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Product Information

BCA Protein Assay Kit

Catalog Number: P011

Unit Size: 2500 assays

Kit Contents

Component A: BCA Reagent A, 500 mL;

Component B: BCA Reagent B, 25 mL;

Component C: BSA standard, 2 mg/mL, 10 mL.

Storage upon receipt:

- Store BCA Reagent A and B at RT
- Store BSA Standard at 4°C

Product Description

BCA Protein Assay Kit is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colormetric detection and quantitation of total protein. This method combines the well-known reduction of Cu^{2+} to Cu^{1+} by protein in an alkaline medium with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{1+}) using a unique reagent containing bicinchoninic acid. The purple-colored reaction product of this assay is formed by the chelation of two molecules of BCA with one cuprous ion. This water-soluable complex exhibits a strong absorbance at 562 nm that is nearly linear with increasing protein concentrations over a broad working range (20-2000 µg/mL).

Protein concentrations are determined with reference to standards of a common protein such as BSA. A series of dilutions of known concentration are prepared from the protein and assayed alongside the unknowns before the concentration of each unknown is determined based on the standard curve. If precise quantitation of an unknown protein is required, it is advisable to select a protein standard that is similar in quality to the unknown.

Preparation of BSA Standard

Use Table 1 (see table in next page) as a guide to prepare a set of protein standards.

Preparation of the BCA Working Reagent

1. Use the following formula to determine the total volume of working reagent required:

   (#standards + #unknowns)×(#replicates)×(volume of working reagent per sample) = total volume

2. Prepare working reagent by mixing 50 parts of BCA reagent A with 1 part of BCA Reagent B (50:1, Reagent A:B).

Test Tube Protocol

1. Pipette 0.1 mL of each standard or unknown sample into appropriately labeled test tubes.

2. Add 2 mL of the BCA working reagent to each tube and mix well.

3. Cover and incubate tubes at selected temperature and time:

- Standard Protocol: 37 °C for 30 min (20-2000 µg/mL);
- RT Protocol: RT for 2 hr (20-2000 µg/mL);
- Enhanced Protocol: 60 °C for 30 min (5-250 µg/mL).

4. Cool all tubes to RT.

5. With the spectrophotometer set to 562 nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples within 10 min.

6. Substrate the average 562 nm absorbance measurement for the Blank replicates from the 562 nm absorbance measurements of all other individual standard and unknown sample replicates.

7. Prepare a standard curve by plotting the average blank-corrected 562 nm measurements for each BSA standard vs, its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

Microplate Protocol

1. Pipette 20 µL of each standard or unknown sample into appropriate microplate wells.

2. Add 200 µL of the BCA working reagent to each well and mix with plate shaker for 30 seconds.

3. Cover plate and incubate at 37 °C for 30 min.

4. Cool plate to RT. Measure the absorbance at or near 562 nm with a plate reader.

5. Substrate the average 562 nm absorbance measurement for the Blank replicates from the 562 nm measurements of all other individual standard and unknown sample replicates.

6. Prepare a standard curve by plotting the average blank-corrected 562 nm measurements for each BSA standard vs, its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

   Note: If using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) or best-fit curve will provide more accurate results than a purely linear fit. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points.

Technical Notes

1. Reducing agents, such as 2-Mercaptoethanol, Dithiothreitol (DTT), and Dithioerythritol (DTE), in concentrations above 1mM; and also chelating agents like EDTA in concentrations greater than 10mM, will interfere with the assay. If your Unknowns contain these agents, check the effect on a Standard Curve assayed in the same buffer as the Unknown samples.

2. If possible, dilute your Unknowns so the agent(s) in question no longer interfere. If you can not dilute your samples enough to lower the interference caused by chelating reagents in your Unknowns, you may increase the

proportion of copper sulfate solution (**BCA Reagent B**) in the prepared **BCA Working Solution** up to 3 times.

3. If you still cannot reduce interference to acceptable levels, try the **Bradford Protein Assay Kit** (Cat. No. P010).

Table 1. Preparation of Diluted BSA Standards

Dilution Scheme for Standard Test Tube and Microplate Protocol (Working range: 200-2000 µg/mL)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	0	300 µL of Stock	2000 µg/mL
B	125 µL	375 µL of Stock	1500 µg/mL
C	325 µL	325 µL of Stock	1000 µg/mL
D	175 µL	175 µL of vial B dilution	750 µg/mL
E	325 µL	325 µL of vial C dilution	500 µg/mL
F	325 µL	325 µL of vial E dilution	250 µg/mL
G	325 µL	325 µL of vial F dilution	125 µg/mL
H	400 µL	100 µL of vial G dilution	25 µg/mL
I	400 µL	0	0 µg/mL (Blank)

Dilution Scheme for Enhanced Test Tube Protocol (Working range: 5-250 µg/mL)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	700 µL	100 µL of Stock	250 µg/mL
B	400 µL	400 µL of vial A dilution	125 µg/mL
C	450 µL	300 µL of vial B dilution	50 µg/mL
D	400 µL	400 µL of vial C dilution	25 µg/mL
E	400 µL	100 µL of vial D dilution	5 µg/mL
F	400 µL	0	0 µg/mL (Blank)

Related Products

Catalog No.	Product
P010	**Bradford Protein Assay Kit**

References:

1. Wiechelman, K. et al, *Anal. Biochem.*, 175, 231-237 (1988).
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3. Brown, R.E. et al, *Anal. Biochem.*, 180, 136 –139,(1989).
4. Lowry, O.H. et al, *J. Biol. Chem.*, 193, 265-275 (1951).
5. Smith, P.K. et al, *Anal. Biochem.*, 150, 76-85, (1985).
6. Bradford, MM. *Analytical Biochemistry*, 72,248-254. (1976).