SMART™ BCA Protein Assay Kit
[for Standard Assay]

Cat. No. 21071

Test Tube : 250 Tests
Microplate : 2,500 Tests

DESCRIPTION

The SMART™ BCA Protein Assay Kit (for Standard Assay) is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein. The purple-colored reaction product of this assay is formed by the chelation of two molecules of BCA with one cuprous ion. This water-soluble complex exhibits a strong absorbance at 562 nm that is nearly linear with increasing protein concentrations over a broad working range (20-2,000μg/ml).

The macromolecular structure of protein, the number of peptide bonds and the presence of four particular amino acids (cysteine, cystine, tryptophan and tyrosine) are reported to be responsible for color formation with BCA. Studies with di-, tri- and tetrapeptides suggest that the extent of color formation caused by more than the mere sum of individual colorproducing functional groups. Accordingly, protein concentrations generally are determined and reported with reference to standards of a common protein such as bovine serum albumin (BSA). A series of dilutions of known concentration are prepared from the protein and assayed alongside the unknown(s) before the concentration of each unknown is determined based on the standard curve.

Two assay procedures are presented. Of these, the Test Tube Procedure requires a larger volume (0.1 ml) of protein sample; however, because it uses a sample to working solution ratio of 1:20 (v/v), the effect of interfering substances is minimized. The Microplate Procedure affords the sample handling ease of a microplate and requires a smaller volume (10-25 μl) of protein sample; however, because the sample to working solution ratio is 1:8 (v/v), it offers less flexibility in overcoming interfering substance concentrations and obtaining low levels of detection.

CHARACTERISTICS

- Colorimetric method; read at 562 nm
- Compatible with most ionic and nonionic detergents
- Faster and easier than the Lowry method
- All reagents stable at room temperature for 2 years
- Working Solution stable for 24 hours
- Linear working range for BSA : 20-2,000μg/ml
- Adaptable to microplates
- Less protein-to-protein variation than dye-binding methods

KIT CONTENTS

• Solution A	250 ml x 2 ea
• Solution B	10 ml
• BSA solution (2 mg/ml)	1 ml x 10 ea

STORAGE

Upon receipt, store at room temperature. Product shipped at ambient temperature. **Note :** If either Solution A or Solution 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 evidence of contamination.

PREPARATION of STANDARDS and WORKING SOLUTION

[Preparation of Standard]

Use Table 1 as a guide to prepare a set of protein standards. Dilute the contents of one BSA Solution tube into several clean vials, preferably using the same diluent as the sample(s). Each 1 ml tube of 2.0 mg/ml BSA Solution is sufficient to prepare a set of diluted standards for either working range suggested in Table1.

Table 1. Preparation of Diluted Albumin (BSA) Standards

Tube	Volume of Diluent	Volume & Source of BSA	Final Conc.
1	0	300 μl of Stock	2,000 μg/ml
2	125 μl	375 μl of Stock	1,500 μg/ml
3	325 μl	325 μl of Stock	1,000 μg/ml
4	325 μl	325 μl of tube 3 dilution	500 μg/ml
5	325 μl	325 μl of tube 4 dilution	250 μg/ml
6	325 μl	325 μl of tube 5 dilution	125 μg/ml
7	400 μl	100 μl of tube 6 dilution	25 μg/ml
8	400 μl	0	0 μg/ml = Blank

[Preparation of Working Solution]

Before use, prepare Working Solution (WS) by mixing 50 parts of Solution A with 1 part of Solution B (50:1, Solution A:B).

For the above example, combine 50 ml of Solution A with 1 ml of Solution B.

Note : When Solution B is first added to Solution A, a turbidity is observed that quickly disappears upon mixing to yield a clear, green WS. Prepare sufficient volume of WS based on the number of samples to be assayed. The WS is stable for several days when stored in a closed container at room temperature(RT).

