BCA Protein Quantification Kit

Product Information

Product Name	Cat#	Size
BCA Protein Quantification Kit	20201ES76	500 T
	20201ES86	2500 T
	20201ES90	5000 T

Product Description

BCA (Bicinchoninic acid) method is a widely used method for protein concentration determination at present. Based on the biuret reaction, that is, in an alkaline environment, the protein reduces Cu^{2+} to Cu^{+}, producing a purple blue complex, which has a high absorbance value at 562 nm. The amount of the reaction product is proportional to the protein concentration. BCA protein concentration determination method is simple, sensitive, fast and stable. The protein standard provided in the kit provides convenience for users to make standard curves.

The BCA protein concentration determination kit can be used for colorimetric assay and microplate assay. The former requires a large amount (100 μL) of protein sample, The ratio of protein sample to BCA working solution is 1:20 (v/v), which reduces the impact of interfering substances. The latter is simple and convenient to operate, requiring only a small amount (10-25 μL) of protein sample. However, because the ratio of protein sample to BCA working solution is 1:8 (v/v), the tolerance concentration of interfering substances is limited to some extent and the minimum detection level is reduced. Our company provides three sizes of BCA protein concentration detection kits, the colorimetric method can be used for 50 times, 250 times and 500 times respectively. The enzyme labeling method can be used for 500 times, 2500 times and 5000 times respectively.

Product Features

1. High sensitivity, with a minimum detection protein of 0.2 μg. The lower limit of detection concentration reaches 10 μg/mL.
2. It is faster and takes less time to develop color than the general BCA protein concentration determination kit. It is about 4 times faster than the traditional Lowry method.
3. Wide linear range, There is a good linear range in the concentration range of 20-2000 μg/mL.
4. It is not affected by the chemicals in most of the samples. Attached table 1 for details.

Product components

Component		20201ES76 (500 T)	20201ES86 (2500 T)	20201ES90 (5000 T)
20201-A	BCA Reagent A	100 mL	500 mL	2×500 mL
20201-B	BCA Reagent B	3 mL	15 mL	2×15 mL
20201-C	Protein standard(BSA)	5×1 mL（2 mg/mL）	10×1 mL（2 mg/mL）	10×2 mL（2 mg/mL）

Shipping and Storage

The products are shipped with ice pack. Reagent A and Reagent B in the kit can be stored at room temperature or 4℃. Protein standard (BSA) can be stored at -20℃ for 12 months if it is not used for a long time.

Cautions

1. When using BCA protein determination method, the color will deepen with the extension of time, and the speed of color reaction is related to temperature. Therefore, attention should be paid to maintaining timing and temperature to ensure accurate quantification.

2. If BCA reagent A or reagent B is found to precipitate after low temperature or long-term storage. Please incubate at 37℃ and stir to make it fully dissolved. If bacterial contamination is found, it should be discarded to avoid affecting the experimental results.

3. The experimental operation is standardized to improve the accuracy of sample loading.

4. The corresponding standard curve should be made for each determination, because the color reaction is related to the change of temperature and time, and the standard curve should be made for accurate protein quantification every time.

5. For your safety and health, please wear lab coat and disposable gloves to operate.

6. For research use only.

Instructions for use

1.Prepare standard and working solution

1.1 Prepare BSA standard system.

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 and table 2 for the preparation of BSA standard system.

Table 1 The preparation of BSA standard system（colorimetric assay, linear range of 20-2000 μg/mL）

Vial	Diluent volume（μL）	2 mg/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

If the cuvette is used for detection, 100 μL standard should be added to each tube, calculated by 3 repetitions, each concentration needs to be prepared at least 300 μL.

Table 2 The preparation of BSA standard system（microplate assay, linear range of 20-2000 μg/mL）

Vial	Diluent volume（μL）	2mg/mL BSA volume（μL）	final concentration of BSA（μg/mL）
A	0	30	2000
B	9	21	1400
C	12	18	1200
D	15	15	1000
E	21	9	600
F	24	6	400
G	27	3	200
H	49	1	40
I	30	0	0=Blank

1.2 Prepare BCA working solution

1.2.1 Calculate the total volume of BCA working solution required.

The total volume of BCA working solution = (standard + sample to be tested) × number of repetitions × BCA working solution required for each sample

【Notes】: add 2.0 mL BCA working solution to each sample during cuvette detection, and add 200 μL BCA working solution to each sample during microplate detection.

1.2.2 Prepare BCA working solution: add 1 volume of BCA reagent B (A:B=50:1) to 50 volume of BCA reagent A, and mix well.

【Notes】: after BCA reagent B is added to BCA reagent A, it becomes turbid rapidly and becomes clear immediately after mixing. BCA working solution is put into a sealed container and stabilized at room temperature for 24h.

2.Test method

2.1 Colorimetric assay method (sample: BCA working solution =1:20)

2.1.1 Take 100 μL standard or sample to be tested to add into the reaction tube.

2.1.2 Add 2.0 mL BCA working solution into each tube and mix well. Incubate at 37℃ for 30 min.

【Notes】: It can also be incubated at room temperature for 2 h, or at 60 ℃ for 30min. BCA detects the protein concentration, and prolonging the incubation time will deepen the color response. Increasing the temperature will accelerate the color reaction. However, temperature rise and time extension will reduce the lower detection limit and reduce the working linear range. If the protein concentration is very low, it can be incubated at a higher temperature or the incubation time can be extended appropriately.

