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Protein Biology Products

Instruction Manual

Micro BCA Protein Assay

Catalog No.: 18-444 and 18-445

INTRODUCTION

Our Micro BCA Protein Assay is a ready-to-use, detergent-compatible, bicinchoninic acid based ultra-sensitive colorimetric quantitation of total protein and has been optimized for dilute protein solutions of 0.5 µg/ml to 20 µg/ml. The assay utilizes bicinchoninic acid (BCA) as the detection reagent for Cu^{+1}, which is formed when Cu^{+2} is reduced by protein in an alkaline environment[1]. A purple color reaction product is formed by the chelation of two molecules of BCA with one cuprous ion (Cu^{+1}). This water-soluble complex exhibits a strong absorbance at 562nm that is linear with increasing protein concentrations. It has been reported that the macromolecular structure of proteins, the number of peptide bonds and the presence of four amino acids (cysteine, cystine, tryptophan and tyrosine) are responsible for color formation with BCA[2]. Our Micro BCA Protein Assay uses concentrated reagents and a protocol with extended incubation time at an elevated temperature, which results in an extremely sensitive colorimetric protein assay, enough for 480 test tube and about 3,200 microplate assay formats. The assay kit can be used for assessing protein concentration in whole cell lysates, studying protein-protein interactions, measuring column fractions after affinity chromatography and high-throughput screening of fusion proteins, etc.

Key Features:

- **Highly Sensitive**, detects down to 0.5µg/ml protein
- **Colorimetric method**, measured in a spectrophotometer or microplate reader at 562nm
- **Accurate** and exhibits less protein-to-protein variation than dye-binding methods
- **Compatible** – with typical concentrations of most ionic and nonionic detergents
- **High linearity** – linear working range for BSA equals 0.5 µg/ml to 20µg/ml

Items Supplied:

Item Name	Cat. No. 18-444	Cat. No. 18-445	Storage Condition*
Micro BCA Reagent A	240 ml	240 ml	Room Temp
Micro BCA Reagent B	240 ml	240 ml	Room Temp
Micro BCA Reagent C	15 ml	15 ml	Room Temp
BSA Standard (2 mg/ml)	N/A	2 x 5.0 ml	4°C

**The kit is shipped at ambient temperature and upon receipt, store it at 4°C and the kit components are stable for 12 months, if stored and used as recommended.*

NOTE: *If either Micro BCA Reagent A or B precipitates when received, due to cold weather or during long-term storage, dissolve the precipitates by gently warming and stirring solutions.*

Additional Items Needed:

1. 60°C water bath or incubator
2. Spectrophotometer or Microplate reader, capable of accurately measuring absorbance at 562nm

Interfering substances

Certain substances are known to interfere with the 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** these substances as components of the sample buffer: **Ascorbic acid, Catecholamines, Cysteine, EGTA, Hydrogen Peroxide, Hydrazides, Impure Glycerol, Impure Sucrose, Iron, Lipids, Phenol Red, Reducing Agents, Reducing Sugars (Melibiose), Tryptophan, Tyrosine, and Uric acid, etc**.

Maximum compatible concentrations for many substances in the Test Tube Procedure are listed in Table 3. 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 562nm absorbance measurements (for the 10µg/mL BSA standard + substance) were compared to the net 562nm readings of the same standard prepared in 0.9% saline.

Strategies for eliminating or minimizing the effects of interfering substances

The effects of interfering substances in the BCA Protein Assay may be eliminated or overcome by one of several methods as below:

- 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 10% Trichloro acetic acid (TCA). The liquid containing the substance that interfered is discarded and the protein pellet is easily solubilized in ultrapure water or directly in a suitable compatible buffer.
- Increase the amount of copper in the Micro BCA Working Reagent (WR) (prepare, using higher proportion of Reagent C in A:B:C dilution as 24:24:2 or 25:24:3), which may eliminate interference by copper chelating agents.

Protocols:

This section describes the preparation of diluted BSA Standards and Working Reagent for both Test Tube and Microplate Assays as below:

A. Preparation of Albumin (BSA) Standards

Dilute the supplied 2mg/ml BSA standard, preferably in the same buffer (diluent) as the test sample(s) for preparing a set of protein standards and follow the Table 1 below to prepare a fresh set of standards.

