Total Protein (TP) Colorimetric Assay Kit (BCA Method)

**Catalog No:** E-BC-K318-M  
**Method:** Colorimetric method  
**Specification:** 96T (Can detect 80 samples without duplication)  
**Measuring instrument:** Microplate reader  
**Sensitivity:** 0.0165 mg/mL  
**Detection range:** 0.0165-1 mg/mL

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA)  
Fax: 240-252-7376(USA)  
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Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.
Application
This kit can measure protein content of a variety of animal serum, plasma, culture cells, tissue and other samples.

Detection significance
The BCA protein concentration Kit is an ideal protein quantification method which is superior to the Lowry method. This method is fast and sensitive, stable and reliable to different types of protein with small variation coefficient, which is greatly favored by professionals. The BCA method is not affected by the chemicals for most samples.

Detection principle
Cu$^{2+}$ can be reduced to Cu$^{+}$ by protein in alkaline condition. Cu$^{+}$ can combine with BCA reagent and form purple complex, which has a maximum absorption peak at 562 nm. The absorbance value is proportional to the protein concentration. Therefore, the protein concentration can be calculated according to the OD value.

Kit components

<table>
<thead>
<tr>
<th>Components</th>
<th>Specifications</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>BCA Reagent</td>
<td>25 mL × 1 vial</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>Copper Salt Solution</td>
<td>0.5 mL × 1 vial</td>
</tr>
</tbody>
</table>

Preparation of BCA working solution: Mix the Reagent 1 and Reagent 2 fully at a ratio of 50:1. Prepare the needed amount solution before use. The prepared working solution can be stored at 4°C for 24 h.

<table>
<thead>
<tr>
<th>Components</th>
<th>Specifications</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 3</td>
<td>Protein BSA Standard</td>
<td>1 mg × 1 vial</td>
</tr>
<tr>
<td>Reagent 4</td>
<td>Standard Diluent</td>
<td>15 mL × 1 vial</td>
</tr>
</tbody>
</table>

Preparation of 1 mg/mL Standard solution: dissolve a vial of reagent 3 powder with 1 mL reagent 4 and mix fully before use. It is recommended to aliquot the prepared solution and it can be store at -20°C for 3 months.

Experimental instruments
Test tube, Micropipettor, Vortex mixer, Water bath, Microplate reader (562 nm), Centrifuge
Sample pretreatment

1. Serum sample:
   Fresh blood was collected and placed at 25°C for 30 min to clot the blood. Centrifuge the sample at 4°C for 15 min at 2000 g, the upper yellowish clear liquid was taken as serum. Place the serum on ice for detection. If not detected on the same day, stored the serum at-80°C, which can be stored for a month.

2. Plasma sample:
   The fresh blood was added into the test tube containing anticoagulant and mixed upside down. Centrifuge the sample at 4°C for 10 min at 700~1000 g, the upper yellowish transparent liquid was taken as the plasma, and the middle white interference layer (white blood cells and platelets) could not be absorbed. Place the plasma on ice for detection. If not detected on the same day, stored the serum at-80°C, which can be stored for a month.

3. 10% tissue homogenate sample:
   Accurately weigh the tissue sample, add 9 times the volume of PBS (0.01 M, pH7~7.4) according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection.

4. Culture cell sample:
   Wash the cells with PBS (0.01 M, pH7~7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add PBS at a ratio of cell number (10^6): PBS (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection.

Note: The detection ranges is from 16.5 to 1000μg/ml, please take 1-2 preliminary experiments before sample tests, and then diluted the samples with normal saline.

Recommended dilution multiple:
Serum (plasma) sample: 50-70 times;
Animal tissue homogenate: 8-12 times;
Plant tissue homogenate: 3-6 times;
Cell sample: 2-3 times.
### Operation steps

1. **Preparation of standard**: Dilute 1 mg/mL BSA standard solution with normal saline to a serial concentration. The recommended dilution gradient is as follows: 1, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, 0 mg/mL. Then take the assay strictly according to the operation steps.

2. **Standard tube**: add 20 μL of standard solution with different concentrations.
   
   **Sample tube**: add 20 μL of tested Samples.
   
3. Add 200 μL of BCA working solution to the wells of Step 1.

4. Oscillate for 20 s to mix fully and incubate at 37°C for 30 min.

5. Measure the OD value of each well at 562 nm with Microplate Reader.

### Note: The following operating table could be as a reference.

<table>
<thead>
<tr>
<th>Standard solution with different concentrations (μL)</th>
<th>Standard well</th>
<th>Sample well</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples (μL)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>BCA working solution (μL)</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

Oscillate for 20 s to mix fully and incubate at 37°C for 30 min. Measure the OD values of each well at 562 nm with Microplate Reader.

### Calculation of results

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample.

The standard curve is: \( y = ax + b \).

- \( y \): The absolute OD value of standard (\( \text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}} \));
- \( x \): The concentration of Standard;
- \( a \): The slope of standard curve;
- \( b \): The intercept of standard curve.

**Protein content (mg/mL) = \( \Delta A_{562} - b \div a \times f \)**

[Note]

\( \Delta A_{562} \): Absolute OD (\( \text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}} \)).

\( f \): Dilution factor of sample before test.

### Technical parameter

1. The sensitivity of the kit is 0.0165 mg/mL.
2. The intra CV is 2.2% and the inter CV is 4.5%.
3. The recovery of the kit is 100%.
4. The detection range of the kit is 0.0165-1 mg/mL.
Notes
1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.
5. Control the incubation time strictly.
6. The concentration of the sample protein must be diluted to 1 mg/mL or less with normal saline, and it will show a good linear range below this concentration.
7. Prevent the formulation of bubbles when adding the reagents to the microplate.