Instructions for Use of BCA Rapid Protein Quantitative Detection Kit

This kit is intended for scientific use and not for diagnostic use

Cat. No. HG- BC001

Product introduction

BCA Rapid Protein Quantitative Detection Kit in the BlueKit® series has the characteristics of high sensitivity, stable results, and simple operation. The principle of this kit is that Cu^{2+} is reduced by protein to Cu^{+} under alkaline conditions, and then Cu^{+} and BCA interact to form a purple reaction complex, showing a strong absorbance at 562 nm, and presenting a good linear relationship with protein concentration.

Assay range : 10-2000 μg/mL

Limit of detection : 0.39 μg/mL

Specification

250T

Usage

This kit is applicable to the quantitative detection of total protein in various types of samples.

Kit composition

Components	Specification	Preparation
Protein standards S1 - S7, S0	1.5 mL/tube	Ready-to-use
Solution A	10 mL x 5 vials	Ready-to-use
Solution B	1 mL x 1 tube	Ready-to-use
Sample lysate	50 mL x 1 vial	Ready-to-use

Notes: All kit components shall be stored at room temperature. Stable for 12 months of storage.

Materials to be self-prepared:

1. Microplate reader and constant temperature incubator.
2. Micropipettes and tips.
3. Deionized water, brand-new filter paper and vortex oscillator.

Sample preparation

Samples are diluted with lentiviral sample lysate. Lentiviral sample is recommended to be diluted 40-fold, that is, 195 μL of lentiviral sample lysate is added to 5 μL of lentiviral sample. Adjust dilution factor if not within standard curve range.

Operating steps

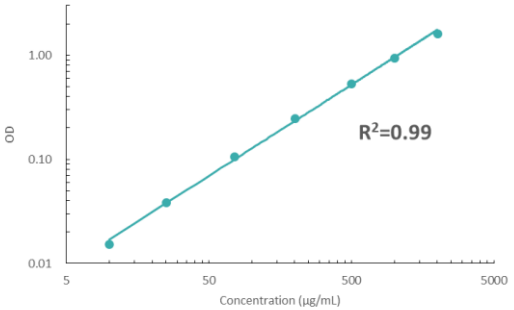
1. Prepare an appropriate amount of BCA working solution as needed. Reagent A is mixed 50:1 by volume with Reagent B and mixed thoroughly.
2. Add 50 μL of protein standards and samples to be tested into the microplate.
3. Add 200 μL of BCA working solution.
4. Allow the solution to react at room temperature for 20 minutes.
5. Absorbance is read at 562 nm using a microplate reader.
6. Calculate the actual absorbance (i.e., absorbance per well - mean blank absorbance) of the protein standards and the samples to be tested.
7. A protein standard curve is plotted and regression equations are fitted to calculate the protein concentrations of samples to be tested.

Result processing

1. OD processing of the standard curve (See the following example. For example only, please refer to the actual measurement for details).

Concentration of standard (ng/mL)	OD value (1)	OD value (2)	Mean value
0.00	0.1039	0.1039	0.1039
10	0.1196	0.1190	0.1193
25	0.1424	0.1430	0.1427
75	0.2098	0.2094	0.2096
200	0.3519	0.3540	0.3530
500	0.6408	0.6380	0.6394
1000	1.0423	1.0500	1.0462
2000	1.7229	1.7210	1.7220

2. The standard curve is obtained by linear fitting with log-log of the theoretical concentration of the standard to the corresponding OD value (as shown in the following figure).



Attached table

Compatible interfering substances and concentrations

Interfering substances	Maximum anti-interference concentration	Interfering substances	Maximum anti-interference concentration
SDS	5%	Sodium azide	0.2%
Dithiothreitol (DTT)	1 mM	2-Mercaptoethanol	0.01%
CHAPS	5%	EDTA	10 mM
Glycerol	10%	Urea	3 M
DMSO	5%	NaOH	0.25 M
(NH4)2SO4	1.5 M	Guanidine HCl	10 mM
Tris, pH 8.0	250 mM	Acetone	10%
Potassium thiocyanate	3.0 M	TritonX-100/X- 114	5%

Precautions

1. Check the reagent for precipitation before each use. If there is precipitation, dissolve the precipitation in a 37℃ warm bath before use. Discard any reagents if discoloration or microbial contamination occurs.

2. When detecting protein concentration by BCA, the absorbance deepens with time, so the determination of all samples needs to be completed within 10 minutes, otherwise it will affect the accuracy of protein quantification.

3. If the sample to be tested contains a higher concentration of non-ionic surfactant, normal Lowry method cannot be applied due to precipitation of reaction solution. This will not occur for BCA method, but instead, BCA will lead to deepening of color reaction of the sample to be tested and may still lead to determination error. Effect of most ionic and non-ionic surfactants in the sample.

4. Negative absorption values may be obtained if the sample to be tested contains chelating agents or is under strong acid or base conditions.

5. Absorption values can be significantly increased if lipids are present.

6. If a detection error is caused by the presence of the above interfering factors, dilution, dialysis, or other treatment can be applied to bring the interfering substance concentration below the maximum compatible concentration for BCA detection.

7. If the microplate reader does not have a detection wavelength of 562 nm, wavelengths between 540 and 590 nm are also acceptable.

8. For your safety and health, please wear lab protective clothing, gloves, masks, and other necessary personal protective equipment.

Disclaimer

In all cases, our liability for this product is limited to the value of the product itself.

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