BCA Protein Quantification Kit

For the rapid, sensitive and accurate measurement of Proteins in various samples.

Description:

BCA Protein Quantification Kit provides a simple, procedure for determining the concentration of proteins in solution. The method utilizes a copper (Cu2+) salt which can be reduced to the cuprous state by protein(s). The generated Cu2+ ion forms an intensely colored complex with the bicinchoninic acid reagent with a very strong absorbance band centered at 562 nm. The intensity of the blue complex is proportional to the amount of protein in the sample. The BCA Protein Assay is suitable for measuring protein concentration in the range of 5-800 ug/ml.

Components and Storage

BCA Reagent	50,**100, 250 mL**
Copper Reagent	1.5, **3, 5 mL**
BSA Standard	**1 mL (10 mg/ml)**

* Store kit at +4°C. The BCA and Copper Reagents are stable at room temperature. The BSA Standard should be aliquoted after the first thaw and stored at -20°C. All reagents are stable for up to 12 months under proper storage conditions.

Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96 well plate
- Orbital shaker

Assay Protocol

Notes:

a) The BCA protocol is very flexible. Both the incubation time and temperature can be varied over a rather wide range. Lower protein samples can be more easily quantified using higher temperatures and longer incubation times.

b) When assaying protein in solutions containing detergent, best results are obtained by adding the same amount of detergent to the wells containing the protein standard.

1. **Reagent Preparation**:

Prepare Working Solution by adding 1 part of Copper Reagent to 50 parts of BCA Reagent. The total volume made will depend upon the number of samples and standards to be quantified. Each sample and standard will require 250 µl or 75 µl of working reagent depending on the protocol. Once made, the working solution is stable for a week at +4°C .

2. **Standard Curve Preparation:**

Label 9 tubes 1-9. Dilute the BSA Standard to 1 mg/ml Stock Solution (i.e., 50 µl + 450 µl buffer). Add 250 µl buffer or distilled water to the rest of the tubes (tube 2-8). Ideally, use the same buffer contained in your samples. Prepare below serial dilution by transferring 250 µl from tube 1 to tube 2. Continue the series of two-fold dilutions until the *last tube.*

Tube 1	1000 µg/ml
Tube 2	500 µg/ml
Tube 3	250 µg/ml
Tube 4	125 µg/ml
Tube 5	62.5 µg/ml
Tube 6	31.25 µg/ml
Tube 7	15.6 µg/ml
Tube 8	7.6 µg/ml
Tube 9	Distilled water

3. If your sample has a high content of total protein, dilute samples to fall within 0.015-1 mg/ml range.

4. Pipette 25 µl Standards or samples into duplicate wells in a clear bottom 96 well plate

5a. Micro-assay 5-250µg (1:3 sample to working reagent ratio): Add 75 µl of working reagent to each standards and sample tube/well.

5b. High range assay 15-1000 µg (1:9 sample to working reagent ratio): Add 225 µl of working reagent to each standards and sample tube/well.

6. Shake gently to mix. Incubate for 60 min at 60°C. Cool to room temperature.

7. Measure OD at 562 nm (or 545 nm, if your spectrophotometer does not support 562 nm). The signal is stable for at least 1 hour. For unknown samples, several dilutions of a sample should be tested to ensure the OD reading is within the standard curve range.
Figure 1 and 2 show representative curves for **the BCA Micro-assay and High range BCA assay,** respectively.

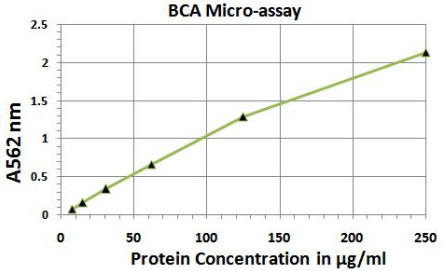

Figure 1. Color response curves obtained with the BCA Micro-assay using bovine serum albumin.

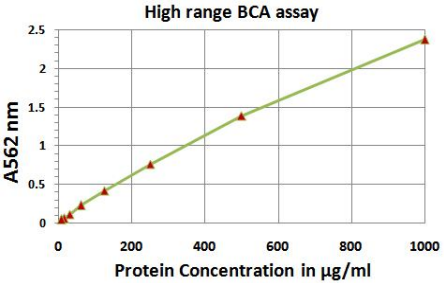

Figure 2. Color response curves obtained with the BCA High range assay using bovine serum albumin.

Data Analysis

Subtract the blank OD (zero standard) from all standard and sample OD values. Plot the corrected OD against standard protein concentrations. Use the standard curve to determine the sample protein concentration. Figure 1 and 2 show representative curves for **the BCA Micro-assay and High range BCA assay,** respectively.

Alternatively, the equation for the best line fitting the standards can be used to determine the protein concentration of your samples.
Standard curves carried out according to assay protocol.
For more information visit our website.