

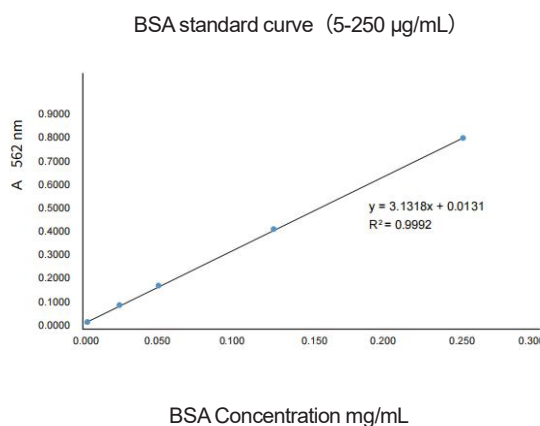
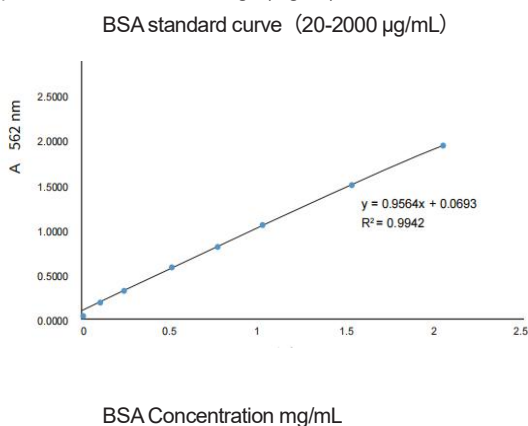
BCA Protein Assay Kit

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1. Product Description

The BCA (Bicinchoninic acid) assay is currently one of the two most commonly used methods for protein concentration measurement in the world. BCA protein quantification consisted of two steps: first, copper divalent ion (Cu²⁺) was reduced to copper monovalent ion (Cu⁺) by the peptide bond of the protein under alkaline conditions; Second, the BCA of both molecules complexate a monovalent copper ion (Cu⁺) to form a purple complex with a strong absorption value at 562 nm, and the absorption value of the complex is linearly related to the concentration of the protein within a certain range (Figure).



Protein quantification using the BCA method has the following features:

1. It is not affected by the type of protein, and has good linearity in the concentration range of 20-2000 µg/mL.
2. The surfactant has less interference to the concentration detection. However, this product is not suitable for quantification of protein samples containing reducing agents or chelating agents because reducing agents and chelating agents hinder the reaction.

Table 1 shows the basic composition of the kit, and we offer two detection protocols: test tubes and microplates. The test tube scheme required a large sample volume (0.1 mL), but the dilution ratio of sample and working solution was 1:20 (V/V), so the influence of distractors was relatively small. The microplate protocol required less working solution (200 µL) and less sample volume (25 µL), but the dilution ratio of sample and working solution was 1:8 (V/V), which was less tolerant to distractors.

Table 1. BCA Protein Assay Kit Composition

Reagent Name	250T	1250T
Solution A	50ml	250ml
Solution B	1ml	5ml
Protein Standard (2 mg/mL BSA)	1ml	1ml*5
Instruction manual	1 份	1 份

2. Operation Procedure

2.1 Dilution gradients of BSA standards

A set of protein standards was prepared according to Table 2. It is preferable to use the same dilution as the sample to be tested, and the volume of the standard at each dilution concentration is sufficient for three duplicate assays.

Table 2. Diluted bovine serum protein (BSA) standards

Dilution methods used in standard protocols (Detection Range = 20-2000 µg/mL)

Tube	Diluent Volume	Standard Volume	Final Concentration
A	0	300 µL of stock	2000 µg/mL
B	125µL	375 µL of stock	1500 µg/mL
C	325µL	325 µL of stock	1000 µg/mL
D	175µL	175 µL of B dilution	750 µg/mL
E	325µL	325 µL of C dilution	500 µg/mL
F	325µL	325 µL of F dilution	250 µg/mL
G	325µL	325 µL of F dilution	125 µg/mL
H	400µL	100 µL of G dilution	25 µg/mL
I	400µL	0	0 = Blank

Dilution method used for tube enhancement protocols (Detection Range = 5-250 µg/mL)

Tube	Diluent Volume	Standard Volume	Final Concentration
A	700µL	100 µL of stock	250 µg/mL
B	400 µL	400 µL of A dilution	125 µg/mL
C	450 µL	300 µL of B dilution	50 µg/mL
D	400 µL	400 µL of C dilution	25 µg/mL
E	400 µL	100 µL of D dilution	5 µg/mL
F	400 µL	0	0 = Blank

2.2 Preparation of BCA Working Reagent

2.2.1 Use the following formula to determine the total volume of working reagent required:

(Number of Standards + Number of Unknown Samples) × (Number of Replicates) × (Working Reagent Volume per Sample) = Total Volume of Working Reagent Required .

