

## 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.

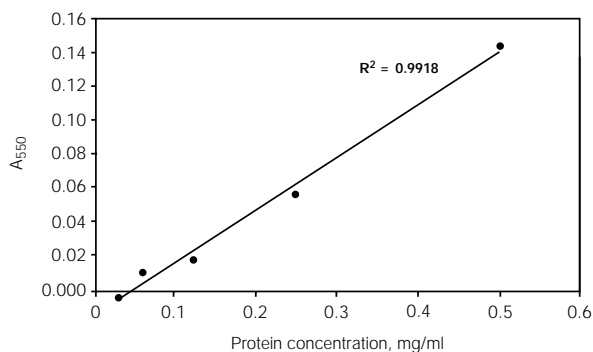


Fig. 1. Absorbance vs. protein concentration using Bio-Rad protein assay (Bradford method) with a 96-well plate.

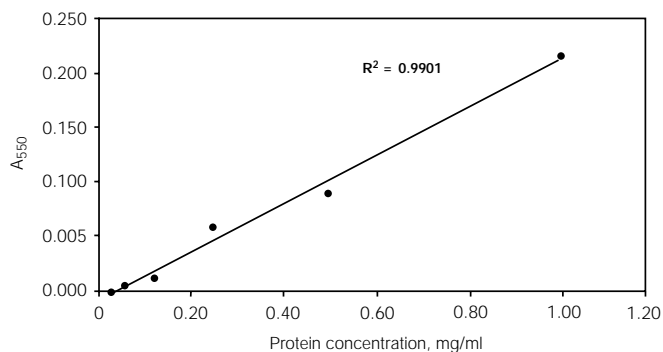


Fig. 2. Absorbance vs. protein concentration using Bio-Rad protein assay (Bradford method) with a 384-well plate.

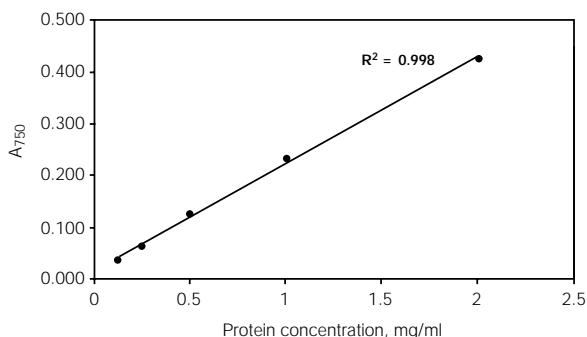


Fig. 3. Absorbance vs. protein concentration using Bio-Rad detergent compatible (DC) protein assay with a 96-well plate.

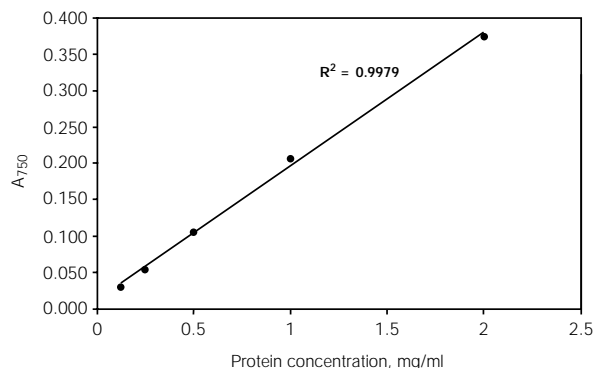


Fig. 4. Absorbance vs. protein concentration using Bio-Rad detergent compatible (DC) protein assay with a 384-well plate.

## 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  $\geq 0.99$ .

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  $\geq 0.99$ .

## 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  $\geq 0.99$ , 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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