Product Description

The Biopioneer Bradford reagent is a quick and well-known Bradford Coomassie-binding, colorimetric method for total protein quantitation. When Coomassie dye binds protein in an acidic medium, an immediate shift in absorption maximum occurs from 465 nm to 595 nm with a concomitant color change from brown to blue.

Performing the assay in either a test tube or microplate format is simple: combine a small amount of protein sample with the assay reagent, mix well, incubate briefly, and then measure the absorbance at 595 nm. Protein concentrations are estimated by reference to absorbances obtained for a series of standard protein dilutions, which are assayed alongside the unknown samples. Because the color response with Coomassie is non-linear with increasing protein concentration, a standard curve must be completed with each assay.

Contents, Storage, and shelf life

Bradford Reagent, 500/100 mL containing Coomassie G-250 dye, ethanol, and phosphoric acid.

The 500/100 ML of Bradford reagent (Cat. No. BPBOI017) contains sufficient reagents for around 525 test tube assays.

Recommended storage is 4˚C

This reagent has a 1-year shelf life at 4°C.

Preparation of standards and assay reagent

Mix, then equilibrate the Bradford reagent

1. Mix the Bradford reagent solution immediately before use by gently inverting the bottle several times. Do not shake the bottle to mix the solution.
2. Remove the amount of reagent needed and equilibrate it to room temperature before use.

Procedures

Standard test tube protocol

Working range = 2.5-100 µg/mL

1. Pipette 0.05 mL (50 µL) of each standard or unknown sample into appropriately labelled test tubes.

2. Add 0.95 mL of the Bradford reagent to each tube, then mix well.

3. Incubate the reaction mixture for 20-30 minutes at room temperature (RT).

4. With the spectrophotometer/microplate reader set to 595 nm, zero the instrument on a cuvette filled only with water.

5. Measure the absorbance of all the samples.

6. Subtract the average 595 nm measurement for the blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.

7. Prepare a standard curve by plotting the average blank-corrected 595 nm measurement for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample

Srl No.	Concentration of Known protein (2mg/ML)	Volume of protein	Volume of diluent	Volume of Bradford reagent
1	Blanck	0	50 µl	950 µl
2	2.5 µg/ml	1.75 µl	48.25 µl	950 µl
3	05 µg/ml	2.5 µl	47.50 µl	950 µl
4	10 µg/ml	5 µl	45 µl	950 µl
5	20 µg/ml	10 µl	40 µl	950 µl
6	30 µg/ml	15 µl	35 µl	950 µl
7	50 µg/ml	25 µl	25 µl	950 µl
8	80 µg/ml	40 µl	10 µl	950 µl
9	100 µg/ml	50 µl	0 µl	950 µl
10	Unknown sample			950 µl

Table 1: Preparation of BSA/Known protein standards

Data Analysis

1. Take the OD from the spectrophotometer or microplate reader, and subtract the average blank value from the standard and unknown sample values.
2. Create a standard curve by plotting the abserbance value at 595 nm on the y-axis versus their corresponding concentration in µg/ml on the x-axis. Determine the unknown sample concentration using the standard curve. If the samples were diluted, adjust the final concentration of the unknown samples by multiplying by the dilution factor used.
3. The microplate procedure may yield lower values than the standard and microassay procedures due to a shorter light path used in the microplate reader. This may decrease the level of detection of the assay.
4. The typical standard curve of the microassay procedure is listed in Figure 1.

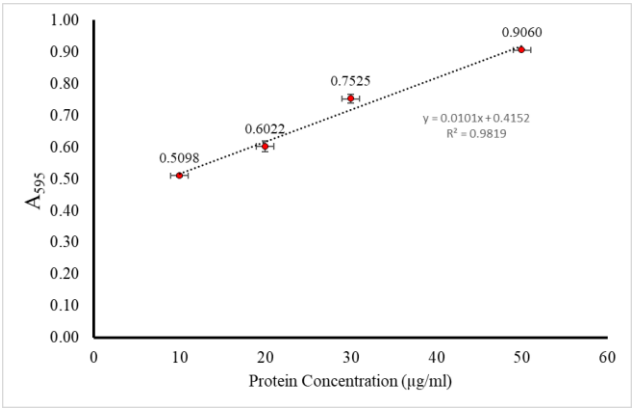

Figure 1: Typical color response curves for BSA and bovine gamma globulin (BGG) using the standard test tube protocol of the Bradford assay.

Reagents compatible with the Bradford reagent

Acetone	10%
Acetonitrile	10%
Ammonium sulfate,	1M
Ampholytes,	3–10, 0.5%
ASB-14	0.03%
Ascorbic acid,	50 mM
Bis-Tris, pH 6.5,	0.2 M
b-mercaptoethanol,	1 M
Calcium chloride,	40 mM
CHAPS,	10%
CHAPSO,	10%
Deoxycholic acid,	0.20%
Octyl b-glucoside,	0.50%
Octyl b-thioglucopyranoside,	1%
PBS	
Phenol Red	0.5 mg/ml
PIPES	0.2 M
PMSF	2 mM
Potassium chloride	2 M
Potassium phosphate	0.5 M
SB 3–10	0.10%
SDS	0.03%
Sodium acetate, pH 4.8	0.2 M
Sodium azide,	0.50%
Sodium bicarbonate,	0.2 M

Sodium carbonate,	0.1 M
Sodium chloride,	2.5 M
Sodium citrate, pH 4.8 or6.4,	0.2 M
Sodium hydroxide,	0.1 M
Sodium phosphate,	0.5 M
Sucrose,	10%
TBP,	5 mM
TBS (25 mM Tris, 0.15 M NaCl, pH 7.6),	0.5x
TCEP,	20 mM
Thio-urea,	1 M
Tricine, pH 8, 50 mM	
Triethanolamine, pH 7.8,	50 mM
Tris,	1 M
Tris-glycine (25 mM Tris, 192 mM glycine)	
Tris-glycine-SDS, (25 mM Tris, 192 mM glycine, 0.1% SDS),	0.5x
Triton X-100,	0.05%
Tween 20,	0.01%
Urea,	4 M

References

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