BCA Protein Quantification Kit

E112

Version 22.1

Product Description

BCA Protein Quantification Kit is one of the most sensitive methods for measuring protein concentration. The protein reduces Cu^{2+} to Cu^{+} under alkaline conditions. Then, Cu^{+} interacts with the unique BCA Reagent A (containing BCA) to preform a sensitive color reaction, forming coordination complexs. The water-soluble complex has strong absorbance at A562 nm. Its absorbance and protein concentration have a good linear relationship in a wide range, and the protein concentration can be calculated according to the absorbance value. Therefore, the concentration of the protein to be tested can be calculated by measuring its absorption value at A562 nm using a microplate reader and comparing it with the standard curve.

Common concentrations of detergents (SDS, Triton X-100, Tween-20, etc.) do not affect the detection results, but they are affected by chelating agents (EDTA, EGTA), reducing agents (DTT, mercaptoethanol) and lipids. In the experiment, if sample dilution or lysis solution with high background values, it is recommended to use Bradford protein assay.

Components

Components	E112-01 250 rxns	E112-02 500 rxns
BSA Standard (1 mg/ml)	2 × 1 ml	4 × 1 ml
BCA Reagent A	50 ml	100 ml
BCA Reagent B	1 ml	2 × 1 ml

Storage

BCA Reagent A/B: Store at 15 ~ 25℃ and transport at room temperature.

BSA Standard: Store at -30 ~ -15℃ and transport at ≤0℃.

Applications

It is applicable for protein samples extracted by common lysis solution. The performance is not affected at common concentrations of most ionic and non-ionic detergents.

Notes

For research use only. Not for use in diagnostic procedure.

1. A 96-well microtiter plate and a microplate reader are required, and the best measurement wavelength is A562 nm. It can also be measured by a spectrophotometer, but when measuring, it is necessary to appropriately increase the amount of BCA working solution used, so that it is not less than the minimum detection volume of the cuvette. In addition, the amount of samples and standards can be scaled up accordingly or not. When measuring protein concentration using a spectrophotometer, the amount of samples that can be measured may be significantly reduced.
2. If not used for a long time, store the Cu reagent and PBS diluent at 2 ~ 8℃, and should be discarded if bacterial contamination is found. If crystal precipitation of BCA reagent occurs at low temperature, incubate at 37℃ to dissolve it completely, which will not affect the use.
3. If the sample dilution or lysing solution with high background values, it is recommended to use Bradford protein assay manufactured by Vazyme.
4. When using the BSA standard protein to draw the standard curve, it is recommended to dissolve the BSA in the lysis solution of the sample protein.
5. BCA reagent: not sensitive to detergents, compatible with SDS 3%, Tween-20 5%, EDTA 15 mM and EGTA-free, Triton X-100 5%; sensitive to reducing agents, compatible with DTT 0.5 mM and β-Mercaptoethanol 0.07%.
6. During the preparation of BCA working solution, be sure to replace the tip when aspirating BCA Reagent A and BCA Reagent B. The working solution should be used immediately after being freshly prepared, as long-term storage will affect the detection sensitivity.
7. Plastic cuvettes are recommended. If using glass cuvettes or quartz cuvettes, wash with a small amount of 95% ethanol immediately after use.
8. For your safety and health, please wear a lab coat and disposable gloves.

Experiment Process

1. 96-well Microplate Assay

a. Prepare BCA working solution. According to the amount of samples, mix BCA Reagent A and BCA Reagent B at a volume ratio of 50:1 to prepare an appropriate amount of BCA working solution.

b. Plot the standard curve. Take a microplate and add reagents according to the following table:

Well No.	0	1	2	3	4	5	6	7
Protein Standard (μl)	0	1	2	4	8	12	16	20
ddH_2O (μl)	20	19	18	16	12	8	4	0
BCA Working Solution (μl)	200	200	200	200	200	200	200	200
Corresponding Protein Content (μg)	0	1	2	4	8	12	16	20

c. Sample preparation: Dilute the protein sample to be tested to an appropriate concentration with ddH_2O, take 20 μl of the sample, and add 200 μl of BCA working solution.

d. Mix by shaking, and place at 37℃ for 20 - 30 min.

e. Measure the absorbance at A562 nm with a microplate reader. The absorbance without BSA is a blank control.

f. Plot a standard curve: Take the protein content (μg) as the X-axis, and the absorbance value as the Y-axis.

g. According to the measured absorbance value, the protein content of the sample can be calculated by the standard curve.

h. Calculate the protein concentration: Divide the detected protein content by the sample volume of 20 μl, and multiply by the corresponding dilution factor to obtain the actual concentration of the sample to be tested.

2. Cuvette assay

In the same way as the above method, according to the size of the cuvette, increase the volume of each solution in proportion appropriately.

Appendix

The following are the upper limits of the concentration of substances that do not interfere with the BCA protein assay:

Components	Compatible Concentration
Glucose	10 mM
Octyl Glycoside	5.0%
Sodium acetate, pH 5.5	200 mM
Sucrose	40%
Ammonium Sulfate	1.5 M
Brij-35	5.0%
CHAPS	5.0%
DTT	0.5 mM
EDTA	15 mM
Emulgen	1.0%
Glycine，pH 2.8	100 mM
Guanidine•HCl	4.0 M
Hepes	100 mM
Tween-20	5.0%
NaOH	0.1 M
NP-40	5.0%
SDS	3.0%
Sodium Chloride	1.0 M
Triton X-100	5.0%
Urea	3.0 M