Cyrusbioscience

Bradford Protein Assay Reagent (5X)

Product No.: BRDK

Introduction

Bradford Protein Assay Reagent is a fast protein quantification method. It's based on the method of Bradford, Coomassie-binding with protein in an acidic solution. The measurement of absorbance shifts from 645nm (brown color) to 595nm (blue color) when binding to protein occurs. In addition, the coloration differs greatly depending on the basic and aromatic amino acid residues of protein. Bradford Protein Assay Reagent provides a wide protein quantification range from 100-1,400 µg/mL or 1-25 µg/mL and the measured absorbance at 595nm is stable for 5 to 60 minutes after the binding reaction starts.

Product component

Bradford Protein Assay Reagent (5X): 500 mL
BSA standard(2mg/mL): 1mL x 10
User's Manual

Storage / Stability

Bradford Protein Assay Reagent should be stored at 4°C.

Materials needed but not provided

1. 96 well plate
2. Test tubes
3. Vortex mixer
4. Plate shaker
5. Spectrophotometer capable of measuring absorbance in the region of 595 nm
6. Microplate Reader capable of measuring absorbance in the region of 595 nm

   Note: *If a 595nm filter is not available, perform measurement with a 575-615nm filter, please note that the slope of standard curve and overall assay sensitivity will be reduced.*

Instruction

A. Preparation of the Bradford Reagent

1. Prepare Bradford Reagent by mixing 1 part of **Bradford Reagent (5X)** and 4 parts of ddH_2O.
2. The required Bradford Reagent for each samples of Test Tube Procedure is 5.0 mL and that of the Microplate Procedure is 200 µL.

   Note: *The Bradford Reagent is a light brown solution and is stable for several days when stored in a closed container at room temperature.*

   Note: *Certain substances are known to interfere with the Bradford assay and it must be avoided in the sample's buffer. The maximum compatible concentrations for these substances are listed in* ***Table 5.***

B. Preparation of the Protein Standards

1. Preparation of diluted protein standards
2. For "**Test Tube Procedure**", use standard guide of 100-1,400 µg/mL or 20-1,000 µg/mL in **Table 1 or Table 2** for the standard protocol and 1-25 µg/mL in **Table 3** for the micorassay protocol.
3. For "**Microplate Procedure**", use standard guide of 100-1,400 µg/mL or 20-1,000 µg/mL in **Table 4 or Table 5** for the standard protocol and 1-25 µg/mL in **Table 6** for the micorassay protocol.

- Preparation of diluted BSA Standards for Test Tube Procedure

Table 1. Working range: 100-1,400 µg/mL

Tube	Volume of Diluent (µL)	Volume and source of 2000ug/ml BSA standard(µL)	Final BSA Standard Concentration (µg/mL)
A	125	375 of Stock	1,500
B	325	325 of Stock	1,000
C	175	175 of tube A dilution	750
D	325	325 of tube B dilution	500
E	325	325 of tube D dilution	250
G	200	200 of tube E dilution	125
H	400	0	0

Table 2. Working range: 20-1,000 µg/mL

Tube	Volume of Diluent (µL)	Volume and source of 2000ug/ml BSA standard(µL)	Final BSA Standard Concentration (µg/mL)
A	500	500 of Stock	1,000
B	125	375 of tube A dilution	750
C	325	325 of tube A dilution	500
D	325	325 of tube C dilution	250
E	325	325 of tube D dilution	125
F	400	100 of tube E dilution	25
G	400	0	0

Table 3. Working range: 1-25 µg/mL

Tube	Volume of Diluent (µL)	Volume and source of 2000ug/ml BSA standard(µL)	Final BSA Standard Concentration (µg/mL)
A	3,160	40 of Stock	25
B	3,960	40 of Stock	20
C	1,000	1,000 of tube A dilution	12.5
D	2,000	2,000 of tube B dilution	10
E	2,000	2,000 of tube D dilution	5
F	2,000	2,000 of tube E dilution	2.5
G	2,000	0	0

