

# SMART™ micro BCA Protein Assay Kit [ for Micro Assay ]

Cat. No. 21072 Test Tube : 200 Tests  
Microplate : 2,000 Tests

## DESCRIPTION

SMART™ micro BCA Protein Assay Kit (for Micro Assay) is a detergent-compatible bicinchoninic acid formulation for the colorimetric detection and quantitation of total protein. An adaptation of the SMART™ BCA Protein Assay Kit (for Standard Assay), SMART™ micro BCA Protein Assay Kit (for Micro Assay) has been optimized for use with dilute protein samples (2-40 µg/ml).

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. 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 color-producing functional groups.

SMART™ micro BCA Protein Assay Kit (for Micro Assay) uses concentrated solutions 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 microplate or test tube assay format.

## CHARACTERISTICS

- Colorimetric method; read at 562 nm
- Compatible with most ionic and nonionic detergents
- A very sensitive reagent for dilute protein samples
- Linear working range for BSA: 0.5-20 µg/ml
- Less protein-to-protein variation than dye-binding methods
- All kit reagents stable at room temperature for 2 years
- Working solution is stable for 24 hours
- Convenient microplate format

## KIT CONTENTS

- Micro Solution A 100 ml
- Micro Solution B 96 ml
- Micro Solution C 4 ml
- BSA Solution (128 µg/ml) 1 ml x 10 ea

## STORAGE

Upon receipt, store product at room temperature. Product shipped at ambient temperature.

**Note:** If either Micro Solution A or Micro Solution B precipitates upon shipping in cold weather or during longterm storage, dissolve precipitates by gently warming and stirring solutions. Discard any reagent that shows discoloration or evidence of microbial 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 Table 1.

**Table 1.** Preparation of Diluted Albumin (BSA) Standards

Tube	Volume of Diluent	Volume & Source of BSA	Final Conc.
1	300 µl	300 µl of Stock	64 µg/ml
2	300 µl	300 µl of tube 1 dilution	32 µg/ml
3	300 µl	300 µl of tube 2 dilution	16 µg/ml
4	300 µl	300 µl of tube 3 dilution	8 µg/ml
5	300 µl	300 µl of tube 4 dilution	4 µg/ml
6	300 µl	300 µl of tube 5 dilution	2 µg/ml
7	300 µl	300 µl of tube 6 dilution	1 µg/ml
8	300 µl	0	0 µg/ml = Blank

### [ Preparation of Working Solution ]

Before use, prepare Working Solution (WS) by mixing 25 parts of Micro Solution A and 24 parts Micro Solution B with 1 part of Micro Solution C (25:24:1, Micro Solution A:B:C).

*For the above example*, combine 12.5 ml of Micro Solution A and 12 ml Micro Solution B with 0.5 ml of Micro Solution C.

**Note :** When Micro Solution C is initially added to Micro Solution A and Micro Solution B, a turbidity is observed that quickly disappears upon mixing to yield a clear-green solution. Prepare sufficient volume of WS based on the number of samples to be assayed. The WS 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.

## ADDITIONAL INFORMATION

### A. Interfering substances

Certain substances are known to interfere with the SMART™ micro 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

Substances were considered compatible at the indicated concentration 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 WR prepared immediately before each experiment. The Blank-corrected 562 nm absorbance measurements (for the 10 µg/ml BSA standard + substance) were compared to the net 562 nm readings of the same standard prepared in 0.9% saline.

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

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

- Remove the interfering substance by dialysis or gel filtration.
- Dilute the sample until the substance no longer interferes. (This is only effective for relatively concentrated samples.)
- Precipitate proteins with acetone or trichloroacetic acid (TCA).
- Increase the amount of copper in the WS (prepare WS using a greater proportion of Solution µC; e.g., µA:µB:µC equal to 25:24:2 or 25:24:3), 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 I (for Microplate)

1. Pipette 100 µl of each standard or unknown sample replicate into a microplate well (working range = 2 - 40 µg/ml).
2. Add 100 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 120 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. 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 II (for Test Tube)

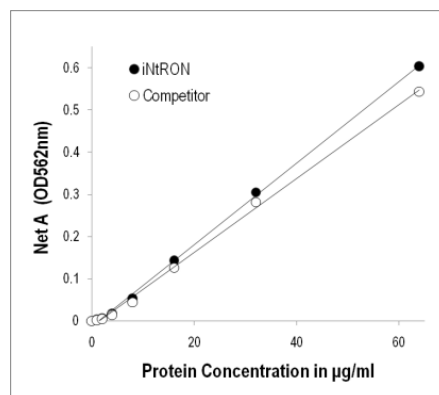
- Pipette 1 ml of each standard and unknown sample replicate into appropriately labeled test tubes.
- Add 1 ml of the Working Solution (WS) to each tube and mix well.
- Cover tubes and incubate at 60°C in a water bath for 1 hour.
- Cool all tubes to room temperature (RT).
- With the spectrophotometer 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 :** Color development continues even after cooling to RT. However, the rate of development at RT is sufficient low that no significant error is introduced if all absorbance measurements are made within a 10 minute period.
- Subtract the average 562 nm absorbance reading of the Blank standard replicates from the 562 nm reading of all other individual standard and unknown sample replicates.
- Prepare a standard curve by plotting the average Blank-corrected 562 nm reading 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
Blank absorbance is OK, but standards and samples show less color than expected	Strong acid or alkaline buffer, alters working reagent pH Color measured at the wrong wavelength	Dialyze, desalt, or dilute sample Measure the absorbance at 562 nm
Color of samples appears darker than expected	Protein concentration is too high Sample contains lipids or Lipoproteins	Dilute sample 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)	Dialyze or dilute sample
Need to measure color at a different wavelength	Spectrophotometer or 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

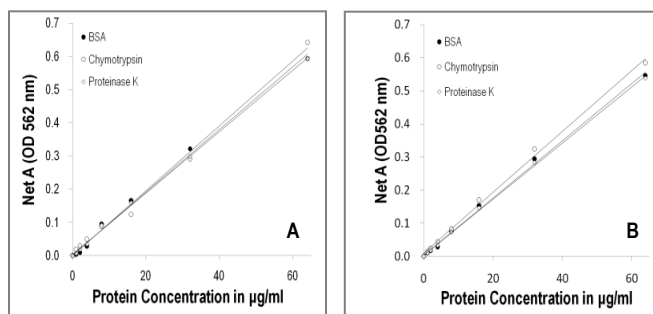


**Fig. 1. Comparison of sensitivity of protein assay.**

SMART™ micro BCA Protein Assay Kit (for Micro Assay) shows improved sensitivity higher than Competitor product as 15~20%.

The color response was estimated with micro well plate reader (37°C/120-minute incubation).

### HIGH FIDELITY



**Fig. 2. Typical Color response curves for BSA, Chymotrypsin and Lysozyme using the Microplate Protocol.**

Absorbance ratios (562 nm) for proteins relative to BSA. The SMART™ micro BCA Protein Assay Kit (for Micro Assay) showed higher sensitivity (slope) than Competitor. However, The Protein-to-Protein variations showed similar patterns.

A: SMART™ micro BCA 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™ BCA Protein Assay Kit (for Standard Assay)	21071
WEST-one™ Western Blot Detection System	16031 ~ 16033
WEST-ZOL® plus Western Blot Detection System	16021