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

Table 1: Preparation of Diluted BSA Standards

Tube No.	Volume of Diluent/Buffer	Volume and Source of BSA	Final BSA Concentration
B (Blank)	8.0 ml	0 µl	0 µg/ml
1	2.25 ml	250 µl of 2 mg/ml	200 µg/ml
2	8.0 ml	2.0 ml of Tube 1	40 µg/ml
3	4.0 ml	4.0 ml of Tube 2	20 µg/ml
4	4.0 ml	4.0 ml of Tube 3	10 µg/ml
5	4.0 ml	4.0 ml of Tube 4	5 µg/ml
6	4.0 ml	4.0 ml of Tube 5	2.5 µg/ml
7	4.8 ml	3.2 ml of Tube 6	1 µg/ml
8	4.0 ml	4.0 ml of Tube 7	0.5 µg/ml

B. Preparation of Micro BCA Working Reagent (WR)

Prepare sufficient volume of the Micro BCA Working Reagent, based on the number of samples to be assayed. The Working Reagent is stable for several days when stored in a closed container at room temperature (RT).

I) Use the following formula to calculate the total volume of working reagent required:
(well numbers for standard + well numbers for sample) × (times of repetition) × (volume of working reagent per well) = total volume of working reagent required
For example: For the standard Test Tube Procedure, each sample needs 3 dilutions and 2 replicates:
(8 standard wells + 3 sample wells) × (2 replicates) × (1 ml) = 22 ml working reagent required (round up to 25 ml)

Note: *Each sample replicate requires 150 µl of BCA working reagent for each well in a microplate assay or 1 ml for a test tube procedure.*

II) Mix Micro BCA Reagent A, B and C in the ratio of 25:24:1. i.e., mix 5 ml of Micro BCA Reagent A and 4.8 ml Reagent B with 0.2ml of Reagent C.

Note: *When Micro BCA Reagent C is first added to Reagent A and B, a turbidity is observed that quickly disappears upon mixing to yield a clear green solution.*

Assay Protocols:

A. Test Tube Protocol

1. Add 1.0 ml of each standard and protein samples into separate labeled test tubes.
2. Add 1.0 ml of Micro BCA working reagent to each tube and mix well.
3. Seal tubes and incubate at 60°C in a water bath for 1 hour.
4. Cool all tubes to room temperature (RT).
5. Set the wavelength of spectrophotometer at OD 562 nm. Calibrate the instrument to zero by using water. Subsequently, measure the absorbance of all samples within 10 minutes.

   Note: *Color development continues even after cooling to RT. However, the subsequent development at RT is too weak to produce significant error if all absorbance measurements are made within 10 minutes.*
6. Subtract OD_{562} of Blank from all readings.
7. Plot the BSA standard curve by putting values of OD_{562} on Y axis Vs BSA Standard concentration on X axis. Use the standard curve to determine the protein concentration of each unknown sample.

B. Microplate Protocol

1. Add 150 µl of each standard and protein sample(s) into separate microplate wells.
2. Add 150 µl of Micro BCA working reagent to each well and mix thoroughly for 30 seconds.
3. Seal plates and incubate at 37°C for 2 hours or at 60°C for 1 hour.
4. Cool plate to room temperature (RT).
5. Measure the absorbance at 562 nm on a plate reader within 10 minutes.
6. Subtract OD_{562} of Blank from all readings.
7. Plot the BSA standard curve by putting the OD_{562} on Y axis Vs BSA Standard concentration on X axis. Use the standard curve to determine the protein concentration of each unknown sample.

Table-2: Troubleshooting

Problem	Possible Cause	Solution
No color in any tubes	Sample contains a copper chelating agent	Dialyze, desalt or dilute sample. Increase copper concentration in Micro BCA working reagent (e.g., Use more Reagent C in the A:B:C dilutions). Remove interfering substances from sample, using acetone or 10% trichloroacetic acid (TCA) precipitation method.
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 measured at the wrong wavelength	Measure the absorbance at 562nm
Color of samples appears darker than expected	Protein concentration is too high	Dilute the sample(s)
	Sample contains lipids or lipoproteins	Add 2% SDS to the sample to eliminate interference from lipids[3]
		Remove interfering substances from sample, using acetone or 10% trichloroacetic acid (TCA) precipitation method[4].
All tubes (including blank) are dark purple	Buffer contains a reducing agent	Dialyze or dilute sample Remove interfering substances from sample, using acetone or 10% trichloroacetic acid (TCA) precipitation method.
	Buffer contains a thiol	
	Buffer contains biogenic amines (catecholamines)	
Need to measure color at a different wavelength	Spectrophotometer or plate reader does not have 562nm filter	Color may be measure at any wavelength between 540nm and 590nm, although the slope of standard curve and overall assay sensitivity will be reduced

