Ver. HB230113

Bradford Protein Quantification Kit

Product description

Bradford method is one of the most sensitive methods used to determine protein concentration. It is based on the Bradford dye (Coomassie brilliant blue G250) and protein combination, the maximum absorption peak of dye from A456 to A595, and the determination of the absorption value and protein concentration is proportional to the principle of design. The method can calculate the protein concentration by the light absorption value and realize the rapidity and simplicity of protein concentration determination. High sensitivity, about four times higher than Lowry method, the lowest protein detection can reach 1μg. The determination is fast and simple, requiring only one reagent, and is not affected by the chemical reagents in most samples.

Our company provides two sizes of Bradford protein concentration detection kits, the colorimetric method can be used for 125 times, and 625 times respectively. The enzyme labeling method can be used for 500 times and 2500 times respectively.

Components

Components No.	Name	20202ES76 (500 T)	20202ES86 (2500 T)
20202-A	Bradford dye	125 mL	5×125 mL
20202-B	Protein standard(BSA)	5×1 mL (2 mg/mL)	5×2 mL (2 mg/mL)

Specifications

Assay	Bradford Assay
Product Type	Protein Quantitation Assay
For Use With (Application)	Solution-based Detection, Absorbance
For Use With (Equipment)	Spectrophotometer, Microplate Reader
Specificity	Not Target-Specific

Storage

Bradford dye in the kit should be stored at 2°C~ 8°C. Protein standard (BSA) can be stored at 2°C~ 8°C for one year.

Instructions

1. Prepare BSA standard.

The diluent of the standard is the solution of the protein sample. In principle, the standard should also be diluted with what solution the protein sample is in. But 0.9% NaCl or 1×PBS can also be used for dilution.

Refer to table 1 for the preparation of BSA standard system.

Table 1 The preparation of BSA standard system（microplate assay, linear range of 100-1500 μg/mL）

Vial	Diluent volume（μL）	2mg/mL BSA volume（μL）	Final concentration of BSA（μg/mL）

A	0	100	2000
B	25	75	1500
C	50	50	1000
D	125	75	750
E	150	50	500
F	350	50	250
G	375	25	125
H	395	5	25
I	400	0	0=Blank

2.Test method

2.1 Colorimetric assay method (linear range of 100-1500 μg/mL)

2.1.1 Take 20 μL standard of different concentration or sample to be tested to add into the reaction tube.

2.1.2 Add 1.0 mL Bradford dye into each tube and mix well. Incubate at room temperature for 10 min.

Notes: Incubation time of samples at room temperature should not exceed 1h.

2.1.3 The wavelength was set to 595 nm. Calibrate the instrument with a cuvette filled with water. And then all samples were tested.

2.1.4 Draw the standard curve (X-protein concentration μg/mL; Y-final $OD_{595\ nm}$) according to the absorbance of BSA standard (the final reading is obtained by subtracting the OD value of the blank well in the standard). The protein concentration of the sample was calculated according to the standard curve and the dilution multiple of the sample.

2.2 Microplate assay method (linear range of 100-1500 μg/mL)

2.2.1 Take 5 μL standard of different concentration or sample to be tested to add into the microplate.

2.2.2 Add 250 μL Bradford dye into each well, shake for 30 sec and mix well. Cover the microplate and incubate at room temperature for 10 min.

Notes: Incubation time of samples at room temperature should not exceed 1h.

2.2.3 The absorbance at 595 nm was measured on the enzyme label instrument. Or other absorbance in the 575-615 nm wavelength range, but with a loss of 0-10% relative to 595 nm.

2.2.4 Draw the standard curve (X-protein concentration μg/mL; Y-final $OD_{595\ nm}$) according to the absorbance of BSA standard (the final reading is obtained by subtracting the OD value of the blank well in the standard). The protein concentration of the sample was calculated according to the standard curve and the dilution multiple of the sample.

Notes: a) Because the light diameter ratio of the enzyme plate to the cuvea is short, the OD595 nm detected by the enzyme plate will be lower than that detected by the cuvea, so the detection limit of this method may be reduced. For higher OD595 nm, 7-10μL standard/sample to be tested, and 250μL Bradford dye can be used for detection. b) If curve fitting algorithm related to the marker is used, then four-parameter or best fitting curve may be fitting with more accurate results than simple linear fitting. If the concentration of each point is marked manually, the result of point-to-point curve is accurate to linear fitting. If the requirement for the accuracy of the results is not very strict, the data can be analyzed using linear fitting.

Notes

1. Bradford dye should be thoroughly mixed before use. At the same time, the enzyme label instrument needs to be preheated for 20 min.
2. Bradford dye should be restored to room temperature before use, which is conducive to improving the sensitivity of detection. In addition, invert several times before use to thoroughly mix.
3. Since the color response of Bradford dye solution is not linear with increasing protein concentration, a standard curve must be established for each test. In addition, for more accurate results, each protein gradient and sample needs to be reperforated.
4. The compatibility of protein concentration measured by Bradford method is relatively good for most chemical substances, such as the compatibility of reducing agent DTT up to 5 mM. However, it is affected by a slightly high concentration of detergent. For example, SDS should be less than 0.01%, Triton X-100 less than 0.05%, and Tween 20/60/80 less than 0.015%. For samples containing detergent, it is recommended to use the BCA Protein Quantification Kit (Cat# 20201ES).
5. Please wear the necessary PPE, such lab coat and gloves, to ensure your health and safety!
6. For research use only!

Attached table: compatibility of Bradford protein concentration determination

Name(Salt/Buffer)	Tolerance 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

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*
Sodium acetate, pH 4.8	180mM
Sodium azide	0.5%
Sodium bicarbonate	100mM
Sodium chloride	5.0M
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*
Zinc chloride in TBS, pH 7.2	10mM

Name(Denaturant)	Tolerance concentration	Reducing agent and sulfhydryl reagent	Tolerance concentration
Brij™-35	0.125%	Dithiothreitol (DTT)	5mM
Brij-56, Brij-58	0.031%	Glucose	1M
CHAPS, CHAPSO	5.0%	Melibiose	100mM
Deoxycholic acid	0.05%	2-Mercaptoethanol	1M
Lubrol™ PX	0.125%	Potassium thiocyanate	3M
Octyl β-glucoside	0.5%	Thimerosal	0.01%
Nonidet P-40 (NP-40)	0.5%	Misc. Reagents & Solvents	Tolerance concentration
Octyl β-thioglucopyranoside	3.0%	Acetone	10%
SDS	0.125%	Acetonitrile	10%
Span™ 20	0.5%	Aprotinin	10mg/L
Triton™ X-100, X-114	0.125%	DMF, DMSO	10%
Triton X-305, X-405	0.5%	Ethanol	10%
Tween™-20	0.062%	Glycerol (Fresh)	10%
Tween-60	0.1%	Hydrochloric Acid	100mM
Tween-80	0.062%	Leupeptin	10mg/L
Zwittergent™ 3-14	0.025%	Methanol	10%
Chelating agent	Tolerance concentration	Phenol Red	0.5mg/mL

EDTA	100mM	PMSF	1mM
EGTA	2mM	Sodium Hydroxide	100mM
Sodium citrate	200mM	Sucrose	10%
Reducing agent and sulfhydryl reagent	Tolerance concentration	TLCK	0.1mg/L
N-acetylglucosamine in PBS, pH 7.2	100mM	TPCK	0.1mg/L
Ascorbic acid	50mM	Urea	3M
Cysteine	10mM	o-Vanadate (sodium salt), in PBS, pH 7.2	1mM
Dithioerythritol (DTE)	1mM		