2.1.3 Cool to room temperature. The wavelength was set to 562 nm. Calibrate the instrument with a cuvette filled with water. All samples were tested within 10 min.

【Notes】: Since the BCA reaction cannot reach the real reaction end point, the chromogenic reaction solution will continue even if the temperature is reduced to room temperature. However, since the color generation ratio is quite low at room temperature, if all samples can be tested for 562 nm absorbance within 10 min, no obvious error will be caused.

2.1.4 Draw the standard curve (X-protein concentration μg/mL; Y-final $OD_{562\ nm}$) 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 (sample: BCA working solution =1:8)

2.2.1 Take 25 μL standard or sample to be tested to add into the microplate.

【Notes】: The ratio of sample to working solution is 1:8. If the sample is limited, 10 μL standard and the sample to be tested (1:20), and the detection range of the kit is 125-2000 μg/mL.

2.2.2 Add 200 μL BCA working solution into each well, shake for 30 sec and mix well. Cover the microplate and incubate at 37℃ for 30min.

2.2.3 Cool to room temperature. The absorbance was detected at the wavelength range of 540-595 nm on the microplate reader, and the wavelength of 562 nm was the best.

【Notes】: a) Because the optical path of the enzyme plate is shorter than that of the cuvette, the enzyme plate needs a better sample: BCA working ratio to obtain the same detection sensitivity. If the detection wavelength is higher than 562nm, it is recommended to extend the incubation time to 2 h. b) Prolonging the incubation time or increasing the sample: the BCA working ratio will increase the $OD_{562\ nm}$ net value of each well, and reduce the lower detection limit and the working linear range.

2.2.4 Draw the standard curve (X-protein concentration μg/mL; Y-final $OD_{562\ nm}$) 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.

Attached table: compatibility of BCA protein concentration determination

Name	Tolerance concentration
Sodium bicarbonate	100mM
Sodium phosphate	25mM
2-Mercaptoethanol	0.01%
Glycercol （pure）	10%
Glycine-HCl, pH2.8	100mM
HEPES	100mM
Hydrochloric acid	100mM
Leupeptin	10mg/L
Nickel chloride （in TBS, pH8.0）	10mM
Nonidet P-40 （NP-40）	5%（w/v）

Octyl β -glucoside	5%（w/v）
Potassium thiocyanate	3.0M
SDS	5%
Sodium acetate, pH4.8	200mM
Sodium azide	0.20%
Sodium hydroxide	100mM
Sucrose	40%
Triton X-100	5%
Triton X-114, X-305,X-405	1%
Tween-20, Tween-60, Tween-80	5%
Zwittergent	1%
ACES, pH7.8	25mM
Acetone	10%
Acetonitrile	10%
Ammonium sulfate	1.5mM
Aprotinin	10mg/L
Bicine, pH8.4	20Mm
Bis-Tris, pH6.5	33mM
Borate, pH8.5	50mM
Brij-35	5%
Brij-52	1%
Brij-56, Brij-58	1%
BugBuster protein Extraction Reagent （Cat. No. 70584）	nointerference（undiluted）
Calcium chloride （in TBS, pH8.0）	10mM
CelLytic B Reagent	nointerference（undiluted）
Cesium bicarbonate	100mM
CHAPS	5%
Cobalt chloride （in TBS, pH8.0）	0.8mM
CytoBuster Protein Extraction Reagent （Cat. No. 71009）	nointerference（undiluted）
Deoxycholic acid	5%
Dithioerythritol （DTE）	1mM
Dithiothreitol （DTT）	1mM
DMF	10%
DMSO	10%
EDTA	10mM
EPPS, pH8.0	100mM
Ethanol	10%
Ferric chloride （in TBS, pH8.0）	10mM
Glucose	10mM
Glycerol	10%
Guanidine-HCl	4M
Imidazole, pH7.0	50mM

MES, PH6.1	100mM
Methanol	10%
MOPS, pH7.2	100mM
N-Acetyglucosamine（10mM）in PBS, pH7.2	10mM
Octyl β-thioglucpyranoside	5%
PIPES, pH6.8	100mM
PMSF	1mM
PopCulture Reagent （Cat. No. 71092）	nointerference（undiluted）
Reportasol Extraction Buffer （Cat. No. 70909）	nointerference（undiluted）
Sodium chloride	1M
Sodium citrate, pH4.8 or pH6.4	200mM
Sodium ortho-vanadate in PBS, pH7.2	1mM
Span 20	1%
TBS （150 mM NaCl, 100 mM Tris-HCl, pH8.0）	nointerference（undiluted）
Thimerosal	0.01%
TLCK	0.1mg/L
TPCK	0.1mg/L
Tricine, pH8.0	25mM
Triethanolamine, pH7.8	25mM
Tris	250mM
Tris（hydroxypropyl）phosphine （THP）	1mM
Urea	3M
Zinc chloride （in TBS, pH8.0）	10mM