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Bradford Protein Assay Reagent

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 1-1,000 $\mu\text{g/mL}$ 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, 1 bottle
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 ddH₂O.
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**.*



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B. Preparation of the Protein Standards

1. Preparation of diluted protein standards
2. For “**Test Tube Procedure**”, use standard guide of 20-1,000 $\mu\text{g/mL}$ in **Table 1** for the standard protocol and 1-25 $\mu\text{g/mL}$ in **Table 2** for the micorassay protocol. For “**Microplate Procedure**”, use standard guide of 20-1,000 $\mu\text{g/mL}$ in **Table 3** for the standard protocol and 1-25 $\mu\text{g/mL}$ in **Table 4** for the micorassay protocol.

- Preparation of diluted BSA Standards for Test Tube Procedure

Table 1. Working range: 20-1,000 $\mu\text{g/mL}$

Tube	Volume of Diluent (μL)	Volume and source of protein Standards (μL)	Final BSA Standard Concentration ($\mu\text{g/mL}$)
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 2. Working range: 1-25 $\mu\text{g/mL}$

Tube	Volume of Diluent (μL)	Volume and source of protein Standards (μL)	Final BSA Standard Concentration ($\mu\text{g/mL}$)
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 3. Working range: 20-1,000 $\mu\text{g/mL}$

Tube	Volume of Diluent (μL)	Volume and source of protein Standards (μL)	Final BSA Standard Concentration ($\mu\text{g/mL}$)
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



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Table 4. Working range: 1-25 µg/mL

Tube	Volume of Diluent (µL)	Volume and source of protein Standards (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: 20- 1,000 µg/mL)

1. Pipet 100µL of each standard (Table 1) and unknown sample replicate into an appropriately labeled test tube.
2. Add 5.0 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.

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

1. Pipet 800µL of each standard (Table 2) 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: 20- 1,000 µg/mL)

1. Pipet 10µL of each standard (Table 3) 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.



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- Microassay Protocol (Working range: 1-25 µg/mL)
1. Pipet 160µL of each standard (Table 4) 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		Salts or Buffers	
EDTA	100mM	ACES, pH 7.8	100mM
EGTA	50mM	Acetate	600mM
Sodium citrate	200mM	Adenosine	1mM
Detergents		Ammonium sulfate	1M
Brij-35	0.12%	Asparagine	10mM
Brij-56, Brij -58	0.03%	ATP	1mM
CHAPS, CHAPS O	5.00%	Bicine, pH 8.4	100mM
Deoxycholic acid	0.05%	Bis-Tris, pH 6.5	100mM
Octyl β-glucoside	0.50%	Borate, pH 9.5	50mM
Nonidet P-40 (NP-40)	0.50%	Calcium chloride in TBS, pH 7.2	10mM
Octyl β-thioglucopyranoside	3.00%	Cesium bicarbonate	100mM
SDS	0.12%	CHES, pH 9.0	100mM
Span 20	0.50%	Cobalt chloride in TBS, pH 7.2	10mM
Triton X-100, X-114	0.12%	EPPS, pH 8.0	100mM
Triton X-305, X-405	0.50%	Ferric chloride in TBS, pH 7.2	10mM
Tween-20, Tween-80	0.06%	Glycine	100mM
Tween-60	0.10%	Guanidine • HCl	3.5M
Zwittergent 3-14	0.02%	HEPES, pH 7.5	100mM
Reducing & Thiol-Containing Agents		Imidazole, pH 7.0	200mM
N-acetylglucosamine in PBS, pH 7.2	100mM	MES, pH 6.1	100mM
Ascorbic acid	50mM	MOPS, pH 7.2	100mM
Cysteine	10mM	Nickel chloride in TBS, pH 7.2	10mM
Dithioerythritol (DTE)	1mM	PBS; Phosphate (0.1 M), NaCl (0.15 M), pH 7.2	undiluted
Dithiothreitol (DTT)	5mM	PIPES, pH 6.8	100mM
Glucose	1M	RIPA lysis buffer; 50mM Tris, 150mM NaCl,	
Melibiose	100mM	0.5% DOC, 1% NP- 40, 0.1% SDS, pH 8.0	1/10 dilution
2-Mercaptoethanol	1M	Sodium acetate, pH 4.8	180mM
Potassium thiocyanate	3M	Sodium azide	0.50%
Thimerosal	0.01%	Sodium bicarbonate	100mM
Misc. Reagents & Solvents		Sodium chloride	5M
Acetone	10%	Sodium citrate, pH 4.8 or pH 6.4	200mM
Acetonitrile	10%	Sodium phosphate	100mM
Aprotinin	10mg/L	Tricine, pH 8.0	100mM
DMF, DMSO	10%	Triethanolamine, pH 7.8	100mM
Ethanol	10%	Tris	2M
Glycerol (Fresh)	10%	TBS; Tris (25mM), NaCl (0.15 M), pH 7.6	undiluted
(~continued)		Tris (25mM), Glycine (192mM), pH 8.0	undiluted



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Hydrochloric Acid	100mM	
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