ELK Biotechnology

BCA Protein concentration determination kit

CAT/NO.: BC016

Item	Component	Specification
BC016A	BCA solution A	100mL
BC016B	BCA solution B	5mL
BC016C	Protein standard	1mL*3
	Manual	1copy

Storage conditions: solution A and solution B are stored at room temperature, and protein standards are stored at -20° C, valid for one year.

Product introduction:

Under alkaline conditions, the protein reduces Cu2+ to Cu+, and Cu+ forms a purple complex with the BCA reagent, and the light absorption intensity is proportional to the protein concentration.Measure its absorbance at 562nm and compare it with the standard curve to calculate the concentration of the protein to be tested.The protein determination range of this product is 25-2000 μg/mL, and the determination can be completed in about 1 hour. The BCA method is not affected by the chemical substances in most samples, and can be compatible with up to 5% SDS, 5% Triton X-100, and 5% Tween 20 in the sample.However, due to the influence of chelating agents and slightly higher concentrations of reducing agents, it is necessary to ensure that EDTA is less than 10mM, without EGTA, dithiothreitol is less than 1mM, and β-mercaptoethanol is less than 0.01%.

Instructions for use:

1.Preparation of working solution: According to the number of standards and samples, 50 volumes of BCA solution A plus 1 volume of BCA solution B (50:1) are used to prepare an appropriate amount of BCA working solution, and mix thoroughly.

2. Dilute the standard product to 125-2000 μg/mL according to the following table. The diluent should be PBS.

Tube number	Diluent volume(μL)	Standard volume(μL)	Final
1	150	100	2000
2	30	90（Take from tube 1）	1500
3	60	60（Take from tube 1）	1000
4	60	60（Take from tube 2）	750
5	60	60（Take from tube 3）	500
6	60	60（Take from tube 5）	250
7	60	60（Take from tube 6）	125
8	60	0	0

3. Add 25 μ L of standard and appropriate volume of sample (if the sample volume is less than 25 μ L, make up to 25 μ L with PBS) respectively into the microwells of the 96-well plate.

4.Add 200 μ L of BCA working solution to each well and mix well.

5.Cover the 96-well plate and incubate at 37° C for 30 minutes.

6.Cool to room temperature, measure A562 with a microplate reader, and calculate the protein concentration according to the standard curve.

Precautions:

1、 When precipitation occurs under low temperature conditions or long-term storage, it can be stirred or incubated at 37° C. If reagent contamination is found, it should be discarded.

2、 If the sample contains EDTA, EGTA, DTT, ammonium sulfate, and lipids, the test results will be affected.

3、 To obtain more accurate protein concentration results, each protein gradient and sample need to be replicated, and the standard and sample processing should be as the same as possible, and a standard curve should be drawn each time.

4、 A 37° C water bath or incubator and a microplate reader should be prepared. The measurement wavelength is between 540-595nm, and 562nm is the best. When using the incubator for incubation, care should be taken to prevent water evaporation from affecting the test results.

5、 When determining the protein concentration, the absorbance will continue to deepen with the extension of the incubation time, and the color reaction will be accelerated due to the increase in temperature. If the concentration is lower, it is suitable to incubate at a higher temperature or extend the incubation time.

6、 For your safety and health, please wear lab coats and gloves for operation.