2.2.2 Prepare the working reagent by mixing 50 parts of Solution A with 1 part of Solution B (A:B = 50:1).

2.3 Test Tube Protocol (Sample-to-Working Reagent Ratio = 1:20)

2.3.1 Pipette 0.1 mL of each protein standard dilution and each unknown protein sample into appropriately labeled test tubes.

2.3.2 Add 2.0 mL of the working reagent to each tube and mix thoroughly.

2.3.3 Seal the tubes and incubate according to the selected protocol at the appropriate temperature and time:

Standard Protocol: 37°C for 30 minutes

Enhanced Protocol: 60°C for 30 minutes

2.3.4 Cool all tubes to room temperature.

2.3.5 Set the spectrophotometer wavelength to 562 nm. Zero the instrument using the blank standard (Tube I for Standard Protocol; Tube F for Enhanced Protocol). Then measure the absorbance of all samples within 10 minutes.

2.3.6 Plot the blank-corrected absorbance values (at 562 nm) of the BSA standards versus their concentrations (µg/mL) to generate a standard curve. Use this curve to determine the concentration of each unknown protein sample.

2.4 Microplate Protocol (Sample-to-Working Reagent Ratio = 1:8)

2.4.1 Pipette 25 µL of each protein standard dilution and each unknown protein sample into the wells of a microplate.

2.4.2 Add 200 µL of the working reagent to each well. Mix thoroughly by shaking on a plate shaker for 30 seconds.

2.4.3 Seal the plate and incubate at 37°C for 30 minutes.

2.4.4 Cool the plate to room temperature. Measure the absorbance of the samples at 562 nm using a microplate reader.

2.4.5 Subtract the average absorbance value (at 562 nm) of the blank standard wells from the absorbance of each standard and unknown sample.

2.4.6 Plot the blank-corrected average absorbance values (at 562 nm) of the BSA standards versus their concentrations (µg/mL) to generate a standard curve. Use this curve to determine the concentration of each unknown protein sample.

2.5 Precautions

2.5.1 When Solution B is added to Solution A, turbidity may be observed initially. Upon stirring, the turbidity quickly disappears, yielding a clear greenish-colored working reagent. Prepare sufficient volume of working reagent according to the number of samples to be assayed. The prepared working reagent is stable for up to 24 hours in a sealed container at room temperature.

2.5.2 This assay is not an endpoint method. Color development continues after incubation, although the rate is slower at room temperature. Complete the measurement of all samples within 10 minutes to avoid significant error.

2.5.3 For the Standard Protocol, if a 37°C incubator is not available, incubation can be performed at room temperature for 2 hours.

2.5.4 Extending the incubation time or increasing the temperature will cause the reaction color to deepen and the absorbance at 562 nm to increase, affecting reading accuracy and reducing the detection sensitivity of the reagent.

2.5.5 The Enhanced Protocol is not recommended for the microplate format because the smaller sample volume in the wells is prone to evaporation during heating, affecting detection accuracy.

2.5.6 For the tolerance concentration of various reagents in the BCA assay, please consult ACE's technical support.

2.5.7 Different proteins exhibit unique absorbance responses when assayed with the BCA kit. BSA is commonly used as the standard for quantifying unknown proteins. However, for precise quantitation, it is recommended to use a highly purified target protein as the standard. For the absorbance coefficient of other proteins relative to BSA, please consult ACE's technical support.

3. Troubleshooting

Problem	Possible Cause	Solution
No color develops after mixing working reagent with sample	Sample contains copper-chelating reagents	Dialyze, desalt, or dilute the sample
Sample color is deeper than expected	Sample concentration is too high	Dilute the sample
Blank standard absorbance is normal, but standards and unknowns show lower color than expected	Incorrect detection wavelength	Measure absorbance at 562 nm
	Buffer is strongly acidic or alkaline, altering the pH of the working reagent	Dialyze, desalt, or dilute the sample
All tubes (including blank) appear dark purple	Buffer contains reducing agents	Dialyze or dilute the sample
	Buffer contains carboxyl groups	
	Buffer contains biogenic amines (catecholamines)	
Spectrophotometer or microplate reader lacks a 562 nm filter	Take readings at 540 nm~590 nm	Color change can be detected at any wavelength between 540 nm and 590 nm, but sensitivity will be reduced.

4. Related products

Product	Cat.No.	Size
BCA Protein Assay Kit	BK0001-01	250 T
	BK0001-02	1250 T