BCA Protein Quantification Kit

E112

Version 20.1

Introduction

BCA Protein Quantification Kit is one of the most sensitive methods for protein assays. In an alkaline condition, Cu^{2+} can be reduced to Cu^{+} by protein and interacts with the unique BCA Reagent A(containing BCA)forming a purple complex, known as the biuret reaction. The water-soluble complex has a strong absorbance at 562 nm, which has well linear correlation with protein concentration in a wide range. Therefore, a standard curve can be drawn by measuring the absorbance of BSA standard with Microplate reader at 562 nm and the concentration of protein samples can be calculated.

The test results will not be affected by commonly used detergents such as SDS, Triton X-100 and Tween-20, but affected by chelating agents(EDTA, EGTA), deoxidizer(DTT, mercaptoethanol)and lipids. In the assay, if the background of the sample diluent buffer or lysis buffer is high, Barford Protein Assay Kit can be tried.

Package Information

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

Store BCA Reagent A/B Protein at 2 ~ 8℃;

Store BSA Standard at -30 ~ -15℃.

Protocol

1. 6-well ELISA Plate Determination:

1) Preparation of BCA working solution. According to sample size, prepare an appropriate volume of BCA working solution, in a ratio of 50 volumes of BCA Reagent A to 1 volume of BCA Reagent B(50:1), and fully mix.
2) Draw the standard curve. Take an ELISA plate and add reagents according to the following table:

Well No.	0	1	2	3	4	5	6	7
Molecular Weight Marker(μl)	0	1	2	4	8	12	16	20
ddH_2O(μl)	20	19	18	16	12	8	4	0
Working fluid(μl)	200	200	200	200	200	200	200	200
Corresponding protein content(μg)	0	1	2	4	8	12	16	20

3) Sample preparation: Dilute the protein sample with ddH_2O to the appropriate concentration, and pipette 20 μl of the diluted samples into 200 μl of BCA working solution and fully mix.
4) Incubation at 37℃ for 20 - 30 min.
5) Measure the absorbance of the solution at 562 nm by the Microplate reader, and the absorbance without BSA is used as the control.
6) Draw the standard curve by plotting the BSA protein standards and set the protein contents(μg)as the abscissa and the absorbance measurement as the ordinate.
7) Determine protein concentration by comparison of the absorbance of the unknown samples to the standard curve prepared using the BSA protein standards.
8) Calculate protein concentration: Divide the measured protein content by the sample volume 20 μl, and then multiply by the corresponding dilution factor to get the actual concentration of the sample.

2. Cuvette Determination

Follow the same steps as described above, just increase the volume of each solution in proportion based on the cuvette specifications for using.

Notes

1) A 96-well ELISA plate and a Microplate reader are needed. The best wavelength measured is at 562 nm. Spectrophotometer can also be used for determination. However, the use amount of BCA working solution should be appropriately increased according to the minimum detection volume of the cuvette, so that it is not less than the minimum detection volume. The volume of samples and standards can be scaled up accordingly or without adjustment. When using a spectrophotometer for measuring, the number of samples that can be measured by each kit might be significantly reduced.
2) The Cu reagent and PBS diluent can be stored at 2 ~ 8℃ when not in use for a long time. It should be discarded if contaminated by bacteria. When the BCA reagent crystallized or precipitated in the low-temperature condition, incubation at 37℃ to completely dissolve will not be affecting its use.
3) If the background of the sample diluent buffer or lysis buffer is high, please try Barford Protein Assay Kit produced by Vazyme.
4) When using BSA Standard protein to draw the standard curve, it is best to use the dissolving solution of the sample protein to dissolve the BSA to draw the standard curve.
5) BCA reagents are not sensitive to detergents and are compatible with SDS 3%, Tween-20 5%, EDTA 15 mM, EGTA free, Triton X-100 5%; BCA reagents are sensitive to deoxidizer and compatible with DTT 0.5 mM and β-mercaptoethanol 0.07%.
6) When preparing the BCA working solution, please change the tips for pipetting the Reagent A or the Reagent B. The working solution should be used immediately after preparation. The detection sensitivity will be affected if stored for a long time.
7) It is best to use plastic cuvettes. If use the glass cuvettes or quartz cuvettes, please wash them immediately with 95% ethanol after using.
8) This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Attached lists

The amount listed is the maximum amount of material allowed in the protein sample without causing a noticeable interference.

Incompatible Substances	E112-02(500 rxns)
Glucose	10 mM
Octyl glucoside	5.0%
Sodium acetate, pH 5.5	200 mM
Saccharose	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
TritonX-100	5.0%
Urea	3.0 M