ADDITIONAL INFORMATION

A. Interfering substances

Certain substances are known to interfere with the SMART™ BCA Protein Assay including those with reducing potential, chelating agents, and strong acids or bases. Because they are known to interfere with protein estimation at even minute concentrations, avoid the following substances as components of the sample buffer:

Ascorbic Acid	EGTA	Iron	Impure Sucrose
Catecholamines	Impure Glycerol	Lipids	Tryptophan
Creatinine	Hydrogen Peroxide	Melibiose	Tyrosine
Cysteine	Hydrazides	Phenol Red	Uric acid

Other substances interfere to a lesser extent with protein estimation using the SMART™ BCA Protein Assay, and these have only minor (tolerable) effects below a certain concentration in the original sample. Substances were compatible at the indicated concentration in the Standard Test Tube Protocol if the error in protein concentration estimation caused by the presence of the substance in the sample was less than or equal to 10%. The substances were tested using WS prepared immediately before each experiment. Blank-corrected 562 nm absorbance measurements (for a 1,000 μg/ml BSA standard + substance) were compared to the net 562 nm measurements of the same standard prepared in 0.9% saline. In the Microplate Procedure, where the sample to WS ratio is 1:8 (v/v), maximum compatible concentrations will be lower.

B. Strategies for eliminating or minimizing the effects of interfering substances

The effects of interfering substances in the SMART™ BCA Protein Assay may be eliminated or overcome by one of several methods.

- Remove the interfering substance by dialysis or gel filtration.
- Dilute the sample until the substance no longer interferes. This strategy is effective only if the starting protein concentration is sufficient to remain in the working range of the assay upon dilution.
- Precipitate the proteins in the sample with acetone or trichloroacetic acid (TCA). The liquid containing the substance that interfered is discarded and the protein pellet is easily solubilized in ultrapure water or directly in the alkaline SMART™ BCA WS.
- Increase the amount of copper in the Ws (prepare WS as 50:2 or 50:3, Solution A:B), which may eliminate interference by copper chelating agents.

Note: For the greatest accuracy, the protein standards must be treated identically to the sample(s).

PROTOCOL (for Microplate, Sample to WS ratio = 1:8)

1. Pipette 25 μl of each standard or unknown sample replicate into a microplate well (working range = 20-2,000 μg/ml).
   Note : If sample size is limited, 10 μl of each unknown sample and standard can be used (sample to Working Solution ; WS ratio = 1:20). However, the working range of the assay in this case will be limited to 125-2,000 μg/ml.
2. Add 200 μl of the Working Solution (WS) to each well and mix plate thoroughly on a plate shaker for 30 seconds.
   Note : To preparation of Working Solution (WS), see the "PREPARATION of STANDARDS and WORKING SOLUTION"
3. Cover plate and incubate at 37°C for 30 minutes.
4. Cool plate to room temperature(RT).
5. Measure the absorbance at or near 562 nm on a plate reader.
   Note : Wavelengths from 540-590 nm have been used successfully with this method. If higher 562 nm measurements are desired, increase the incubation time to 2 hours.
6. Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562 nm measurements of all other individual standard and unknown sample replicates.

7. Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562 nm measurements of all other individual standard and unknown sample replicates.
8. Prepare a standard curve by plotting the average Blank-corrected 562 nm measurement for each BSA standard vs. its concentration in µg/ml. Use the standard curve to determine the protein concentration of each unknown sample.
   Note : If using curve-fitting algorithms associated with a microplate reader, a four- parameter (quadratic) or best-fit curve will provide more accurate results than a purely linear fit. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points.

PROTOCOL (for Test Tube, Sample to WS ratio = 1:20)

1. Pipette 0.1 ml of each standard and unknown sample replicate into an appropriately labeled test tube.
2. Add 2 ml of the Working Solution (WS) to each tube and mix well.
3. Cover and incubate tubes at 37°C for 30minutes.
4. Cool all tubes to room temperature(RT).
5. With the spectrophotometer set to 562 nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples within 10 minutes.
   Note : Because the SMART™ BCA Assay does not reach a true end point, color development will continue even after cooling to RT. However, because the rate of color development is low at RT, no significant error will be introduced if the 562 nm absorbance measurements of all tubes are made within 10 minutes of eachother.
6. Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562 nm absorbance measurement of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 562 nm measurement for each BSA standard vs. its concentration in µg/ml. Use the standard curve to determine the protein concentration of each unknown sample.

