Bradford protein assay (5X)

Cat No# A1034-500 ml
Storage 4˚C

INTRODUCTION

Bradford protein assay (5X) is a fast protein quantification method. It based on the method of Bradford, coomassie-binding with protein in an acidic solution. The measurement of absorbance shifts from 465 nm (brown color) to 595 nm (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 (5X)** provides a wide protein quantification range from 1-1,000 µg/ml and the measured absorbance at 595 nm is stable for 5 to 60 minutes after the binding reaction starts

CONTENTS

No	Component	A1040– 500 ml	
AA	Bradford Reagent (5X)	500 ml	1 bottles

SAFETY INFORMATION

Please wear gloves, lab coat and goggles while operating. Prevent contact product directly. In case of contacting, wash with large amount of water

STORAGE

Bradford protein assay (5X) should be stored at 2-8 °C. Expiration date is labeled on the bottle or box

MATERIALS NEEDED BUT NOT PROVIDED

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

Note：
If a 595 nm filter is not available, perform measurement with a 575-615 nm 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 sample of Test Tube Procedure is 5.0 ml and that of the Micro plate 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.
- 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 Standard**

1. Preparation of diluted protein standards.
2. For "**Test Tube Procedure**", use standard guide of 20-1,000 μg/ml in **Table** 1 for the standard protocol and 1-25 μg/ml in **Table** 2 for the enhanced protocol. For "**Microplate Procedure**", use standard guide of 20-1,000 μg/ml in Table 3 for the standard protocol and 1-25 μg/ml in **Table** 4 for the enhanced protoco

Table 1. Preparation of Diluted Albumin (BSA) Standards for Test tube Procedure (working range: 20-1,000 μg/ml)

Tube	Volume of diluent (μl)	Volume and source of protein standards (μl)	Final BSA standard c oncentration (μg/ml)
A	500	500 of stock	1000
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. Preparation of Diluted Albumin (BSA) Standards for Test tube Procedure (working range: 1-25 μg/ml)

Tube	Volume of diluent (μl)	Volume and source of protein standards (μl)	Final BSA standard c oncentration (μg/ml)
A	3160	40 of stock	25
B	3960	40 of stock	20
C	1000	1000 of tube A dilution	12.5
D	2000	2000 of tube B dilution	10
E	2000	2000 of tube D dilution	5
F	2000	2000 of tube E dilution	2.5
G	2000	0	0

Table 3. Preparation of Diluted Albumin (BSA) Standards for Microplate Procedure (working range: 20-1,000 μg/ml)

Tube	Volume of diluent (μl)	Volume and source of protein standards (μl)	Final BSA standard c oncentration (μg/ml)
A	50	50 of stock	1000
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 4. Preparation of Diluted Albumin (BSA) Standards for Microplate Procedure (working range: 5-250 μg/ml)

Tube	Volume of diluent (μl)	Volume and source of protein standards (μl)	Final BSA standard c oncentration (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 D dilution	5
F	400	400 of tube E dilution	2.5
G	400	0	0

C. **Test tube Procedure**

- **Standard Protocol (Working range: 20-1,000 μg/ml)**

1. Pipet 100 μl of each standard (Table 1) and unknown sample replicate into an appropriately labeled test tube.
2. Add 5.0 ml of the Bradford Reagent to each tube and vortex well.
3. Incubate at room temperature for at least 5 minutes.
4. Turn on the spectrophotometer and set to 595 nm 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 595 nm and determine the protein concentration of each unknown sample by standard curve.

- **Enhanced Protocol (Working range: 1-25 μg/ml)**

1. Pipet 800 μl of each standard (Table 2) and unknown sample replicate into an appropriately labeled test tube.
2. Add 200 μl 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 595 nm 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 595 nm 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 10 µl of each standard (Table 3) and unknown sample replicate into a microplate well.
2. Add 200 µl of the Bradford Reagent 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 595 nm on a microplate reader within 1 hour of the reaction.
5. Prepare a standard curve by measurement the absorbance of BSA at 595 nm and determine the protein concentration of each unknown sample by standard curve.

