INSTRUCTIONS

Micro BCA Protein Assay Kit

23235 0412.6

Number	Description
23235	**Micro BCA Protein Assay Kit**, sufficient reagents for 480 tube assays or 3200 microplate assays

Kit Contents:

Micro BCA Reagent A (MA), 240mL

Micro BCA Reagent B (MB), 240mL

Micro BCA Reagent C (MC), 12mL

Bovine Serum Albumin Standard Ampules, 2mg/mL, 10 × 1mL ampules containing bovine serum albumin (BSA) at 2.0mg/mL in a solution of 0.9% saline and 0.05% sodium azide

Storage: Upon receipt store product at room temperature. Product shipped at ambient temperature.

Note: If either Reagent MA or Reagent MB precipitates upon shipping in cold weather or during long-term storage, dissolve precipitates by gently warming and stirring solutions. Discard any reagent that shows discoloration or evidence of microbial contamination.

Table of Contents

Introduction

The Thermo Scientific™ Micro BCA™ Protein Assay Kit is a detergent-compatible bicinchoninic acid formulation for the colorimetric detection and quantitation of total protein. An adaptation of the Thermo Scientific™ BCA Protein Assay Kit (Product No. 23225), the Micro BCA Kit has been optimized for use with dilute protein samples (0.5-20µg/mL). The unique, patented method uses 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-colored 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.

The macromolecular structure of protein, the number of peptide bonds and the presence of four amino acids (cysteine, cystine, tryptophan and tyrosine) are reported to be responsible for color formation with BCA.[2] Studies with di-, tri- and tetrapeptides suggest that the extent of color formation is caused by more than the mere sum of individual color-producing functional groups.[2]

The Micro BCA Protein Assay Kit uses concentrated reagents 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 test tube or microplate assay format.

Note: For peptide sample concentration measurements, use the Thermo Scientific™ Pierce™ Quantitative Fluorometric Peptide Assay or the Pierce™ Quantitative Colorimetric Peptide Assay (see Related Thermo Scientific Products).

Preparation of Standards and Working Reagent

A. Preparation of Diluted Albumin (BSA) Standards

Use Table 1 as a guide to prepare a set of protein standards. Dilute the contents of one Albumin (BSA) Standard ampule into several clean vials, preferably using a diluent that is similar to the sample buffer. Each 1mL ampule of 2.0mg/mL Albumin Standard is sufficient to prepare a set of diluted standards such that three replicates of each dilution may be included in the Test Tube Procedure.

Table 1. Preparation of Diluted Albumin (BSA) Standards

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	4.5mL	0.5mL of Stock	200µg/mL
B	8.0mL	2.0mL of vial A dilution	40µg/mL
C	4.0mL	4.0mL of vial B dilution	20µg/mL
D	4.0mL	4.0mL of vial C dilution	10µg/mL
E	4.0mL	4.0mL of vial D dilution	5µg/mL
F	4.0mL	4.0mL of vial E dilution	2.5µg/mL
G	4.8mL	3.2mL of vial F dilution	1µg/mL
H	4.0mL	4.0mL of vial G dilution	0.5µg/mL
I	8.0mL	0	0µg/mL = Blank

B. Preparation of the Micro BCA Working Reagent (WR)

1. Use the following formula to determine the total volume of WR required:

   (# standards + # unknowns) × (# replicates) × (volume of WR per sample) = total volume WR required

   Example: for the standard Test Tube Procedure with 3 unknowns and 2 replicates of each sample:

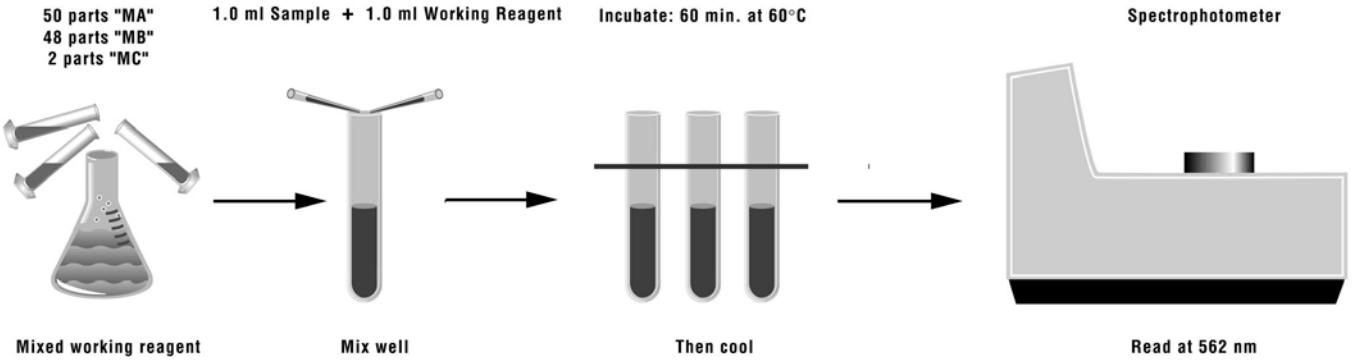
   (9 standards + 3 unknowns) × (2 replicates) × (1mL) = 24mL WR required (round up to 25mL)

   Note: 1mL of the WR is required for each sample in the Test Tube Procedure, while only 150µL of WR is required for each sample in the Microplate Procedure.

