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Protein Biology Products

Instruction Manual

Bradford Protein Assay with BSA Protein Standard — Catalog No.: 18-442 and 18-443

INTRODUCTION

Our Bradford Protein Assay is an improved, ready to use quick colorimetric method for total protein quantitation. The assay method involves binding of the Coomassie Brilliant Blue (CBB) G-250 dye to proteins (Bradford, MM 1976). The CBB G-250 dye exists in three forms: Cationic (red), Neutral (green), and Anionic (blue) and under acidic conditions, the dye is predominantly in the doubly protonated red cationic form with A_{max} at 470 nm. However, when the dye binds to protein, an immediate shift in absorption maximum occurs from 470nm to 595nm with a concomitant color change from brown to blue, which can be measured using a spectrophotometer or microplate reader.

The Coomassie Brilliant Blue G-250 dye binds primarily to basic (arginine) and aromatic amino acid residues and shows constant extinction coefficient of a dye-albumin complex solution over a 10-fold concentration range. Thus, Beer Lambert's law is very well applied for accurate quantitation of protein by selecting an appropriate ratio of dye volume to sample concentration. Performing the assay in either cuvette or microplate format is very simple, which requires combining a small amount of protein sample with the assay reagent with mixing and brief incubation, then measuring the absorbance at 595nm. The two most common protein standards used for protein assays are bovine serum albumin (BSA) and gamma-globulin. Since, dye color development in Bradford assay is significantly greater with BSA than with most other proteins, including gamma-globulin, we supply BSA standard with our kit. The supplied reagent is sufficient for 500 standard test tube assays and 2,500 standard microwell assays

Items Supplied:

Item Name	Cat. No. 18-442	Cat. No. 18-443	Storage Condition*
Bradford Protein Assay Reagent	2 x 250 ml	2 x 500 ml	4°C
BSA Standard (2 mg/ml)	5.0 ml	2 x 5.0 ml	4°C

**The kit is shipped at ambient temperature and upon receipt, store it at 4°C. The kit components are stable for 12 months, if stored and used as recommended.*

Preparation before Use:

Remove the Bradford Protein Assay Reagent from the refrigerator and gently mix it by inverting the bottle several times. Do not shake the bottle vigorously. Remove the required volume of the dye reagent needed and equilibrate it to room temperature (RT) before use. ***NOTE:*** *The dye-dye and dye-protein aggregates tend to form in all Coomassie dye-based reagents and if left undisturbed, the aggregates will become large enough over time to be visible. However, gentle mixing completely disperses the dye-dye aggregates, therefore, it is highly recommended to mix the Coomassie Dye Reagent before use and to immediately mixing each tube or plate before measuring absorbances.*

Protocol:

Preparation of Diluted BSA Protein Standards

Dilute the supplied 2mg/ml BSA standard, preferably in the same buffer (diluent) as the test sample(s). Use the table below as a guide for preparing a set of protein standards.

Dilution Scheme for Standard Test Tube and Microplate Protocols

(Working Range 100 to 1500 µg/ml):

Tube No.	Buffer/Diluent Volume (µl)	BSA Standard (2mg/ml) Volume (µl)	Final Protein Concentration
Blank	400	0	0
1	0	400	2000 µg/ml
2	100	300	1,500 µg/ml
3	200	200	1,000 µg/ml
4	250	150	750 µg/ml
5	300	100	500 µg/ml
6	350	50	250 µg/ml
7	375	25	125 µg/ml
8	395	5	25 µg/ml

Dilution Scheme for Micro Test Tube or Microplate Protocols:

(Working Range 1 to 25 µg/ml):

Tube No.	Buffer/Diluent Volume (µl)	BSA Standard (2mg/ml) Volume (µl)	Final Protein Concentration
Blank	4,000	0	0
1	3,950	50	25 µg/ml
2	3,960	40	20 µg/ml
3	3,970	30	15 µg/ml
4	3,980	20	10 µg/ml
5	3,990	10	5 µg/ml
6	3,995	5	2.5 µg/ml
7	3,997.5	2.5	1.25 µg/ml