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
No color in any tubes	Sample contains a copper chelating agent	Dialyze, desalt, or dilute sample Increase copper concentration in working solution (e.g., use 50:2, Solution A:B)
Blank absorbance is OK, but standards and samples show less color than expected	Strong acid or alkaline buffer, alters working reagent pH	Dialyze, desalt, or dilute sample
Color of samples appears darker than expected	Color measured at the wrong wavelength Protein concentration is too high	Measure the absorbance at 562nm Dilute sample
	Sample contains lipids or Lipoproteins	Add 2% SDS to the sample to eliminate interference from lipids
All tubes (including blank) are dark purple	Buffer contains a reducing agent Buffer contains a thiol Buffer contains biogenic amines (catecholamines) Spectrophotometer or	Dialyze or dilute sample
Need to measure color at a different wavelength	plate reader does not have 562 nm filter	Color may be measure at any wavelength between 540 nm and 590 nm, although the slope of standard curve and overall assay sensitivity will be reduced

EXPERIMENTAL DATA

IMPROVED SENSITIVITY & LINEAR WORKING RANGE

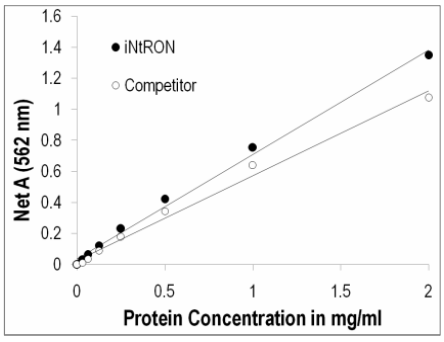

Fig. 1. Comparison of sensitivity of protein assay.
SMART™ BCA Protein Assay Kit (for Standard Assay) shows improved sensitivity higher than Competitor product as 15~20%.
The color response was estimated micro well plate reader (37°C/30 minute incubation).

HIGH FIDELITY

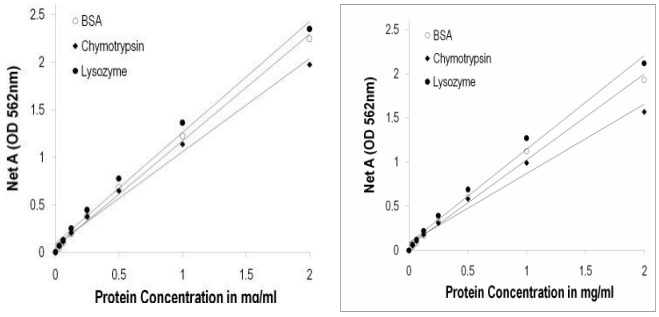

Fig. 2. Typical Color response curves for BSA, Chymotrypsin and Lysozyme using the Micro plate Protocol.
Absorbance ratios (562 nm) for proteins relative to BSA. The SMART™ BCA Protein Assay Kit (for Standard Assay) showed higher sensitivity (slope) than Competitor. However, The Protein-to-Protein variations showed similar patterns
A; SMART™ BCA Protein Assay Kit, **B**; Competitor A Product

RELATED PRODUCT

Product Name	Cat. No.
PRO-PREP™ Protein Extraction Solution (C/T)	17081
SMART™ Bacterial Protein Extraction Solution	17511
PRO-MEASURE™ Protein Measurement Solution	21011
PRO-STAIN™ (I) Prestained Protein Marker	24051
SMART™ micro BCA Protein Assay Kit (for Micro Assay)	21072
WEST-one™ Western Blot Detection System	16031 ~16033
WEST-ZOL® plus Western Blot Detection System	16021