2. Prepare WR by mixing 25 parts of Micro BCA Reagent MA and 24 parts Reagent MB with 1 part of Reagent MC (25:24:1, Reagent MA:MB:MC). For the above example, combine 12.5mL of Reagent MA and 12.0mL Reagent MB with 0.5mL of Reagent MC.

   Note: When Reagent MC is initially added to Reagents MA and MB, turbidity occurs that quickly disappears upon mixing to yield a clear-green solution. Prepare sufficient volume of WR based on the number of samples to be assayed. The WR 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.

Procedure Summary (Test Tube Procedure)



Test Tube Procedure (linear working range of 0.5-20µg/mL)

1. Pipette 1.0mL of each standard and unknown sample replicate into appropriately labeled test tubes.
2. Add 1.0mL of the WR to each tube and mix well.
3. Cover tubes and incubate at 60°C in a water bath for 1 hour.
4. Cool all tubes to room temperature (RT).
5. With the spectrophotometer set to 562nm, 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.
6. Subtract the average 562nm absorbance reading of the Blank standard replicates from the 562nm reading of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 562nm reading for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

Microplate Procedure (linear working range of 2-40µg/mL)

1. Pipette 150µL of each standard or unknown sample replicate into a microplate well (Product No. 15041**)**.
2. Add 150µL of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds.
3. Cover plate using Sealing Tape for 96-Well Plates (Product No. 15036) and incubate at 37°C for 2 hours.

   Note: Limit incubations of microplate to less than or equal to 37°C, otherwise high background and aberrant color development may result. Most polystyrene assay plates deform, leach, and become cloudy at 60°C.
4. Cool plate to room temperature (RT).
5. Measure the absorbance at or near 562nm on a plate reader.
6. Subtract the average 562nm absorbance reading of the Blank standard replicates from the 562nm reading of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 562nm reading 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 software, use a best-fit polynomial equation rather than a linear equation for the standard curve. If plotting results by hand, a point-to-point fit is preferable to a linear fit to the standard points.

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 working reagent (e.g., use more Reagent MC)
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 sample
	Sample contains lipids or lipoproteins	Add 2% SDS to the sample[3]
All tubes (including blank) are dark purple	Buffer contains a reducing agent	Dialyze or dilute sample
	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	Wavelengths between 540nm and 590nm can be used, but standard curve slope and overall assay sensitivity will be decreased. See Tech Tip on website

Additional Information

A. Interfering Substances

Certain substances are known to interfere with the Micro BCA Assay including those with reducing potential, chelating agents, and strong acids or bases. Avoid the following substances as components of the sample buffer:

Ascorbic Acid	Hydrogen Peroxide	Iron	Reducing Sugars
Catecholamines	Hydrazides	Lipids	Tryptophan
Cysteine	Impure Glycerol	Phenol Red	Tyrosine
EGTA	Impure Sucrose	Reducing Agents	Uric Acid

Maximum compatible concentrations for many substances in the Test Tube Procedure are listed in Table 2 (see last page). 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.

B. Strategies for Eliminating or Minimizing the Effects of Interfering Substances

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

- Remove the interfering substance by dialysis or gel filtration (see Related Thermo Scientific Products).
- 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).[4]
- Increase the amount of copper in the WR (prepare WR using a greater proportion of Reagent MC; e.g., MA:MB:MC equal to 25:24:2 or 25:24:3), which may eliminate interference by copper chelating agents.
  Note: For greatest accuracy, the protein standards must be treated identically to the sample(s).

C. Response Characteristics for Different Proteins

Each of the commonly used total protein assay methods exhibits some degree of varying response toward different proteins (Table 3). These differences relate to amino acid sequence, pI, structure and the presence of certain side chains or prosthetic groups that can dramatically alter the protein's color response. Most protein assay methods use BSA or immunoglobulin (IgG) as the standard against which the concentration of protein in the sample is determined. Pierce Albumin Standard (BSA) (Product No. 23209) provides a consistent standard for protein estimations. Nevertheless, individual proteins, including BSA and IgG, differ slightly in their color responses in the Micro BCA Assay (Figure 1). Therefore, for maximum accuracy use a purified (known concentration) sample of the target protein as the assay standard.

