BCA Protein Assay Kit

Catalog No.: abx090640

Size: 200 tests / 500 tests

Storage: Store the kit components at 4 °C for up to one year.

Introduction

Abbexa's BCA Protein Assay Kit provides a sensitive method for determining protein concentrations. It is suitable for use with samples containing chemical substances such as SDS, Triton X-100 and Tween.

Principle of the assay

Proteins in alkaline medium reduce Cu^{2+} to Cu^{+}, the latter of which chelates with bicinchoninic acid (BCA) to form a complex with a strong linear absorbance at 562 nm. The concentration can be calculated by measuring the absorbance at 562 nm.

Kit components	200 tests	500 tests
1. BCA Reagent A	40 ml	100 ml
2. BCA Reagent B	1.2 ml	3 ml
3. Lyophilized Albumin Standard (BSA)	5 mg	5 mg

Materials Required But Not Provided

1. Microplate reader or spectrophotometer (562 nm)
2. 96-well microplate and plate sealer, or spectrophotometer cuvettes and test tubes
3. High-precision pipette and sterile pipette tips
4. 37 °C incubator or water bath
5. Distilled water
6. PBS or 0.9% NaCl solution

Protocol

Reagent Preparation

- **BCA Working solution:** To a tube, prepare the BCA Working solution by adding BCA Reagent A and BCA Reagent B at a ratio of 50:1 (e.g. add 25 ml of BCA Reagent A to 0.5 ml of BCA Reagent B).
- **5 mg/ml BSA stock standard solution:** Reconstitute the BSA Standard with 1 ml of distilled water to prepare 1 ml of BSA standard solution with a concentration of 5 mg/ml. Ensure that the standard is fully dissolved before use.
- **Standard solutions:** Label 9 tubes with 2000 µg/ml, 1500 µg/ml, 1000 µg/ml, 750 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 25 µg/ml, and 0 µg/ml. Dilute the standard with PBS or 0.9% NaCl

solution as shown in the table below, starting with the 2000 µg/ml standard tube. Mix each standard tube gently by pipetting up and down before transferring the solution to the next tube.

Standard Concentration (µg/ml)	Distilled Water (µl)	Standard (µl)
2000	360	240 (from 5 mg/ml tube)
1500	280	120 (from 5 mg/ml tube)
1000	300	300 (from 2000 µg/ml tube)
750	200	200 (from 1500 µg/ml tube)
500	300	300 (from 1000 µg/ml tube)
250	300	300 (from 500 µg/ml tube)
125	300	300 (from 250 µg/ml tube)
25	400	100 (from 125 µg/ml tube)

Assay Procedure (Test Tube)

1. Set the sample, standard and blank tubes. We recommend setting up each standard and sample in duplicate.
2. Add 100 µl of prepared standards to each standard tube.
3. Add 100 µl of sample to each sample tube.
4. Add 100 µl of PBS or 0.9% NaCl solution (same diluent used to dilute the standard solutions) to each blank tube.
5. Add 2 ml of BCA Working solution to each tube. Mix thoroughly by pipetting up and down.
6. Incubate the tubes at 37 °C for 30 minutes. The incubation time and temperature are for reference only, the optimal time should be determined by the end user. Increasing the incubation time or temperature can increase the absolute OD_{562} absorbance value for each test and decreases the minimum detection level of the reagent and working range of the protocol.
7. Allow the tubes to stand and cool to room temperature.
8. Using a spectrophotometer set to 562 nm, zero the instrument using a cuvette filled with PBS or 0.9% NaCl solution (same diluent used to dilute the standard solutions), and then measure the absorbance of the tubes. The measurements should be carried out within 10 minutes of cooling to room temperature.
9. Prepare a standard curve by plotting the average blank-corrected OD_{562} measurements of the standard solutions against the standard concentration. The standard curve can be used to determine the protein concentration in the samples.

Assay Procedure (Microplate)

1. Set the sample, standard and blank wells on the 96-well microplate. We recommend setting up each standard and sample in duplicate.
2. Add 25 µl of prepared standards to each standard well.
3. Add 25 µl of sample to each sample well.
4. Add 25 µl of PBS or 0.9% NaCl solution (same diluent used to dilute the standard solutions) to each blank well.

5. Add 200 µl of BCA Working solution to each well. Tap the plate gently to mix thoroughly.
6. Cover the microplate with a plate sealer and incubate at 37 °C for 30 minutes.
7. Allow the microplate to stand and cool to room temperature.
8. Remove the plate sealer and measure the absorbance using a microplate reader set to 562 nm.
9. Prepare a standard curve by plotting the average blank-corrected OD_{562} measurements of the standard solutions against the standard concentration. The standard curve can be used to determine the protein concentration in the samples.

Notes

- If a precipitate is observed in any of the reagents, mix, incubate at 37 °C, or microwave for a few seconds to dissolve the precipitate. Do not use reagents which are contaminated with bacteria.
- Turbidity may be observed when preparing the BCA Working solution, after adding BCA Reagents A and B together. If this occurs, mix the solution thoroughly.
- It is recommended that samples containing chelating agents (EDTA, EGTA), deoxidizers (DTT, mercaptoethanol), ammonium sulfate and lipids are tested using Bradford Protein Assay Kit (abx090644), as these components can interfere with the BCA assay.
- Samples containing high concentration of detergents should be pretreated with trichloroacetic acid (TCA) precipitation to remove the detergents.
- Avoid evaporation from the microplate or tubes during the incubation steps.
- Use personal protective equipment (PPE) such as lab coats, lab goggles, gloves when using this kit.