Use of the Bradford Protein Assay in a Microtiter Plate Format

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Introduction

The Bradford protein assay is a simple procedure for determination of protein concentrations in solutions that depends upon the change in absorbance in Coomassie Blue G-250 upon binding of protein (Bradford, *Anal. Biochem. 72*: 248, 1976). Unlike many other assays, including the Lowry procedure, the Bradford assay is not susceptible to interference by a wide variety of chemicals present in samples. The notable exception is high concentrations of detergents. There is significant protein-to-protein variation in absorbance values obtained with the Bradford procedure and it is advisable to choose a protein standard that is likely to give absorbance values close to those for the protein samples of interest.

The assay here is designed for use in microtiter plates. This is an easy assay format for those with access to multiple channel pipettors and microtiter plate spectrophotometers.

Materials

Bradford Reagent - Bio-Rad sells a ready-to-use reagent (cat#500-0006) that can be stored at 4 C. Alternatively, the reagent can be made by dissolving 100 mg Coomassie Blue G-250 (available from several sources) in 50 ml 95% ethanol, adding 100 ml 85% (w/v) phosphoric acid to this solution and diluting the mixture to 1 liter with water.

Bovine serum albumin (BSA) (1 mg/ml) - we dissolve BSA in saline and store it frozen in 1 ml aliquots for quick use. The standard should be dissolved in a buffer similar to that the unknowns will be dissolved in. Note: other standards are acceptable and may be preferred for certain applications (for example, use an IgG standard when measuring concentrations of immunoglobulins.

Microtiter plates - we use Micro Test III Flexible assay plate from Falcon because they are cheap and work well.

Any multi-well microtiter plate reader equipped with a 595 nm filter.

Procedure

Note: do all determinations in duplicate or triplicate

1. Pipette 0, 2, 4, 6, 10, 15 and 20 µl of BSA (1 mg/ml) into assigned wells of a 96-well plate.

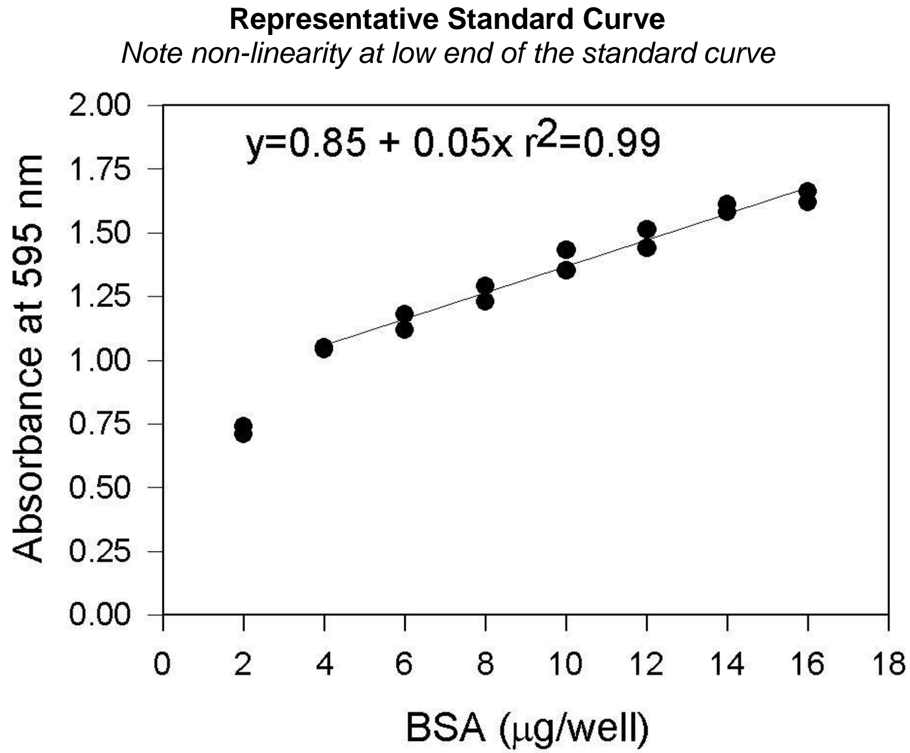
2. Pipette up to 20 µl of unknown samples into individual wells of a 96-well plate.

3. Add 40 ml of Bradford Reagent into all wells containing standard or sample.

4. Add dd H_2O to all wells to bring the final volume to 200 µl.

5. Read absorbance at 595 nm without any prior incubation.

Representative Standard Curve
Note non-linearity at low end of the standard curve



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