Ver.250101

BCA Protein Assay Kit

PC0020

Microplate reader: 500 Tests

Spectrophotometer: 50 Tests

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

BCA protein assay is used for quantitation of total protein in a sample. The principle of this method is that proteins can reduce Cu^{2+} to Cu^{+} in an alkaline solution. The result is the formation of a purple colour by bicinchoninic acid having an absorption at 562nm.

Kit components

Reagent	Volume	Storage
BCA Reagent	100mL × 1	RT
Cu Reagent	3mL × 1	RT
PBS Dilution	30mL × 1	RT
BSA Standard (5mg/mL)	1mL × 1	-20°C

BSA standard is stable for 3 months at 4ºC or for 1 year at -20ºC. Other reagents can be kept at room temperature for 1 year.

Reagents and Equipment Required but Not Provided

Centrifuge, micropipette, cooling centrifuge, spectrophotometer / microplate reader, micro glass cuvette / 96-well flat bottom plates and distilled water.

Protocol

Prepare Working Solution

1. BCA Working Solution.
   Mix 50 parts of BCA Reagent with 1 part of Cu Reagent. The BCA working reagent could be kept at RT for 24 hours.

2. BSA standard working solution.
   Dilute 10µL BSA standard (5mg/mL) to 100µL with PBS to obtain working standard of concentration 0.5mg/mL.

Microplate Reader

- Add different volume of BSA standard working solution (0.5mg/m1) to 96 well plates, make up to 20µL with PBS as mentioned in the table below.

BSA Working Standard (0.5mg/mL) (µL)	0	2	4	6	8	10	12	14	16	18	20
PBS (µL)	20	18	16	14	12	10	8	6	4	2	0
Final Amount of BSA (µg)	0	1	2	3	4	5	6	7	8	9	10

- Add 20µL sample to each well.
- Add 200µL BCA working solution to each wells, incubate at 37°C for 30 minutes. Cover the plate properly to avoid evaporation during incubation.
- Measure absorbance at 562nm.
- Draw standard curve with X axis as amount of protein and Y axis as the absorbance.
- Determine sample concentration from standard graph.

Spectrophotometer

- Prepare standards and samples as mentioned in the table below.

	Standard						Sample		
	1	2	3	4	5	6	S1	S2	S3
BSA Working Standard (0.5mg/mL) (µL)	0	40	80	120	160	200	-	-	-
Sample (µL)	-	-	-	-	-	-	200	200	200
PBS (µL)	20	18	16	14	12	10	8	6	4
BCA Working Solution (mL)	2	2	2	2	2	2	2	2	2
Final Amount of BSA (µg)	0	20	40	60	80	100	-	-	-

- Incubate at 37°C for 30 minutes. Cover the tubes properly to avoid evaporation during incubation.
- Measure absorbance at 562nm.
- Draw standard curve with X axis as amount of protein and Y axis as the absorbance.
- Determine sample concentration from standard graph.

Note:

- If the absorption of the sample is high, dilute appropriately with PBS and repeat the assay.
- BCA Protein Assay is affected by the presence of chelating agents (EDTA, EGTA), reducing agents (DTT, mercaptoethanol) and lipids.