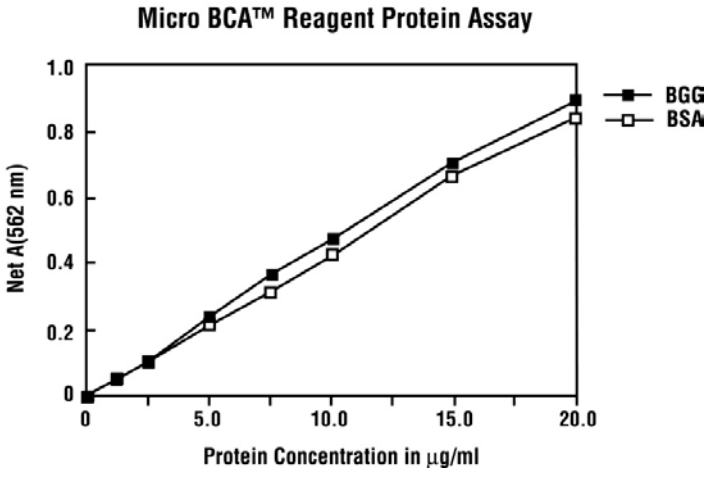


Figure 1. Typical color response curves for BSA and BGG using the Test Tube Procedure.

Table 3. Protein-to-Protein Variation. Absorbance ratios (562nm) for proteins relative to BSA using the Test Tube Procedure.

Ratio = (avg. "test" net Abs.) / (avg. BSA net Abs.)

Protein Tested	Ratio
Albumin, bovine serum	1.00
Aldolase, rabbit muscle	0.80
α-Chymotrypsinogen, bovine	0.99
Cytochrome C, horse heart	1.11
Gamma globulin, bovine	0.95
IgG, bovine	1.12
IgG, human	1.03
IgG, mouse	1.23
IgG, rabbit	1.12
IgG, sheep	1.14
Insulin, bovine pancreas	1.22
Myoglobin, horse heart	0.92
Ovalbumin	1.08
Transferrin, human	0.98
Average Ratio	**1.05**
Standard Deviation	**0.12**
Coefficient of Variation	**11.4%**

D. Alternative Total Protein Assay Reagents

If interference by a reducing substance or metal-chelating substance contained in the sample cannot be overcome, try the Thermo Scientific™ Coomassie Plus™ **(**Bradford) Protein Assay Kit (Product No. 23236), which is less sensitive to such substances.

E. Cleaning and Re-using Glassware

Care must be exercised when re-using glassware. The Micro BCA WR is sensitive to metal ions, especially copper ions. All glassware must be cleaned and then given a thorough final rinse with ultrapure water.

Related Thermo Scientific Products

15041	**Pierce™ 96-Well Plates,** 100/pkg
15075	**Reagent Reservoirs,** 50mL, 200/pkg
15036	**Sealing Tape for 96-Well Plates,** 100/pkg
23209	**Bovine Serum Albumin (BSA) Standard Ampules, 2mg/mL**, 10 × 1mL
23212	**Bovine Gamma Globulin (BGG) Standard Ampules, 2mg/mL**, 10 × 1mL
23290	**Pierce Quantitative Fluorometric Peptide Assay**
23275	**Pierce Quantitative Colorimetric Peptide Assay**
23236	**Coomassie Plus™ (Bradford) Protein Assay Kit**
89882	**Zeba™ Spin Desalting Columns, 0.5mL**
89889	**Zeba Spin Desalting Columns, 2mL**
69576	**Slide-A-Lyzer™ MINI Dialysis Units**
69576	**Slide-A-Lyzer Dialysis Cassettes**

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2. Wiechelman, *et al.* (1988). Investigation of the bicinchoninic acid protein assay: Identification of the groups responsible for color formation. *Anal Biochem* **175**:231-7.
3. Kessler, R. and Fanestil, D. (1986). Interference by lipids in the determination of protein using bicinchoninic acid. *Anal Biochem* **159:**138-42.
4. Brown, R., *et al.* (1989). Protein measurement using bicinchoninic acid: elimination of interfering substances. *Anal Biochem* **180**:136-9.

General References

Gembitsky, D.S., *et al.* (2004). A prototype antibody microarray platform to monitor changes in protein tyrosine phosphorylation. *Mol Cell Proteomics* **3:**1102-1118.

O'Nuallain, B. and Wetzel, R. (2002). Conformational Abs recognizing a generic amyloid fibril epitope. *Proc Natl Acad Sci USA* **99:**1485-90.

Paratcha, G., *et al.* (2003). The neural cell adhesion molecule NCAM is an alternative signaling receptor for GDNF family ligands. *Cell* **113:**867-79.