- Preparation of diluted BSA Standards for Microplate Procedure

Table 4. Working range: 100-1,400 µg/mL

Tube	Volume of Diluent (µL)	Volume and source of 2000ug/ml BSA standard(µL)	Final BSA Standard Concentration (µg/mL)
A	25	75 of Stock	1,500
B	50	50 of Stock	1,000
C	30	30 of tube A dilution	750
D	30	30 of tube B dilution	500
E	30	30 of tube D dilution	250
F	20	20 of tube E dilution	125
G	40	0	0

Table 5. Working range: 20-1,000 µg/mL

Tube	Volume of Diluent (µL)	Volume and source of 2000ug/ml BSA standard(µL)	Final BSA Standard Concentration (µg/mL)
A	50	50 of Stock	1,000
B	10	30 of tube A dilution	750
C	30	30 of tube A dilution	500
D	30	30 of tube C dilution	250
E	30	30 of tube D dilution	125
F	40	10 of tube E dilution	25
G	40	0	0

Table 6. Working range: 1-25 µg/mL

Tube	Volume of Diluent (µL)	Volume and source of 2000ug/ml BSA standard(µL)	Final BSA Standard Concentration (µg/mL)
A	790	10 of Stock	25
B	990	10 of Stock	20
C	200	200 of tube A dilution	12.5
D	400	400 of tube B dilution	10
E	400	400 of tube C dilution	5
F	400	400 of tube D dilution	2.5
G	400	0	0

C. Test tube Procedure

■ Standard Protocol (Working range: 100- 1,400 µg/mL)

1. Pipet 100µL of each standard (Table 1) and unknown sample replicate into an appropriately labeled test tube.
2. Add 3.0 mL of the **Bradford Reagent (1X)** to each tube and vortex well.
3. Incubate at room temperature for at least 5 minutes.

4. Turn on the spectrophotometer and set to 595nm to measure the absorbance of all the samples and the BSA standard within 1 hour of the reaction.
5. Prepare a standard curve by measurement the absorbance of BSA at 595nm and determine the protein concentration of each unknown sample by standard curve.

■ Standard Protocol (Working range: 20- 1,000 µg/mL)

6. Pipet 100µL of each standard (Table 2) and unknown sample replicate into an appropriately labeled test tube.
7. Add 5.0 mL of the **Bradford Reagent (1X)** to each tube and vortex well.
8. Incubate at room temperature for at least 5 minutes.
9. Turn on the spectrophotometer and set to 595nm to measure the absorbance of all the samples and the BSA standard within 1 hour of the reaction.
10. Prepare a standard curve by measurement the absorbance of BSA at 595nm and determine the protein concentration of each unknown sample by standard curve.

■ Microassay Protocol (Working range: 1-25 µg/mL)

1. Pipet 800µL of each standard (Table 3) and unknown sample replicate into an appropriately labeled test tube.
2. Add 200µL of the **Bradford Reagent (5X)** to each tube. Mix the sample and **Bradford Reagent (5X)** thoroughly using vortex mixer.
3. Incubate at room temperature for at least 5 minutes.
4. Turn on the spectrophotometer and set to 595nm to measure the absorbance of all the samples and the BSA standard within 1 hour of the reaction.
5. Prepare a standard curve by measurement the absorbance of BSA at 595nm and determine the protein concentration of each unknown sample by standard curve.

D. Microplate Procedure

■ Standard Protocol (Working range: 100- 1,400 µg/mL)

1. Pipet 5µL of each standard (Table 4) and unknown sample replicate into a microplate well.
2. Add 250µL of the **Bradford Reagent (1X)** to each well. Mix the sample and the reagent thoroughly using plate shaker.
3. Incubate at room temperature for at least 5 minutes.
4. Measure the absorbance at 595nm on a microplate reader within 1 hour of the reaction.
5. Prepare a standard curve by measurement the absorbance of BSA at 595nm and determine the protein concentration of each unknown sample by standard curve.

■ Standard Protocol (Working range: 20- 1,000 µg/mL)

1. Pipet 10µL of each standard (Table 5) and unknown sample replicate into a microplate well.
2. Add 200µL of the **Bradford Reagent (1X)** to each well. Mix the sample and the reagent thoroughly using plate shaker.

3. Incubate at room temperature for at least 5 minutes.
4. Measure the absorbance at 595nm on a microplate reader within 1 hour of the reaction.
5. Prepare a standard curve by measurement the absorbance of BSA at 595nm and determine the protein concentration of each unknown sample by standard curve.

