Benchmark Plus™ Microplate Reader Quantitation of Protein Concentration Using 2 Different Colorimetric Assay Kits

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The Bradford and Lowry assays are 2 colorimetric assays that are commonly used to quantitate protein concentration. Bio-Rad's protein assay (catalog #500-0001), based on the Bradford dye-binding assay (Bradford 1976), measures total protein concentration. The standard assay is used for protein concentrations ranging from 50 µg/ml to 1.0 mg/ml. This assay is based on the change of color of Coomassie Brilliant Blue G-250 dye with different protein concentrations, in which the dye binds to basic and aromatic amino acid residues. This assay is measured between 465 and 595 nm. Bio-Rad's detergent compatible (*DC*) protein assay (catalog #500-0111) is similar to the Lowry assay (Lowry et al. 1951), but only requires a 15 min incubation. The reaction between protein and copper in the alkaline solution and the Folin reagent reduced by the copper-treated protein leads to color development. This protein assay is measured between 650 and 750 nm, with protein concentrations ranging from 200 µg/ml to 1.0 mg/ml.

Procedure

Bradford Method (Bio-Rad Protein Assay) Using Clear 96- and 384-Well Plates

1. Dilute dye reagent with 1 part of dye reagent concentrate and 4 parts of distilled water. Filter through a Whatman #1 filter. The diluted solution is good for about 2 weeks when stored at room temperature.
2. Serially dilute standard bovine IgG protein 1:2, ranging from 31 µg/ml to 1.0 mg/ml.
3. Aliquot 10 µl of each diluted standard in triplicate into a clear 96-well microplate.
4. Add 200 µl of diluted dye reagent. Mix thoroughly using a microplate mixer.
5. Incubate 5 min at room temperature.
6. Measure absorbance at 595 nm.
7. For a 384-well microplate, the protein standard and diluted dye reagent volumes are reduced to one quarter of the total volume used in a 96-well plate; that is, 50 µl per well.
8. For 384-well measurements, aliquot 10 µl of each diluted standard into a tube.
9. Add 200 µl of diluted dye reagent. Vortex for about 5 sec.
10. Let incubate for 5 min at room temperature.
11. Aliquot 52.5 µl per well. Make sure no bubbles are present, as this may cause reading errors.
12. Measure absorbance at 595 nm.

Lowry Method (Bio-Rad Detergent Compatible (*DC*) Protein Assay) using 96- and 384-Well Plates

1. Prepare working reagent as follows: Add 20 µl of reagent S to 1 ml of reagent A as needed for assay.
2. Serially dilute the standard bovine IgG protein 1:2, from 125 µg/ml to 2.0 mg/ml.
3. Aliquot 30 µl of each of the diluted standards into a separate 2 ml tube.
4. Add 150 µl of the working reagent to each tube and then add 1.2 ml of reagent B.
5. Vortex for about 5 sec.
6. Incubate at room temperature for 15 min.
7. Aliquot 230 µl of each sample in triplicate into a clear 96-well microplate.
8. Measure absorbance at 750 nm.
9. For a 384-well microplate, aliquot 6.25 µl of diluted standard into a 1.5 ml microfuge tube.
10. Add 31.25 µl of working reagent and 250 µl of reagent B.
11. Vortex for about 5 sec.
12. Incubate at room temperature for 15 min.
13. Aliquot 57.5 µl of each sample in triplicate into a clear 384-well microplate.
14. Measure at 750 nm.

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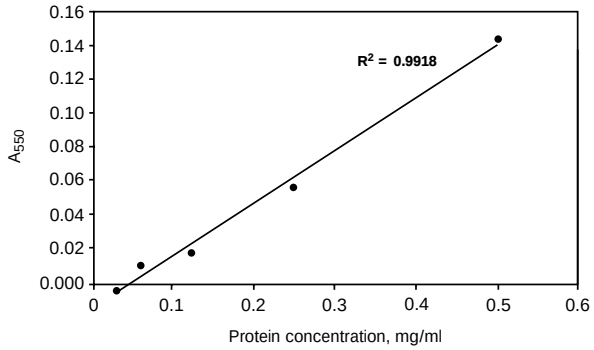

Fig. 1. Absorbance vs. protein concentration using Bio-Rad protein assay (Bradford method) with a 96-well plate.