Yang, G., *et al*. (2005). Activation-induced deaminase cloning, localization, and protein extraction from young Vh-mutant rabbit appendix. *Proc Natl Acad Sci USA* **102:**17083-8.

Table 2. Compatible Substance Concentrations in the Micro BCA Protein Assay (see text for details).

Substance	Compatible Concentration
Salts/Buffers	
ACES, pH 7.8	10mM
Ammonium sulfate	--------
Bicine, pH 8.4	2mM
Bis-Tris, pH 6.5	0.2mM
Borate (50mM), pH 8.5 (#28384)	1:4 dilution*
B-PER® Reagent (#78248)	1:10 dilution*
Calcium chloride in TBS, pH 7.2	10mM
Na-Carbonate/Na-Bicarbonate (0.2 M), pH 9.4 (#28382)	undiluted
Cesium bicarbonate	100mM
CHES, pH 9.0	100mM
Na-Citrate (0.6 M), Na-Carbonate (0.1 M), pH 9.0 (#28388)	1:600 dilution*
Na-Citrate (0.6 M), MOPS (0.1 M), pH 7.5 (#28386)	1:600 dilution*
Cobalt chloride in TBS, pH 7.2	--------
EPPS, pH 8.0	100mM
Ferric chloride in TBS, pH 7.2	0.5mM
Glycine	n/a
Guanidine•HCl	4M
HEPES, pH 7.5	100mM
Imidazole, pH 7.0	12.5mM
MES, pH 6.1	100mM
MES (0.1M), NaCl (0.9%), pH 4.7 (#28390)	1:4 dilution*
MOPS, pH 7.2	100mM
Modified Dulbecco's PBS, pH 7.4 (#28374)	undiluted
Nickel chloride in TBS, pH 7.2	0.2mM
PBS; Phosphate (0.1M), NaCl (0.15M), pH 7.2 (#28372)	undiluted
PIPES, pH 6.8	100mM
RIPA lysis buffer; 50mM Tris, 150mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0	1:10 dilution*
Sodium acetate, pH 4.8	200mM
Sodium azide	0.2%
Sodium bicarbonate	100mM
Sodium chloride	1M
Sodium citrate, pH 4.8 (or pH 6.4)	5mM (16.7mM)
Sodium phosphate	100mM
Tricine, pH 8.0	2.5mM
Triethanolamine, pH 7.8	0.5mM
Tris	50mM
TBS; Tris (25mM), NaCl (0.15M), pH 7.6 (#28376)	1:10 dilution*
Tris (25mM), Glycine (192mM), pH 8.0 (#28380)	1:10 dilution*
Tris (25mM), Glycine (192mM), SDS (0.1%), pH 8.3 (#28378)	undiluted
Zinc chloride in TBS, pH 7.2	0.5mM

Substance	Compatible Concentration
Detergents**	
Brij™-35	5.0%
Brij-56, Brij-58	1.0%
CHAPS (CHAPSO)	1.0% (5.0%)
Deoxycholic acid	5.0%
Nonidet P-40 (NP-40)	5.0%
Octyl β-glucoside	0.1%
Octyl β-thioglucopyranoside	5.0%
SDS	5.0%
Span™ 20	1.0%
Triton™ X-100	5.0%
Triton X-114	0.05%
Triton X-305, X-405	1.0%
Tween™-20, Tween-80	5.0%
Tween-60	0.5%
Zwittergent™ 3-14	--------
Chelating agents	
EDTA	0.5mM
EGTA	--------
Sodium citrate, pH 4.8 (or pH 6.4)	5mM (16.7mM)
Reducing & Thiol-Containing Agents	
N-acetylglucosamine in PBS, pH 7.2	--------
Ascorbic acid	--------
Cysteine	--------
Dithioerythritol (DTE)	--------
Dithiothreitol (DTT)	--------
Glucose	1mM
2-Mercaptoethanol	1mM
Thimerosal	--------
Misc. Reagents & Solvents	
Acetone	1.0%
Acetonitrile	1.0%
Aprotinin	1mg/L
DMF, DMSO	1.0%
Ethanol	1.0%
Glycerol (Fresh)	1.0%
Hydrazide	--------
Hydrides (Na_2BH_4 or $NaCNBH_3$)	--------
Hydrochloric Acid	10mM
Leupeptin	10mg/L
Methanol	1.0%
Phenol Red	--------
PMSF	1mM
Sodium Hydroxide	50mM
Sucrose	4%
TLCK	0.1mg/L
TPCK	0.1mg/L
Urea	3M
o-Vanadate (sodium salt), in PBS, pH 7.2	1mM

* Diluted with ultrapure water
** Detergents were tested using high-purity Thermo Scientific™ Surfact-Amps™ Products, which have low peroxide content
--- Dashed-line entry indicates that the material is incompatible with the assay