T-Pro Bradford Protein Assay reagent (5X)

Store at 2~8℃

(JB04-D003) 500 ml

T-Pro Biotechnology

This product is for laboratory research ONLY and not for diagnostic use.

Description	The T-Pro Bradford protein assay (5X) was based on the Bradford method, is a simple and accurate procedure for determining concentration of solubilized protein. It involves the addition of an acidic dye to protein solution, and subsequent measurement at 595 nm with a spectrophotometer or microplate reader. Comparison to a standard curve provides a relative measurement of protein concentration.
Detection Limitations	micro assay : 1-25 µg macro assay : 20-1,000 µg
Advantages	• Fast and inexpensive • Highly specific for protein • Very sensitive • Compatible with a wide range of substances • Extinction co-efficient for the dye-protein complex is stable over 10 orders of magnitude (assessed in albumin) • Dye reagent is complex is stable for approximately one hour
Disadvantages	• Non-linear standard curve over wide ranges • Response to different proteins can vary widely, choice of standard is very important
Standard BSA	Solution (2 mg/ml): 0.2 g BSA is dissolved and made up to 100 ml with PBS.
Notes	• Dye binds to quartz cuvettes quite strongly; therefore, glass or plastic cuvettes should be used. • It should be noted that the assay primarily responds to arginine residues (eight times as much as the other listed residues) so if you have an arginine rich protein, you may need to find a standard that is arginine rich as well. • Hydrophobic, membrane or sticky proteins tend to precipitate in the presence of dyes; it is therefore recommended that small amount of sodium hydroxide is used in the assay to help in their solubility.
Storage	T-Pro Bradford Protein Assay Regent (5X) is stable for 2~8℃

Preparation of Standards and Working Reagent

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 Microplate Procedure is 200 µl.

B. Preparation of the Protein Standards

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.
3. 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 protocol.

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 Concentration (µg/ml)
A	1000	1000 of Stock	1000
B	150	450 of tube A dilution	750
C	600	600 of tube A dilution	500
D	600	600 of tube C dilution	250
E	600	600 of tube D dilution	125
F	600	600 of tube E dilution	62.5
G	900	600 of tube F dilution	25
H	600		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 Concentration (µg/ml)
A	3160	40 of Stock	25
B	3960	40 of Stock	20
C	1500	1500 of tube A dilution	12.5
D	2000	2000 of tube B dilution	10
E	1000	1000 of tube C dilution	6.25
F	2000	2000 of tube D dilution	5
G	2000	2000 of tube F dilution	2.5
H	2000		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 Concentration (µg/ml)
A	150	150 of Stock	1000
B	20	60 of tube A dilution	750
C	100	100 of tube A dilution	500
D	100	100 of tube C dilution	250
E	100	100 of tube D dilution	125
F	100	100 of tube E dilution	62.5
G	150	100 of tube F dilution	25
H	100		0

Table 4. Preparation of Diluted Albumin (BSA) Standards for Microplate Procedure (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	400	400 of tube A dilution	12.5
D	400	400 of tube B dilution	10
E	400	400 of tube C dilution	6.25
F	400	400 of tube D dilution	5
G	400	400 of tube F dilution	2.5
H	400		0

Test tube Procedure

a. Standard Protocol (Working range: 20-1,000 µg/ml)	
1	Pipet 200 µl of each standard (Table 1) or 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 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.

b. Enhanced Protocol (Working range: 1-25 µg/ml) use JB04-D003	
1	Pipet 800 µl of each standard (Table 2) or 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.

Microplate Procedure

c. Standard Protocol (Working range: 20-1,000 µg/ml)	
1	Pipet 20 µl of each standard (Table 3) or 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 plate by measurement the absorbance of BSA at 595 nm and determine the protein concentration of each unknown sample by standard plate.

d. Enhanced Protocol (Working range: 1-25 µg/mL) use JB04-D003	
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 plate by measurement the absorbance of BSA at 595 nm and determine the protein concentration of each unknown sample by standard plate.

Bradford Reagent	5X	
Standard Protocol: 20-1,000 μg/ml		
Dilrtion		Prepare 1 part of dye reagent with 4 parts of ddH_2O
test tube(sample/reagent)	100 μl / 5 ml (reagent 1X)	
microplate(sample/reagent)	10 μl / 200 μl (reagent 1X)	
Enhanced protocol: 1-25 μg/ml		
test tube(sample/reagent)	800 μl / 200 μl (reagent 5X)	
microplate(sample/reagent)	160 μl / 40 μl (reagent 5X)	
Reaction	Incubate for 5 mins at RT	
Measurement	Measure absorbance at 595 nm; color will be stable for 1 hr.	

Compatible concentration of common substances

Salts/Buffers			
ACES, pH 7.8	100mM	MES, pH 6.1	100mM
Acetate	600mM	MOPS, pH 7.2	100mM
Adenosine	1mM	Nickel chloride in TBS, pH 7.2	10mM
Ammonium sulfate	1M	PBS; Phosphate (0.1 M), NaCl (0.15 M), pH 7.2	undiluted
Asparagine	10mM	PIPES, pH 6.8	100mM
ATP	1mM	RIPA lysis buffer; 50mM Tris, 150mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0	1/10 dilution
Bicine, pH 8.4	100mM	Sodium acetate, pH 4.8	180mM
Bis-Tris, pH 6.5	100mM	Sodium azide	0.5%
Borate, pH 9.5	50mM	Sodium bicarbonate	100mM
Calcium chloride in TBS, pH 7.2	10mM	Sodium chloride	5M
Cesium bicarbonate	100mM	Sodium citrate, pH 4.8 or pH 6.4	200mM
CHES, pH 9.0	100mM	Sodium phosphate	100mM
Cobalt chloride in TBS, pH 7.2	10mM	Tricine, pH 8.0	100mM
EPPS, pH 8.0	100mM	Triethanolamine, pH 7.8	100mM
Ferric chloride in TBS, pH 7.2	10mM	Tris	2M
Glycine	100mM	TBS; Tris (25mM), NaCl (0.15 M), pH 7.6	undiluted
Guanidine-HCl	3.5M	Tris (25mM), Glycine (192mM), pH 8.0	undiluted
HEPES, pH 7.5	100mM	T-Pro RIPA lysis buffer	1/20 dilution
Imidazole, pH 7.0	200mM		

Chelating agents			
EDTA	100mM	Sodium citrate	200mM
EGTA	50mM		

Misc. Reagents & Solvents			
Acetone	10%	Methanol	10%
Acetonitrile	10%	Phenol Red	0.5mg/L
Aprotinin	10mg/L	PMSF	1mM
DMF, DMSO	10%	Sodium Hydroxide	100mM
Ethanol	10%	Sucrose	10%
Glycerol (Fresh)	10%	TLCK	0.1mg/L
Hydrochloric Acid	100mM	TPCK	0.1mg/L
Leupeptin	10mg/L	Urea	6M

Detergents			
Brij-35	0.12%	SDS	0.12%
Brij-56, Brij-58	0.03%	Span 20	0.5%
CHAPS, CHAPSO	5%	Triton X-100, X-114	0.12%
Deoxycholic acid	0.05%	Triton X-305, X-405	0.5%
Octyl β-glucoside	0.5%	Tween-20, Tween-80	0.06%
Octyl β-thioglucopyranoside	3%	Tween-60	0.1%
Nonidet P-40 (NP-40)	0.5%	Zwittergent 3-14	0.02%

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