Table-3: Reagents/Buffers/Detergents/Reducing & Thiol Containing Agents/Solvents Compatible with BCA Protein Assay if standard recommended procedure is used

Reagents/Buffers	Concentration	Detergents/Reducing & Thiol Containing Agents/Solvents	Concentration
ACES, pH 7.8	10 mM	Brij-35	5.0 %
Ammonium sulfate	Not Compatible	Brij-58	1.0 %
Bicine, pH 8.4	2 mM	CHAPS	5.0 %
Bis-Tris, pH 6.5	0.2 mM	CHAPSO	5.0 %
Borate (50mM), pH 8.5	1:4 dilution	Deoxycholic acid	5.0 %
Calcium chloride in TBS, pH 7.2	10 mM	Nonidet P-40 (NP-40)	5.0 %
Na-Carbonate/Na-Bicarbonate (0.2M), pH 9.4	Undiluted	Octyl β-glucoside	0.1 %
Cesium bicarbonate	100 mM	Octyl β-thioglucopyranoside	5.0%
CHES, pH 9.0	100 mM	SDS	5.0 %
EDTA	0.5 mM	Triton X-100	5.0 %
EGTA	Not Compatible	Triton X-114	0.05 %
Na-Citrate (0.6M), Na-Carbonate (0.1M), pH 9.0	1:600 dilution	Tween-20	5.0 %
Na-Citrate (0.6M), MOPS (0.1M), pH 7.5	1:600 dilution	Tween-80	5.0 %
Cobalt chloride in TBS, pH 7.2	Not Compatible	EDTA	0.5 mM
EPPS, pH 8.0	100 mM	EGTA	Not Compatible
Ferric chloride in TBS, pH 7.2	0.5 mM	Sodium citrate	20 mM
Glucose	1 mM	N-acetylglucosamine in PBS, pH 7.2	Not Compatible
Glycine-HCl, pH 2.8	10 mM	Ascorbic acid	Not Compatible
Guanidine-HCl	4.0 M	Cysteine	Not Compatible
HEPES, pH 7.5	100 mM	Dithioerythritol (DTE)	Not Compatible
Imidazole, pH 7.0	12.5 mM	Dithiothreitol (DTT)	Not Compatible
MES, pH 6.1	100 mM	Melibiose	Not Compatible
MES (0.1M), NaCl (0.9%), pH 4.7	1:4 Dilution	β-Mercaptoethanol	0.01 %
MOPS, pH 7.2	100 mM	Thimerosal	0.01 %
Modified Dulbecco's PBS, pH 7.4	undiluted	Acetone	1 %
Nickel chloride in TBS, pH 7.2	0.2 mM	Acetonitrile	1 %
PBS; Phosphate (0.1M), NaCl (0.15M), pH 7.2	undiluted	Aprotinin	1 mg/L
PIPES, pH 6.8	100 mM	DMF	1 %
RIPA lysis buffer *[50mM Tris, 150mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0]*	1:10 Dilution	DMSO	1 %
		Ethanol	1 %
Sodium acetate, pH 4.8	200 mM	Glycerol (Fresh)	1 %
Sodium azide	0.2 %	Hydrazine	Not Compatible
Sodium bicarbonate	100 mM	Hydrazide (Na2BH4 or NaCNBH3	Not Compatible
Sodium chloride	1.0 M	Hydrochloric acid	10 mM
Sodium citrate	20 mM	Leupeptin	10 mg/L
Sodium citrate, pH 4.8 or pH 6.4	20 mM	Methanol	1%
Sodium phosphate	100 mM	Phenol Red	Not Compatible
Tricine, pH 8.0	2.5 mM	PMSF	1 mM
Triethanolamine, pH 7.8	0.5 mM	Sodium hydroxide	50 mM
Tris	50 mM	Sucrose	4 %
TBS; Tris (25mM), NaCl (0.15M), pH 7.6	1:10 Dilution	TLCK	0.1 mg/L
Tris (25mM), Glycine (192mM), pH 8.0	1:10 dilution	TPCK	0.1 mg/L
Tris (25mM), Glycine (192mM), SDS (0.1%), pH 8.3	undiluted	Urea	3 M
Zinc chloride in TBS, pH 7.2	0.5 mM	o-Vanadate (sodium salt), in PBS, pH 7.2	1 mM

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