Bicinchoninic Acid (BCA) Protein Assay Kit

Protein Chemistry

Catalogue Number: MPK002

Kit Components:

Reagent A	100 mL
Reagent B	1 mL (x2)
Protein Standard (BSA):	1 mL (10 mg/mL)

Description: BCA serves the purpose of the Folin reagent in the Lowry assay, namely to react with complexes between copper ions and peptide bonds to produce a purple end product. The advantage of BCA is that the reagent is fairly stable under alkaline conditions, and can be included in the copper solution to allow a one step procedure. A molybdenum/tungsten blue product is produced as with the Lowry. In addition to standard liquid handling supplies a visible light spectrophotometer is needed with transmission set to 562 nm. Glass or polystyrene (cheap) cuvettes may be used. The BCA is used for the same reasons the Lowry is used. Stoscheck (1990) has suggested that the BCA assay will replace the Lowry because it requires a single step, and the color reagent is stable under alkaline conditions.

Pack Size: 1 kit, 100 standard reactions

Storage: 4-8 ºC

Experimental Procedures:

- Preparation of Standard working solution (SWR): Mix 100 volumes reagent A with 2 volumes reagent B.
  Note：The stock solutions are stable. The working solution is stable for 1 week and should be green.
- Prepare protein standards containing 0.2, 0.5, 1, 2, 5, 10mg/mL BSA. Prepare a standard curve of absorbance versus micrograms protein (vice versa).
- Prepare samples containing 0.2 to 10 mg/mL.
- Add 1 mL SWR to each 20 microliters sample and mix. Incubate 30 min. at 37ºC.
- Read at 562 nm. Color will be stable for at least one hour.
- Determine concentrations of original samples from the standard curve.

Reference: Stoscheck, CM. Quantitation of Protein. Methods in Enzymology 182: 50-69 (1990).

Notes: A longer incubation increases the sensitivity of the assay. The heating can be stopped earlier to prevent the color from becoming too dark. The assay can be performed at room temperature, but there is greater variability among proteins and the assay is less sensitive.