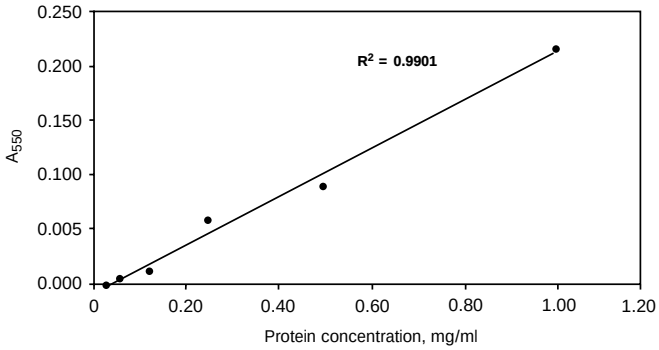

Fig. 2. Absorbance vs. protein concentration using Bio-Rad protein assay (Bradford method) with a 384-well plate.

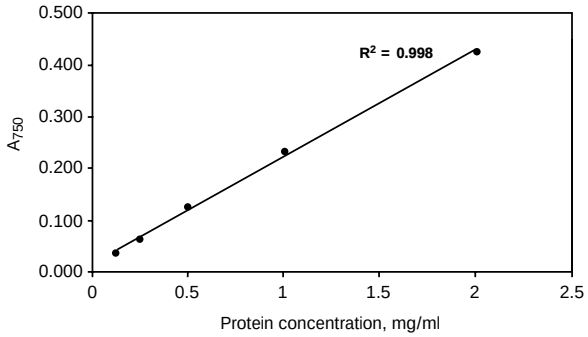

Fig. 3. Absorbance vs. protein concentration using Bio-Rad detergent compatible (*DC*) protein assay with a 96-well plate.

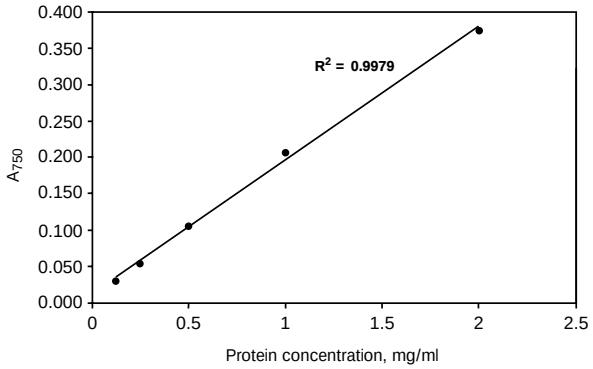

Fig. 4. Absorbance vs. protein concentration using Bio-Rad detergent compatible (*DC*) protein assay with a 384-well plate.

Results

Bio-Rad's protein assay kit gave linear results using the Benchmark Plus microplate reader to quantitate protein concentration. The linear range for Bio-Rad's protein assay was between 50 µg/ml and 1.0 mg/ml in both 96- and 384-well plates (Figures 1 and 2). Readings were linear within their designated ranges with a correlation coefficient of ≥0.99.

Bio-Rad's detergent compatible (*DC*) protein assay kit gave linear results using the Benchmark Plus microplate reader to quantitate protein concentration. The linear range for both plates was between 125 µg/ml and 2.0 mg/ml in both 96- and 384-well plates (Figures 3 and 4). Readings were linear within their designated ranges with a correlation coefficient of ≥0.99.

Conclusion

The Benchmark Plus microplate reader is an excellent instrument to quantitate concentrations of protein samples using 2 of Bio-Rad's protein assay kits. The correlation coefficients of all the graphs were ≥0.99, within the specified detection ranges of 31.25 µg/ml to 1.0 mg/ml. Assays of both 96- and 384-well plate formats gave linear graphs, thus allowing a choice of using these 2 different plate formats with excellent results along with high throughput. The results showed that the Benchmark Plus microplate reader exhibits excellent linearity, repeatability, and accuracy for use in 96- and 384-well plate formats.

References

Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal Biochem 72, 248–254 (1976)

Lowry OH et al., Protein measurement with the Folin phenol reagent, J Biol Chem 193, 265–275 (1951)

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