■ Microassay Protocol (Working range: 1-25 µg/mL)

1. Pipet 160µL of each standard (Table 6) and unknown sample replicate into a microplate well.
2. Add 40µL of the **Bradford Reagent (5X)** to each well. Mix the sample and **Bradford Reagent (5X)** thoroughly using plate shaker.
3. Incubate at room temperature for at least 5 minutes.
4. Measure the absorbance at 595nm on a microplate reader within 1 hour of the reaction.
5. Prepare a standard curve by measurement the absorbance of BSA at 595nm and determine the protein concentration of each unknown sample by standard curve.

Appendix

Table 5. Compatible concentration of common substances

Chelating agents	
EDTA	100mM
EGTA	50mM
Sodium citrate	200mM
Detergents	
Brij-35	0.12%
Brij-56, Brij -58	0.03%
CHAPS, CHAPS O	5.00%
Deoxycholic acid	0.05%
Octyl β-glucoside	0.50%
Nonidet P-40 (NP-40)	0.50%
Octyl β-thioglucopyranoside	3.00%
SDS	0.12%
Span 20	0.50%
Triton X-100, X-114	0.12%
Triton X-305, X-405	0.50%
Tween-20, Tween-80	0.06%
Tween-60	0.10%
Zwittergent 3-14	0.02%
Reducing & Thiol-Containing Agents	
N-acetylglucosamine in PBS, pH 7.2	100mM
Ascorbic acid	50mM
Cysteine	10mM
Dithioerythritol (DTE)	1mM
Dithiothreitol (DTT)	5mM
Glucose	1M
Melibiose	100mM
2-Mercaptoethanol	1M
Potassium thiocyanate	3M
Thimerosal	0.01%

Salts or Buffers	
ACES, pH 7.8	100mM
Acetate	600mM
Adenosine	1mM
Ammonium sulfate	1M
Asparagine	10mM
ATP	1mM
Bicine, pH 8.4	100mM
Bis-Tris, pH 6.5	100mM
Borate, pH 9.5	50mM
Calcium chloride in TBS, pH 7.2	10mM
Cesium bicarbonate	100mM
CHES, pH 9.0	100mM
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
MOPS, pH 7.2	100mM
Nickel chloride in TBS, pH 7.2	10mM
PBS; Phosphate (0.1 M), NaCl (0.15 M), 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
Sodium acetate, pH 4.8	180mM
Sodium azide	0.50%
Sodium bicarbonate	100mM

Misc. Reagents & Solvents			
Acetone	10%	Sodium chloride	5M
Acetonitrile	10%	Sodium citrate, pH 4.8 or pH 6.4	200mM
Aprotinin	10mg/L	Sodium phosphate	100mM
DMF, DMSO	10%	Tricine, pH 8.0	100mM
Ethanol	10%	Triethanolamine, pH 7.8	100mM
Glycerol (Fresh)	10%	Tris	2M
(~continued)		TBS; Tris (25mM), NaCl (0.15 M), pH 7.6	undiluted
Hydrochloric Acid	100mM	Tris (25mM), Glycine (192mM), pH 8.0	undiluted
Leupeptin	10mg/L		
Methanol	10%		
Phenol Red	0.5mg/L		
PMSF	1mM		
Sodium Hydroxide	100Mm		
Sucrose	10%		
TLCK	0.1mg/L		
TPCK	0.1mg/L		
Urea	6M		

Trouble shooting

Problem	Possible cause	Solution
A precipitate forms in all tubes	Sample contains a surfactant (detergent)	Dialyze or dilute the sample.
	Samples not mixed well or left to stand for extended time, allowing aggregates to form with the dye.	Mix samples immediately prior to measuring absorbance
The Protein Standards show unfavorable linear regression	Samples and reagent are not vortexed or mixed well	Mix thoroughly using vortex mixer or plate shaker
Sample color less intense than expected	Reagent still cold	Allow reagent to warm to RT
	Sample protein (peptide) has a low molecular weight (e.g. less than 3,000)	Use BCA Protein Assay Kit (Cyrusbio)
All the tubes are dark blue	Strong alkaline buffer raises pH of formulation	Dialyze or desalt the sample
	Sample volume too large, thereby raising reagent pH	Dialyze or desalt the sample