Test Tube Procedures:

A. Standard Test Tube Protocol (Working Range: 100-1500µg/ml)

1. Pipette 50 µl of each standard or unknown sample into appropriately labeled tubes.
2. Add 1.0 ml of the Bradford Protein Assay Reagent to each tube and mix it well.
3. Incubate the tubes for 10 minutes at room temperature (RT).
4. Set the spectrophotometer at 595nm and zero it, using a cuvette filled only with water and measure the absorbance of all the tubes.
5. Subtract the average 595nm measurement for the Blank replicates from the 595nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank-corrected 595nm measurement for each BSA standard Vs its concentration in µg/ml. Use the standard curve to determine the protein concentration of unknown sample(s).

B. Micro Test Tube Protocol (Working Range: 1-25 µg/ml)

1. Pipette 1.0ml of each standard or unknown sample into appropriately labeled tubes.
2. Add 1.0 ml of the Bradford Protein Assay Reagent to each tube and mix well.
3. Incubate the tubes for 10 minutes at room temperature (RT).
4. Set the spectrophotometer at 595nm and zero it, using a cuvette filled only with water and measure the absorbance of all the tubes.
5. Subtract the average 595nm measurement for the Blank replicates from the 595nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank-corrected 595nm measurement for each BSA standard Vs its concentration in µg/ml. Use the standard curve to determine the protein concentration of unknown sample(s).

Microplate Procedures:

A. Standard Microplate Protocol (Working Range: 100-1500 µg/ml)

1. Pipette 10 µL of each standard or unknown sample into the appropriate microplate wells.
2. Add 200 µl of the Bradford Protein Assay Reagent to each well and mix it using a plate shaker for 30 seconds.
3. Remove plate from shaker and incubate plate for 10 minutes at room temperature (RT).
4. Measure the absorbance at 595nm with a plate reader.
5. Subtract the average 595nm measurement for the Blank replicates from the 595nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank-corrected 595nm measurement for each BSA standard Vs its concentration in µg/ml. Use the standard curve to determine the protein concentration of each unknown sample. If using curve-fitting algorithms associated with a microplate reader, we recommend to use a quadratic (four-parameter) or best-fit curve for more accurate results than a purely linear fit. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points. ***Note****: When compared to the Standard Test Tube Protocol, 595nm measurements obtained with the Microplate Protocols are lower because the light path used is shorter. Consequently, this may increase the minimum detection level of the assay. If higher 595nm measurements are required, use 7-10 µl of standard or sample and 250 µl of Coomassie Dye Reagent per well.*

B. <u>Micro Microplate Protocol</u> (Working Range: 1-25 µg/ml)

1. Pipette 100 µl of each standard or unknown sample into the appropriate microplate wells.
2. Add 100 µl of the Bradford Protein Assay Reagent to each well and mix with plate shaker for 30 seconds.
3. Remove plate from shaker and incubate it for 10 minutes at room temperature (RT).
4. Measure the absorbance at or near 595nm on a plate reader.
5. Subtract the average 595nm measurement for the Blank replicates from the 595nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average blank corrected 595nm measurement for each BSA standard vs. its concentration in µg/ml. Using the standard curve, determine the protein concentration estimate for each unknown sample. *<u>Note</u>: 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 results by hand, a point-to-point curve is preferable to a linear fit to the standard points.*

Reagents compatible with Bradford Protein Assay if standard recommended procedure is used:

