aladdin®

Ready-to-use BCA Protein Assay Kit

R1491648

Introduction:

The BCA method is one of the most widely used techniques for protein quantification. This kit, based on the bicinchoninic acid (BCA) assay, enables rapid, stable, and sensitive measurement of protein concentrations. Its principle relies on the reduction of Cu^{2+} to Cu^{+} by peptide bonds in an alkaline environment, followed by the formation of a purple-colored complex between Cu^{+} and BCA. This complex exhibits strong absorbance at 562 nm, proportional to protein concentration. The kit includes a series of ready-to-use protein standard solutions (BSA), eliminating the need for dilution and simplifying the workflow.

Product Component List and Storage Conditions:

R1491648	Component	500T	500T×5	Storage
R1491648A	Reagent A	100 mL	100 mL×5	2-8℃
R1491648B	Reagent B	3 mL	3 mL×5	2-8℃
R1491648C	Ready-to-use BSA standard①(0μg/mL)	1 mL	1 mL×5	-20℃
R1491648D	Ready-to-use BSA standard②(125μg/mL)	1 mL	1 mL×5	-20℃
R1491648E	Ready-to-use BSA standard③(250μg/mL)	1 mL	1 mL×5	-20℃
R1491648F	Ready-to-use BSA standard④(500μg/mL)	1 mL	1 mL×5	-20℃
R1491648G	Ready-to-use BSA standard⑤(750μg/mL)	1 mL	1 mL×5	-20℃
R1491648H	Ready-to-use BSA standard⑥(1000μg/mL)	1 mL	1 mL×5	-20℃
R1491648I	Ready-to-use BSA standard⑦(1500μg/mL)	1 mL	1 mL×5	-20℃
R1491648J	Ready-to-use BSA standard⑧(2000μg/mL)	1 mL	1 mL×5	-20℃

Key Features:

1. Convenient: Ready-to-use standards avoid tedious dilution steps.
2. High Accuracy: Lower coefficient of variation than Coomassie Brilliant Blue assays.
3. Broad Linear Range: Detection range: 20–2000μg/mL.
4. Excellent Compatibility: Tolerates metal ions, reducing agents, chelators, and detergents.

Protocol (Microplate Reader Example):

1. Prepare Working Reagent:

1.1 Calculate the total volume required:

Working reagent volume = (Number of standard samples + Number of test samples) × Replicates × Volume per well.

Example: 8 standards + 3 test samples with 3 replicates each at 200μL/well:

(8 + 3) × 3 × 200μL = 6.6 mL

1.2 Mix Reagent A and Reagent B at a 50:1 ratio to prepare the working reagent. Vortex thoroughly.

Note:

- Prepare extra volume (1–2 wells) to compensate for pipetting errors.
- Freshly prepared working reagent is stable for 24 hours at room temperature (sealed).

2. Quantification Steps:

2.1 Add 20μL of each ready-to-use BSA standard (①–⑧) to a 96-well plate (mix thoroughly before use):

Well	1	2	3	4	5	6	7	8
Standard	①	②	③	④	⑤	⑥	⑦	⑧
Volume (μL)	20	20	20	20	20	20	20	20
BSA (μg/mL)	0	125	250	500	750	1000	1500	2000

2.2 Dilute test samples in 1× PBS or 0.9% saline (e.g., 2×, 4×, 8× serial dilutions). Add 20 μL to wells.

2.3 Add 200μL working reagent to each well. Mix thoroughly, cover the plate, and incubate at 37°C for 30 min. Cool to room temperature.

Note: Incubation alternatives:

- Room temperature: 2 hours.
- 60°C: 30 minutes.
- Absorbance increases with time/temperature. Extend incubation for low-concentration samples.

2.4 Measure absorbance at 562 nm (or 540–590 nm) using a microplate reader. Subtract the blank control (Standard ① + working reagent) absorbance.

2.5 Plot a standard curve and calculate sample protein concentrations.

Note:

- Exclude obvious outliers.
- Multiply calculated concentrations by dilution factors.
- Use linear regression for computer-based fitting.

Matters needing attention:

1. Compatible with microplate readers (microplate method) or spectrophotometers (cuvette method). For cuvettes, ensure the volume meets the minimum requirement (may reduce total number of assays).
2. If precipitates form during cold/long-term storage, stir or warm to 37°C to dissolve.
3. Always generate a fresh standard curve for each assay to ensure accuracy.
4. For samples with high levels of interfering substances (see Appendix), consider alternative protein quantification methods.
5. Wear a lab coat and disposable gloves during operations.
6. For research use only.