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Product information

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Micro BCA Protein Assay Kit

Catalog #: SK3061
Size: 1250 Prep
Storage: 8°C to -20°C*

*: Product will be shipped at ambient temperature. Upon receipt, store Reagents A and B and C at 2-8°C. Store BSA Standard at -20°C. Components will have a one year shelf life under these conditions.

Product Description:

The Micro BCA Protein Assay Kit is a detergent-compatible bicinchoninic acid formulation for the colorimetric detection and quantitation of total protein. An adaptation of the BCA Protein Assay Kit , the Micro BCA Kit has been optimized for use with dilute protein samples (0.5-20µg/ml). The unique, patented method utilizes bicinchoninic acid (BCA) as the detection reagent for Cu^{+1}, which is formed when Cu^{+2} is reduced by protein in an alkaline environment.

A purple-colored reaction product is formed by the chelation of two moleculesvof BCA with one cuprous ion (Cu^{+1}). This water-soluble complex exhibits a strong absorbance at 562nm that is linear with increasing protein concentrations. The macromolecular structure of protein, the number of peptide bonds and the presence of four amino acids (cysteine, cystine, tryptophan and tyrosine) are reported to be responsible for color formation with BCA , the kit uses concentrated reagents and a protocol that utilizes an extended incubation time at an elevated temperature (60°C, Test Tube Procedure only). The result is an extremely sensitive colorimetric protein assay in a test tube or microplate assay format. the kit is sufficient for 250 test tube or 1250 microplate assays.

Features:

- Accurately detect down to 0.5ug/ml (2ug/ml in microplate format).
- Linear working range for BSA equals 0.5 to 20µg/ml (test tube format) or 2 to 40µg/ml (microplate format).
- Measure with a standard spectrophotometer or plate reader (562nm).
- Unaffected by typical concentrations of most ionic and nonionic detergents.
- Microplate and cuvette protocols provided.

Contents:

Description	Size
Reagent A	60ml
Reagent B	60ml
Reagent C	3ml
BSA Standard (5mg/ml)	2ml

Procedure:

A. Preparation of Standards and Working Reagent

1. Transfer 40ul of BSA standard into a new centrifuge tube and add 4.96ml DDH2O,thus the final concentration of the diluted BSA standard is 40ug/ml.
2. Transfer 0, 12.5, 25, 50, 125, 250, 375, 500, 1000ul of the above diluted BSA standard into each 2ml centrifuge tube, then add DDH2O into each 2ml centifuge tube,so that the total volume in each centrifuge tube is 1000ul and the final concentration of BSA in each centrifuge tube is 0, 0.5, 1, 2, 5, 10, 15, 20, 40ug/ml.
3. Prepare WR by mixing 25 parts of Micro BCA Reagent A and 24 parts Reagent B with 1 part of Reagent C (25:24:1, Reagent A:B:C).

Note: When Reagent C is initially added to Reagents A and B, turbidity occurs that quickly disappears upon mixing to yield a clear-green solution. Prepare sufficient volume of WR based on the number of samples to be assayed. The WR is stable for one day when stored in a closed container at room temperature (RT). It is not necessary to protect the solution from light.

B. Test Tube Procedure (linear working range of 0.5-20µg/mL)

1. Pipette 0.5mL of each standard and unknown sample replicate into appropriately labeled test tubes.
2. Add 0.5mLof the WR to each tube and mix well.
3. Cover tubes and incubate at 60°C in a water bath for 1 hour.
4. Cool all tubes to room temperature (RT).
5. With the spectrophotometer set to 562nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples within 10 minutes.
Note: Color development continues even after cooling to RT. However, the rate of development at RT is sufficiently low such that no significant error is introduced if all absorbance measurements are made within a 10 minute period.
6. Subtract the average 562nm absorbance reading of the Blank standard replicates from the 562nm reading of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 562nm reading for each BSA standard, use the standard curve to determine the protein concentration of each unknown sample.

C. Microplate Procedure (linear working range of 2-40µg/mL)

1. Pipette 100ul of each standard or unknown sample replicate into a microplate well
2. Add 100ul of the WR to each well and mix plate thoroughly on a plate shaker for 30seconds.
3. Cover plate using Sealing Tape for 96-Well Plates and incubate at 37°C for 2 hours.
Note: Limit incubations of microplate to less than or equal to 37°C, otherwise high background and aberrant color development may result. Most polystyrene assay plates deform, leach, and become cloudy at 60°C.
4. Cool plate to room temperature (RT).
5. Measure the absorbance at or near 562nm on a plate reader.
6. Subtract the average 562nm absorbance reading of the Blank standard replicates from the 562nm reading of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 562nm reading for each BSA standard, use the standard curve to determine the protein concentration of each unknown sample.

Additional Notes:

1. If either Reagent A or Reagent B precipitates upon shipping in cold weather or during long-term storage, dissolve precipitates by gently warming and stirring solution. Discard any kit reagent that shows discoloration or microbial contamination.
2. Certain substances are known to interfere with the BCA assay including those with reducing potential, chelating agents, and strong acids or bases, thus should avoid select DTT, Ascorbic Acid , EGTA, Iron, Impure Sucrose, Tyrosine, Uric Acid and so on as components of the sample buffer.
3. The effects of interfering substances in the BCA Assay may be overcome by remove the interfering substance by dialysis or desalting or dilute the sample until the substance no longer interferes or precipitate proteins with acetone or trichloroacetic acid (TCA)
4. Increase the amount of copper in the WR (prepare WR as 25:24:2 or 25:24:3, Reagent A:B:C), which may eliminate interference by copper-chelating agents.