CREATIVE BIOMART®
Assay Kit

BCA Protein Quantification Kit (Enhanced)

Product Information

Cat

Kit-0867

Common Name

BCA

Cat.No. Kit-0867

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

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Albumin (BSA) Standards: 20 ml (2 mg/ml)

Materials Required but Not Supplied

Test tubes or miscroplates; Spectrophotometer; Microplate Reader

Detection method Colorimetric/Spectroscopic

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).

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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.
8. Subtract OD562 of Blank from all readings.

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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

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