BCA Protein Quantification Kit (Enhanced)

Cat. No. Kit-0867 **Lot. No.** (See product label)

SPECIFICATION

Description	BCA Protein Assay Kit is a ready-to-use detergent-compatible Western blot related total protein analysis reagent used for the quick determination of total protein concentration by measuring absorbance at 562 nm and comparing to a protein standard absorption vs. concentration curve, according to Smith.
Applications	Western blotting, protein expression assays, protein profiling and characterization, protein quantitation assays
Usage	Studying protein-protein interactions, Measuring column fractions after affinity chromatography, Assessing protein yields in whole cell lysates, High-throughput screening of fusion proteins
Storage	Store at room temperature for one year.
Size	Tube procedure: 50 assays Microplate procedure: 500 assays
Kit Components	BCA Reagent A: 100 ml BCA Reagent B: 5 ml Albumin (BSA) Standards: 20 ml (2 mg/ml)
Materials Required but Not Supplied	Test tubes or miscroplates; Spectrophotometer; Microplate Reader
Detection method	Colorimetric/Spectroscopic

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

Compatible Sample Types	Serum, Plasma, Cell culture extracts, Tissue Extracts
Assay Protocol	A. Test Tube Procedure 1. Mix BCA Reagent A and BCA Reagent B in the ratio of 50:1. i.e., mix 50 ml of BCA Reagent A with 1 ml BCA Reagent B. 2. Follow Table 1 to prepare a fresh set of standards. (Dilute Albumin (BSA) Standards with 0.9% NaCl or PBS) Table 1: Preparation of Albumin (BSA) Standards 3. Add 0.1 ml of each standard and protein samples into separate labeled test tubes. Tube Number Volume of Diluent (µl) Volume of BSA (µl) Final BSA Concentration (µg/ml) A 0 µl 900 µl of 2 mg/ml Stock 2000 µg/ml B 100 µl 300 µl of tube A 1500 µg/ml C 300 µl 300 µl of tube A 1000 µg/ml D 200 µl 200 µl of tube B 750 µg/ml E 300 µl 300 µl of tube C 500 µg/ml F 300 µl 300 µl of tube E 250 µg/ml G 300 µl 300 µl of tube F 125 µg/ml H 400 µl 100 µl of tube G 25 µg/ml I 300 µl 0 0 (blank) 4. Add 2 ml of BCA working reagent to each tube and mix well. 5. Incubate at 37°C for 30 minutes. Note: Increasing the incubation time and temperature can increase the net 562 nm absorbance for each test and decreases both minimum detection level and test range of the kit. 6. Cool all tubes to room temperature (RT). 7. Set the wavelength of spectrophotometer at OD 562 nm. Calibrate the instrument to zero by using water. Subsequently, measure the absorbance of all samples within 10 minutes.

Note: Color development continues even after cooling to RT. However, the subsequent development at RT is too weak to produce significant error if all absorbance measurements are made within 10 minute.

8. Subtract OD562 of Blank from all readings.

9. Plot the BSA standard curve: OD562 (on Y axis) vs BSA Standard concentration (on X axis). Use the standard curve to determine the protein concentration of each unknown sample.

B. Microplate Procedure

1. Mix BCA Reagent A and BCA Reagent B in the ratio of 50:1. i.e., mix 50 ml of BCA Reagent A with 1 ml BCA Reagent B.

2. Follow Table 2 to prepare a fresh set of standards. (Dilute Albumin (BSA) Standards with 0.9% NaCl or PBS)

Table 2: Preparation of Albumin (BSA) Standards

Tube Number Volume of Diluent (µl) Volume of BSA (µl) Final BSA Concentration (µg/ml)

A 0 µl 200 µl of 2 mg/ml Stock 2000 µg/ml

B 30 µl 90 µl of tube A 1500 µg/ml

C 60 µl 60 µl of tube A 1000 µg/ml

D 60 µl 60 µl of tube B 750 µg/ml

E 60 µl 60 µl of tube C 500 µg/ml

F 60 µl 60 µl of tube E 250 µg/ml

G 60 µl 60 µl of tube F 125 µg/ml

H 100 µl 25 µl of tube G 25 µg/ml

I 60 µl 0 0 (blank)

3. Add 25 µl of each standard and protein samples into separate microplate wells.

4. Add 200 µl of BCA working reagent to each well and mix well.

5. Seal plates and incubate at 37°C for 30 minutes.

6. Cool plate to room temperature (RT).

7. Measure the absorbance at 562 nm on a plate reader within 10 minutes.

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

	8. Subtract OD562 of Blank from all readings. 9. Plot the BSA standard curve: OD562 (on Y axis) vs BSA Standard concentration (on X axis). Use the standard curve to determine the protein concentration of each unknown sample.
Sensitivity	20 - 2000 μg/ml

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA