

PROTOCOL

BCA Assay for Amyloid-Beta Quantitation

Protocol # SOP-212

Description:

The bicinchoninic acid (BCA) assay is the recommended method to obtain accurate assessments of total peptide concentration in commercial samples of Amyloid-beta products. Due to the lack of chromophoric amino acids in Amyloid-beta peptides, direct UV-VIS absorbance measurements are of limited utility at lower peptide concentrations. When used in comparison to a standard curve of protein samples of known quantity, Amyloid-beta samples can be accurately quantitated prior to experimental use. AmideBio recommends that researchers always perform one or more quantitation procedures to determine accurate stock concentrations of Amyloid-beta products prior to performing an experiment.

Reference

Please refer to Pierce BCA Protein Assay Kit, CAT# 23225; (<http://www.piercenet.com/>)

Procedure

1. Protein Standard Curve
 - 1.1. Dilute one 2mg Albumin Standard (BSA) ampule into 1mL of MQ H₂O for initial stock concentration of 2000 µg/ml.
 - 1.2. Serially dilute the BSA stock using MQ H₂O to make 500ul of standard curve samples at concentrations of 200, 150, 100, 50, 25, 10, 5 and 0 µg/mL (blank)
 - 1.3. Pipette 100µL of each standard in duplicate into the wells of a 96-well microplate, making sure to record the locations.
 - 1.4. Dilute 10µl of 1% NH₄OH into 190µl of MQ H₂O as sample control; Pipette 100µl of sample control in duplicate to adjacent wells of the 96-well microplate, making sure to record the locations.
2. Sample preparation
 - 2.1. Resuspend the entire Amyloid-Beta sample into 1% NH₄OH at a final calculated concentration of 1mg/ml; e.g. 50uL solvent to a 50ug sample tube. **NOTE: AmideBio BioPure Amyloid Beta peptide samples nominally contain 10% more material than the labeled amount. This surplus provides sufficient material for the quantitation assay prior to use in a biological experiment.**
 - 2.1.1. Vortex for 1 minute and batch sonicate for 5 minutes until the material has completely solubilized.
 - 2.1.2. Centrifuge at 13,000rpm for 5 minutes.
 - 2.2. Pipette 5µl of the Amyloid-beta sample in duplicate to adjacent wells of the 96-well microplate making sure to record the locations.
 - 2.3. Pipette 95µl of MQ H₂O into each of the above Amyloid-beta sample wells to achieve a calculated concentration of 50µg/ml.

3. Prepare BCA working reagent
 - 3.1. Refer to Pierce BCA Protein Assay Kit, CAT# 23227 instructions.
 - 3.2. Mix 50 parts of BCA reagent A with one part of BCA reagent B (50:1, Reagent A:B)
4. Pipette 100µl of BCA working reagent into each test well of the 96-well microplate.
 - 4.1. Cover plate with Adhesive PCR Plate Seals and incubate at 37°C for 30 minutes.
 - 4.2. Cool plate to RT. Measure the absorbance at 562 nm on a plate reader spectrophotometer according to the manufacturer's instructions.
5. Subtract the average 562nm absorbance measurement of the Blank standard duplicates from the 562nm measurements of all other individual standards.
6. Subtract the average 562nm absorbance measurement of the Sample Control duplicates from the 562nm measurements of all Amyloid-Beta samples.
7. Prepare a standard curve by plotting the average Blank-corrected 562nm measurement for each BSA standard vs. its concentration in µg/mL.
8. Use the standard curve to determine the protein concentration of Amyloid-Beta peptide in the main stock sample, **Figure**:

Actual Amyloid-Beta peptide (µg/mL) = calculated Amyloid-Beta peptide (µg/mL) X **1.51**

NOTE: A correction factor of 1.51 is used to obtain the true Amyloid-peptide concentration due to the decreased chromophoric development of amyloid-peptides compared to BSA. Please see AmideBio's AN-101 for further details.