- **Enhanced Protocol (Working range: 1-25 µg/ml)**

1. Pipet 160 µl of each standard (Table 4) and unknown sample replicate into a microplate well.
2. Add 40 µl 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 595 nm on a microplate reader within 1 hour of the reaction.
5. Prepare a standard curve by measurement the absorbance of BSA at 595 nm and determine the protein concentration of each unknown sample by standard curve.

TROUBLESHOOTING

Problem	Possible cause	Remedy
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 plat 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 Dual-Range™ BCA Protein Assay Kit (Visual Protein)
All the tubes are dark blue	Strong alkaline buffer raises pH of formulation	Dialyze or dilute the sample
	Sample volume too large, thereby raising reagent pH	Dialyze or dilute the sample

APPENDIX

Table 5. Compatible concentration of common substances

Salts/Buffers		Salts/Buffers	
ACES, pH 7.8	100 mM	Ferric chloride in TBS, pH 7.2	10 mM
Acetate	600 mM	Glycine	100 mM
Adenosine	1 mM	Guanidine•HCl	3.5 M
Ammonium sulfate	1 M	HEPES, pH 7.5	100 mM
Asparagine	10 mM	Imidazole, pH 7.0	200 mM
ATP	1 mM	MES, pH 6.1	100 mM
Bicine, pH 8.4	100 mM	MOPS, pH 7.2	100 mM
Bis-Tris, pH 6.5	100 mM	Nickel chloride in TBS, pH 7.2	10 mM
Borate, pH 9.5	50 mM	PBS; Phosphate (0.1 M), NaCl (0.15 M), pH 7.2	undiluted
Calcium chloride in TBS, pH 7.2	10 mM	PIPES, pH 6.8	100 mM
Cesium bicarbonate	100 mM	RIPA lysis buffer; 50mM Tris, 150mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0	1/10 dilution
CHES, pH 9.0	100 mM		
Cobalt chloride in TBS, pH 7.2	10 mM	Sodium acetate, pH 4.8	180 mM
EPPS, pH 8.0	100 mM	Sodium azide	0.50%

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Salts/Buffers		Misc. Reagents & Solvents	
Sodium bicarbonate	100 mM	Acetone	10%
Sodium chloride	5 M	Acetonitrile	10%
Sodium citrate, pH 4.8 or pH 6.4	200 mM	Aprotinin	10 mg/l
Sodium phosphate	100 mM	DMF, DMSO	10%
Tricine, pH 8.0	100 mM	Ethanol	10%
Triethanolamine, pH 7.8	100 mM	Glycerol (Fresh)	10%
Tris	2 M	Hydrochloric Acid	100 mM
TBS; Tris (25mM), NaCl (0.15 M), pH 7.6	undiluted	Leupeptin	10 mg/l
Tris (25mM), Glycine (192mM), pH 8.0	undiluted	Methanol	10%
Chelating agents		Phenol Red	0.5 mg/l
EDTA	100 mM	PMSF	1 mM
EGTA	50 mM	Sodium Hydroxie	100 mM
Sodium citrate	200 mM	Sucrose	10%
Detergents		TLCK	0.1 mg/l
Brij-35	0.12%	TPCK	0.1 mg/l
Brij-56, Brij-58	0.03%	Urea	6 M
CHAPS, CHAPSO	5.00%	Reducing &Thiol-Containing Agents	
Deoxycholic acid	0.05%	N-acetylglucosamine in PBS, pH 7.2	100 mM
Octyl β-glucoside	0.05%	Ascorbic acid	50 mM
Nonidet P-40 (NP-40)	0.05%	Cysteine	10 mM
Octyl β-thioglucopyranoside	3.00%	Dithioerythritol (DTE)	1 mM
SDS	0.12%	Dithiothreitol (DTT)	5 mM
Span 20		Glucose	1 M
Triton X-100, X-114	0.12%	Melibiose	100 mM
Triton X-305, X-405	0.50%	2-Mercaptoethanol	1 M
Tween-20, Tween-80	0.06%	Potassium thiocyanate	3 M
Tween-60		Thimerosal	0.01%
Zwittergent 3-14	0.02%		

Technical support: service@acebiolab.com
Phone: 886-3-2870051
ACE BIOLABS CO., LTD. www.acebiolab.com
6F., No 68, Qingsheng 2nd St., Dayuan Dist., Taoyuan City33743, Taiwan.

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