BCA protein quantification kit

Project number: B665595

Storage conditions: 2-8° C.

Products

individual parts making up a compound	500 microplate assay or 50 tube assay
BCA-A	2 x 50 mL
BCA-B	3 mL
BSA Standard Solution (2mg/mL)	2 mL

Products

BCA protein quantification method is one of the widely used protein quantification methods. It is developed based on the BCA (Bicin-choninic Acid) method, which realizes rapid, stable and sensitive concentration determination of proteins. The principle is that the peptide chain structure of protein molecules in alkaline environment can complex with Cu2+ to form complexes, and at the same time reduce Cu2+ to Cu+. BCA reagent can sensitively and specifically bind to Cu+ to form stable and colorful complexes. It has a high light absorption value at 562 nm, and the depth of color is proportional to the protein concentration, and the protein content can be determined according to the size of the absorption value. This kit contains bovine serum albumin (BSA) solution as the protein standard solution, and the determination range is from 20 to 2000 μg/mL.

caveat

1. This product can be used to determine the protein concentration by spectrophotometer (test tube assay) or enzyme marker (microtiter assay).
2. It is recommended that a standard curve be drawn for each determination of protein samples to obtain accurate data.
3. The dilution of the BSA standard needs to be the same as the dilution of the sample to be tested (can be diluted with 1×PBS or 0.9% saline).
4. If the sample to be tested contains a high level of interfering substances, the Bradford Method Protein Quantification Kit or other protein quantification products may be used.

procedure

1. Dilution of BSA standard: Dilute the BSA standard with a diluent consistent with the protein sample to be measured according to the following table.

tube number	Amount of diluent (μL)	Amount of BSA standard (μL)	Final concentration of BSA standard (μg/μL)
A	0	100	2
B	200	200	1
C	200	200 (from tube B)	0.5
D	200	200 (from tube C)	0.25
E	200	200 (from tube D)	0.125
F	200	200 (from tube E)	0.0625
G	200	0	0

Total BCA working solution = (number of BSA standard samples + number of unknown samples) × number of replicate wells × volume of BCA working solution for each sample

Example: The number of BSA standard samples is 7, the number of unknown samples is 2, and the number of replicate wells is 3.

Test tube assay:

Total BCA working solution = (7 BSA standard samples + 2 unknown samples) x 3 replicate wells x 2 mL/volume of working solution per sample = 54 mL

Microporous assay:

Total amount of BCA working solution = (7 BSA standard samples + 2 unknown samples) x 3 replicate wells x 200 μL/volume of working solution per sample = 5.4 mL 2) Based on the calculated total amount of BCA working solution needed, prepare BCA working solution by mixing BCA-A and BCA-B at a volume ratio of 50:1, and mix thoroughly.

Note: 1) Due to possible errors in adding samples, it is recommended to prepare 1-2 extra wells when preparing the BCA working solution.

(2) The freshly prepared BCA working solution can be stored stably for 24 hours under sealed conditions at room temperature.

3. Quantitative testing

3a. In vitro assay (protein concentration range: 20-2000 μg/mL)

1) According to the above table, add 100 μL of diluted A-G BSA standard and 100 μL of the protein sample to be measured (stock solution or dilution) to the labeled tubes. It is recommended to do 2-3 parallel reactions for each sample.

2) Add 2mL of BCA working solution to each tube, mix thoroughly, and incubate in a 37℃ water bath for 30 minutes, cool each tube to room temperature, and complete the assay within 3-5 minutes.

3) Determine the absorbance value of each sample and BSA standard at 562 nm using a spectrophotometer, and make a record at the same time.

4) Plot the standard curve and calculate the protein concentration in the sample.

3b. Microtiter assay (protein concentration range: 20-2000 μg/mL)

1) According to the above table, add 25μl of diluted A-G BSA standard and 25μl of each of the protein samples to be assayed (stock solution or dilution) into the wells of a well-labeled 96-well plate. It is recommended to do 2-3 parallel reactions for each sample to be measured.

(2) Add 200μL BCA working solution to each well, mix well, cover the 96-well plate, incubate at 37℃ for 30 minutes, cool to room temperature, and the test must be completed within 3-5 minutes.

3) Determine the absorbance value of each sample and BSA standard in the range of 540-590 nm using an enzyme labeling instrument, and make a record at the same time.

4) Plot the standard curve and calculate the protein concentration in the sample.

Note: 1) When the BCA method is used to determine protein concentration, the absorbance value will deepen with time. Therefore, all samples should be analyzed within 3-5 minutes, otherwise the accuracy of protein quantification will be affected.

2) It is recommended that the standard curve be plotted with absorbance readings after removal of background values.

(3) Standard readings that deviate significantly from the linear curve due to operational errors should be discarded.

(4) The concentration of the unknown sample can be calculated from the standard curve equation, the actual concentration needs to be multiplied by the dilution of the sample.

5) If the protein concentration obtained is not within the detection range, please re-dilute the sample and measure again.

4. Examples of quantitative BCA protein analysis results:

Microtiter plate assay results

Protein concentration (μg/μL)	raw absorbance value	Absorbance values after removal of background values
0	0.086 (background value)	0
0.125	0.123	0.037
0.25	0.28	0.194
0.5	0.551	0.465
1	1.068	0.982
2	2	1.913