Enhanced BCA Protein Assay Kit – Instruction Manual

Catalogue Information

Catalogue No.	Product Name	Size / Tests
BL1054A	Enhanced BCA Protein Assay Kit	500 Tests

Product Description

The Enhanced BCA Protein Assay Kit is based on the principle that peptide bonds in proteins, under alkaline conditions, form complexes with Cu^{2+}, reducing them to Cu^{+}. The BCA reagent binds specifically to Cu^{+} to form a stable purple-colored complex with maximum absorbance at 562 nm. The absorbance intensity is proportional to protein concentration. This assay provides a simple, sensitive, rapid, and stable method for protein quantification. The included BSA standard makes it convenient for users to generate a standard curve. The assay can be performed using either cuvettes or microplate formats.

Product Features

1. High sensitivity: detection limit is 10 μg/mL; linear range 20–1000 μg/mL; minimum detectable protein is 0.2 μg.
2. Fast: color development time is short; approximately 4× faster than the Lowry method.
3. Detergent compatible: tolerates up to 5% SDS, 5% Triton X-100, 5% Tween-20/60/80.
4. Limitations: EDTA < 10 mM, DTT < 1 mM, β-mercaptoethanol < 0.01%, EGTA interferes significantly.

Kit Contents

1. Reagent A, 100 mL
2. Reagent B, 3mL
3. BSA Standard Solution (5 mg/mL), 2 x 1 mL
4. PBS Solution, 10 mL

Instructions for Use

Prepare working BCA solution by mixing Reagent A and Reagent B at a ratio of 50:1 (e.g., 50 mL A + 1 mL B). If precipitate forms, mix until dissolved.

Microplate Protocol (20–2000 μg/mL)

1. Prepare BSA standards by serial dilution in PBS.
2. Pipette standards and samples into a 96-well plate.
3. Add working BCA reagent to each well.
4. Incubate at 37 °C for 30 min (alternatively: room temp for 2 h, or 60 °C for 30 min).
5. Measure absorbance at 562 nm.
6. Quantify protein using the standard curve. Dilute and repeat if sample exceeds range.

Tube No.	0	1	2	3	4	5	6	7	8
1 mg/mL BSA Std Solution (μL)	0	0.5	2.5	5.0	10	15	20	–	–
5 mg/mL BSA Std Solution (μL)	–	–	–	–	–	–	–	6	8
PBS Solution (μL)	20	19.5	17.5	15	10	5	0	14	12
Final BSA Conc. (μg/mL)	0	25	125	250	500	750	1000	1500	2000
Total Volume	20 μL								

Test-Tube Protocol (20–1000 μg/mL)

1. Prepare BSA standards by serial dilution in PBS.
2. Add 0.1 mL of standard or sample to a test tube.
3. Add 2 mL of working BCA reagent, mix thoroughly.
4. Incubate at 37 °C for 30 min (alternatively: room temp for 2 h, or 60 °C for 30 min).
5. Measure absorbance at 562 nm against blank.

Tube No.	0	1	2	3	4	5	6	7	8
1 mg/mL BSA Std Solution (μL)	0	2.5	12.5	25.0	50	75	100	–	–
5 mg/mL BSA Std Solution (μL)	–	–	–	–	–	–	–	30	40
PBS Solution (μL)	100	97.5	87.5	75	50	25	0	70	60
Final BSA Conc. (μg/mL)	0	25	125	250	500	750	1000	1500	2000
Total Volume	100 μL								

Storage and Stability

1. Reagent A & B: store at room temperature.
2. BSA standard solution: store at –20 °C.
3. PBS solution: store at 4 °C.
4. Shelf life: 12 months when stored as directed.

Notes

1. The color development in the BCA protein assay will continue to deepen with time. The speed of the color reaction is related to temperature and incubation time. Adjustment should be made in terms of incubation time and temperature to ensure accurate quantification.
2. If precipitate forms in reagents, warm at 37 °C and mix to dissolve.
3. If microbial contamination is found, discard the reagent.
4. Wear a lab coat and disposable gloves when handling reagents.
5. Always run a fresh standard curve for each assay.
6. Do not use contaminated reagents.

Notice

For in vitro research use only, not for diagnostic or therapeutic use. This product is not a medical device.
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