GENCHEM® BCA Protein Quantification Assay Kit

REF : KBCA1166

Ver 1.0

RUO

Biochemical Assays for the Quantitative Determination of BCA Protein Quantification in serum, plasma, Animal Tissues, Cells and Bacteria

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF KBCA1166 Σ 500 tests

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Introduction:

The BCA method for protein concentration determination is not affected by most chemicals present in samples. In tissue and cell lysis experiments, it is highly compatible with detergents such as SDS, Triton X-100, Tween 20, and Tween 80 at concentrations up to 5%. However, it is susceptible to interference from chelating agents (EDTA, EGTA), reducing agents (DTT, β-mercaptoethanol), and lipids.
The kit contains a series of protein standard solutions (BSA solutions) at different concentrations. The standards are ready to use and require no dilution, providing a convenient and rapid assay.
Detection range: A good linear relationship is obtained within the concentration range of 0.02–2 mg/ml.

Intended Use:

GENCHEM® BCA Protein Quantification Assay Kit is intended for Quantitative determination of BCA Protein Quantification in Serum, Plasma, Animal Tissues, Cells and Bacteria.

Principle:

The peptide bonds in protein molecules reduce divalent copper ions (Cu^{2+}) to monovalent copper ions (Cu^{+}) under alkaline conditions. Cu^{+} then reacts with bicinchoninic acid (BCA) to form a purple-colored complex. This complex can be detected at 540–590 nm, with maximum absorbance at 562 nm. Within a certain range, the color intensity is linearly related to protein concentration; therefore, protein content can be determined from the absorbance value. The method is rapid, sensitive, stable, and reliable, with very small coefficients of variation among different types of proteins.

Materials Provided:

1. BCA Reagent A - 100 ml
2. BCA Reagent B – 3 ml
3. BSA Standards (0, 125, 250, 500, 750, 1000, 1500, 2000 ug/ml) – 1 ml each

Materials & Equipment Required But Not Provided:

1. Microplate Reader or Ultraviolet Spectrophotometer capable of measuring absorbance at 340 nm.
2. Incubator, Ice Maker, Freezing Centrifuge.
3. 96-well UV Plate or Microquartz cuvette, precision Pipettes, disposable Pipette Tips.
4. Deionized Water.
5. Homogenizer (for Tissue Samples).

Handling/Storage:

1. All reagents should be stored as indicated on the component label and keep away from the light
2. All the reagents should be used within 12 months from manufacturing date.
3. After receiving the kit, an unopened kit can be stored at -20ºC for 12 months, protected from light. Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Reagent Preparation:

Preparation of BCA Working Solution:
Mix Reagent A and Reagent B at a volume ratio of 50:1 and mix thoroughly to prepare the BCA working solution. Total volume of working solution = (number of BSA standard samples + number of test samples) x 200 ul. Freshly prepared BCA working solution can be stored sealed at room temperature for 24 hours.

Assay Procedure:

I. Microplate Assay

1. Add 20 ul of each of the eight BSA standards to a 96-well plate. Thoroughly dissolve and mix the BSA standards before use.

2. Dilute the protein samples appropriately with 1× PBS or 0.9% normal saline (e.g., 5-fold, 10-fold, or 20-fold dilution), and add 20 ul to the sample wells.

3. Add 200 ul of BCA working solution to each well of the 96-well plate. Mix thoroughly, incubate at 37°C for 30 minutes, and cool to room temperature.

   Note: Alternatively, incubate at room temperature for 2 hours or at 60°C for 30 minutes. In the BCA assay, the color continues to intensify over time, and the color development reaction accelerates as temperature increases. For low protein concentrations, incubation at a higher temperature or extending the incubation time appropriately may be used.

4. Measure the absorbance at 562 nm using a microplate reader and record the readings. Use the absorbance of the BSA standard at 0 μg/mL as the blank control.

5. Plot A562 on the y-axis and BSA concentration on the x-axis to generate the standard curve and calculate the protein concentration in the samples. If the calculated protein concentration is outside the standard curve range, dilute the sample and repeat the assay.

   Note: Data processing: Remove obviously erroneous values and subtract the absorbance of the blank control. The concentration of the test sample can be obtained from the standard curve; the actual concentration should be multiplied by the sample dilution factor.

II. Tube Assay:

1. Add 100 ul of each of the eight BSA standards to separate tubes. Thoroughly dissolve and mix the BSA standards before use.

2. Dilute the protein samples appropriately with 1× PBS or 0.9% normal saline (e.g., 5-fold, 10-fold, or 20-fold dilution), and add 100 ul to the tubes.

3. Add 2 mL of BCA working solution to each tube. Mix thoroughly, incubate at 37°C for 30 minutes, and cool to room temperature.

4. Steps 4 and 5 are the same as described above for the microplate assay.

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Since the kit contains potentially hazardous materials, the following precautions should be observed.
- Do not smoke, eat or drink while handling kit material.
- Always use protective gloves.
- Never pipette material by mouth.
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

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