Salts & Buffers	Concentration
ACES, pH 7.8	100mM
Ammonium sulfate	1M
Asparagine	10mM
Bicine, pH 8.4	100mM
Bis-Tris, pH 6.5	100mM
Borate (50mM), pH 8.5	undiluted
Calcium chloride in TBS, pH 7.2	10mM
Na-Carbonate/Na-Bicarbonate (0.2M), pH 9.4	Undiluted
Cesium bicarbonate	100mM
CHES, pH 9.0	100mM
Na-Citrate (0.6M), Na-Carbonate (0.1M), pH 9.0	Undiluted
Na-Citrate (0.6M), MOPS (0.1M), pH 7.5	undiluted
Cobalt chloride in TBS, pH 7.2	10mM
EPPS, pH 8.0	100mM
Ferric chloride in TBS, pH 7.2	10mM
Glycine	100mM
Guanidine-HCl	3.5M
HEPES, pH 7.5	100mM
Imidazole, pH 7.0	200mM
MES, pH 6.1	100mM
MES (0.1M), NaCl (0.9%), pH 4.7	undiluted
MOPS, pH 7.2	100mM
Modified Dulbecco's PBS, pH 7.4	undiluted
Nickel chloride in TBS, pH 7.2	10mM
PBS; Phosphate (0.1M), NaCl (0.15M), pH 7.2	undiluted
PIPES, pH 6.8	100mM
RIPA lysis buffer; 50mM Tris, 150mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0	1/10 dilution with ultrapure water
Sodium acetate, pH 4.8	180mM
Sodium azide	0.5%
Sodium bicarbonate	100mM
Sodium chloride	5.0M

Detergents	Concentration
Brij-35	0.125%
Brij-58	0.031%
CHAPS	5.0%
CHAPSO	5.0%
Deoxycholic acid	0.05%
Octyl β-glucoside	0.5%
Nonidet P-40 (NP-40)	0.5%
Octyl β-thioglucopyranoside	3.0%
SDS	0.125%
Triton X-100	0.125%
Triton X-114	0.125%
Tween-20	0.062%
Tween-80	0.062%

Chelating agents	Concentration
EDTA	100mM
EGTA	2mM
Sodium citrate	200mM

Reducing & Thiol-Containing Agents	Concentration
N-acetylglucosamine in PBS, pH 7.2	100mM
Ascorbic acid	50mM
Cysteine	10mM
Dithioerythritol (DTE)	1mM
Dithiothreitol (DTT)	5mM
Glucose	1M
Melibiose	100mM
β-Mercaptoethanol	1M
Potassium thiocyanate	3M
Thimerosal	0.01%

Sodium citrate, pH 4.8 or pH 6.4	200mM
Sodium phosphate	100mM
Tricine, pH 8.0	100mM
Triethanolamine, pH 7.8	100mM
Tris	2M
TBS; Tris (25mM), NaCl (0.15M), pH 7.6	Undiluted
Tris (25mM), Glycine (192mM), pH 8.0	Undiluted
Tris (25mM), Glycine (192mM), SDS (0.1%), pH 8.3	1/2 dilution with ultrapure water
Zinc chloride in TBS, pH 7.2	10mM

Other Reagents & Solvents	Concentration
Acetone	10%
Acetonitrile	10%
Aprotinin	10 mg/L
DMF	10%
DMSO	10%
Ethanol	10%
Glycerol (Fresh)	10%
Hydrochloric acid	100 mM
Leupeptin	10 mg/L
Methanol	10%
Phenol Red	0.5 mg/ml
PMSF	1 mM
Sodium Hydroxide	100 mM
Sucrose	10%
TLCK	0.1 mg/L
TPCK	0.1mg/L
Urea	3M
o-Vanadate (sodium salt), in PBS, pH 7.2	1mM

REFERENCE(S):

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)

RELATED PRODUCTS:

1. **Protein Extraction Buffers/ Kits (Cat. No. 18-400, 18-402, 18-404, 18-406, 18-409, 18-411**
   For extracting proteins from Bacteria, Insects Cells, Mammalian cells, Tissues and Yeast samples
2. **RIPA Lysis Buffer (18-415, 18-416 and 18-417**
   For extracting proteins from different species samples.
3. **Protein Loading Buffer [2X], Cat. No. 20-309**
   Non-reducing ready to use buffer for loading protein samples on to the gel. Premixed, just add an equal volume to your protein sample
4. **Protein Loading Buffer [2X], Cat. No. 20-310**
   Reducing ready to use buffer for loading protein samples on to the gel. Premixed, just add an equal volume to your protein sample

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