BIOMEDICAL RESEARCH SERVICE CENTER
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Protein Assay kit/DC (Cat #: A-117)

COMPONENTS:	BCA Solution:	200 ml, store at room temperature (for 1000 assays)
	Copper Solution:	5 ml, store at room temperature
	100 mg/ml Bovine Serum Albumin (BSA):	1 ml, store at -20℃

PRODUCT DESCRIPTION: The detergent compatible Protein Assay Kit combines two famous reactions- the Biuret reaction and the Smith reaction. The Biuret reaction depicts the reduction of cupric ion (Cu^{2+}) to cuprous ion (Cu^{1+}) by protein in an alkaline solution. The intensity of the reaction is proportional to the number of peptide bonds (at least 2 peptide bonds) and therefore to the number of protein molecules present in the sample solution. The Smith reaction depicts the chelation of each Cu^{1+} ion by two molecules of bicinchoninic acid (BCA), resulting in an intense purple-colored product that absorbs light at 562 nm (Anal. Biochem. 150: 76–85, 1985), which can be measured with a spectrophotometer or microplate reader. Unlike the dye-based protein assay system, the BCA-based protein assay system is compatible with detergent-containing protein samples. However, **the BCA assay is incompatible with chelating agents (such as DTT and EDTA) and divalent ions.** Prepare fresh working solution by mixing 50 parts of BCA Solution with 1 part of Copper Solution (50:1). The Protein Assay system has a broad working range of 20 - 2000 μg/ml proteins. The assay kit also includes 1 ml of 100 mg/ml sterilized BSA dissolved in distilled water to be diluted for use as protein standard. The assay kit is stable for at least one year under proper storage and handling conditions.

PROTOCOL:

Preparation of working solution: Estimate the volume of working solution required for each assay (0.2 ml working solution required per well). Mix 50 parts of BCA Solution with 1 part of Copper Solution. The working solution should be prepared fresh for each assay.

Preparation of BSA standards: Dilute the 100 mg/ml BSA to 2 mg/ml (50 fold dilution) using dH_2O or a compatible protein buffer. Perform additional dilutions to obtain 1, 0.5, and 0.25 mg/ml. Store the BSA protein standards at -20℃.

1. To each well of a 96-well plate, add 25 μl dH_2O or a protein diluent (serving as blank), BSA standards, and protein samples. Samples should be assayed at least in duplicate. It may be possible to dilute sample 5 fold prior to protein assay if dealing with precious or limited protein samples.

2. To each well, add 0.2 ml freshly prepared working solution. Agitate the plate gently but thoroughly. Cover the plate and incubate at 37 ℃ for 30 min.

3. Measure the absorbance at 540-590 nm using a plate reader. Generate a standard curve by plotting the measurement for each BSA standard vs. its concentration. Use the standard curve to determine the protein concentration of each sample.

Note:

- The BCA solution contains 2 mM NaOH (sodium hydroxide). Avoid contact with skin.
- The working solution contains NaOH, BCA, potassium sodium tartrate, and cupric sulfate. Please contact us or visit the product webpage for MSDS information.
- The assay kit is incompatible with protein samples containing chelating agents and divalent ions. Use our Coomassie Blue-based Protein Assay kit (Cat# A-116) for these samples.