# Bioepitope$^{R}$ Bicinchoninic Acid Protein Assay Kit

## Introduction and product overview:

Protein quantification is often required before precessing protein samples for analysis. The Bicinchoninic Acid (BCA) Protein Assay is highly sensitive colorimetric assay that is not affected by chemicals in the sample. The BCA Protein Assay primarily reduces $Cu^{2+}$ to $Cu^{1+}$ by proteins in an alkaline environment followed by highly sensitive and selective colorimetric detection of BCA/copper complex. It is water-soluble and strongly absorbs light at 562nm in a linear fashion with increasing protein concentration.

## Kit contents:

Biogot BCA Reagent A: 100ml, stored at room temperature
Biogot BCA Reagent B: 2ml stored at room temperature
Bovine Serum Albumin Standard: **100mg(Power)**, Stored at 4℃.( Recommend to make stock solution(2mg/ml) and aliquot before store at 4℃ )

## Test Tube Procedures:

**A) Standard Preparation:**

Label 9 test tubes with A-I and prepare the standards as indicated below. The diluent used should be the same as used for the protein samples. The following dilutions are suitable for duplicate Standard assays.

| Tube | Bovine Serum Albumin | Diluent(µl) | Final Concentration (µg/ml) |
|---|---|---|---|
| A | 200µl from Stock | 0 | 2,000 |
| B | 120µl from Stock | 40 | 1,500 |
| C | 100µl from Tube A | 100 | 1,000 |
| D | 100µl from Tube B | 100 | 750 |
| E | 100µl from Tube C | 100 | 500 |
| F | 100µl from Tube E | 100 | 250 |
| G | 100µl from Tube F | 100 | 125 |
| H | 20 µl from Tube G | 80 | 25 |
| I | 0 | 100 | 0(Blank) |

**B) Working Solution Preparation:**

1) Use following formula to determine the amount of working solution required. (Total number of standards and samples)*(Number of replicates)*(Volume of working solution sample)= Total volume working solution required.
2) Mix fifty parts of BCA Reagent A with one part of BCA reagent B (50:1, Reagent A:B).

**C) Micro-plate Procedure (Sample to WR ratio = 1:20)**

1. Pipette 10 µl of each standard or unknown sample replicate into a micro-plate well (working range = 20-2,000 µg/ml).
2. Add 200 µl of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds.
3. Cover plate and incubate in the water bath at 37°C for 30 minutes.
4. Cool plate to RT.
5. Measure the absorbency at or near 562 nm on a plate reader.
6. Use the standard curve to calculate the protein concentration of each unknown sample.

## Notes

1) Certain substances including reducing potential, chelating agents, and strong acids or bases are known to interfere with protein estimation and avoid those substances in the sample buffer. (For example, EDTA, EGTA, DTT)
2) Prepare a clear and fresh WR reagent at room temperature when prepping new experiments. After adding WR reagent, it could be incubated for sixty minutes at 37°C or 2 hours at room temperature. Absorbance at 562nm increases with the increasing incubation time. Color development runs faster with the increasing temperature. If sample concentration is too low, it will be better to run the reaction at a higher temperature or increase the incubation time.
3) Good linear range for samples is from 50-2000µg/ml.
4) BCA assay is interfered with by chelating agents and high concentration reducing agents. Make sure EDTA<10mM, no EGTA, DTT<1mM and β-ME <1mMin the sample buffer. Try to remove the interfering substance by dialysis or gel filtration to eliminate or minimize the effects of interfering substances. If interference can not be overcome, it is recommend you use the Bradford protein assay kit.
5) period of validity is 6 months.

Made in CHINA

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