. Use corrected data to generate a standard curve of absorbance versus protein concentration. Then use this standard curve to determine protein concentrations of unknown samples.

   Note: If using curve-fitting algorithms associated with a microplate reader, a four parameter (quadratic) or best-fit curve will provide more accurate results than a purely linear fit. If plotting by hand, a point-to-point curve is preferable to a linear fit to the standard points.

Table 2: Low-Range Dilution

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	2370 µL	30 µL of stock	25 µg/ml
B	4950 µL	50 µL of stock	20 µg/ml
C	3970 µL	30 µL of stock	15 µg/ml
D	2500 µL	2500 µL of vial B dilution	10 µg/ml
E	2000 µL	2000 µL of vial D dilution	5 µg/ml
F	1500 µL	1500 µL of vial E dilution	2.5 µg/ml
G	5000 µL	0	0 µg/ml = Blank



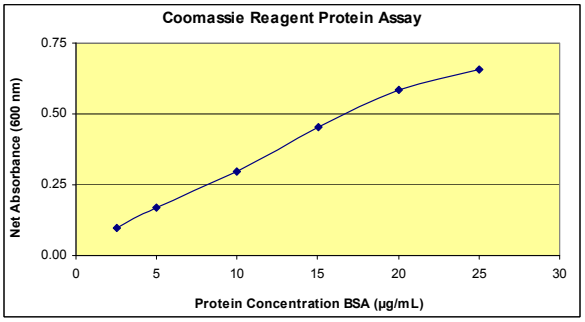


Figure 1. Typical protein concentration curves for BSA when measured with the GloMax® Multi Microplate Reader.

RESULTS

Sensitivity:< 2.5 µg/mL

Instrument Dynamic Range: 0 – 4.0 OD @ 450 - 700 nm

Minimum Detection Limit: 1.18 µg/mL; calculated using 3 x standard deviation of the assay background, n = 24

CONCLUSION

The GloMax® Multi Microplate Reader offers both superior sensitivity and dynamic range. The GloMax® Multi Microplate Reader achieves its superior performance by use of a dedicated fluorescence detector instead of sharing equipment with other detection modes. The individual Optical Kit offers solid-state optics and a powerful wavelength-matched LED to deliver excellent sensitivity and dynamic range.

The modular approach of the GloMax® Multi Microplate Reader allows for instrument capability expansion as needs in the lab change. Luminescence and/or Absorbance Detection Modules as well as other accessories can be added after the initial purchase.

Superior performance, ease of use, and the utmost flexibility of the GloMax® Multi Microplate Reader make it an ideal instrument for today's life science laboratory.

REFERENCES

1. Pierce Data Sheet 23200: Coomassie (Bradford™) Assay Kit

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