

# Micro BCA™ Protein Assay Kit

23235

0412.4

Number	Description
23235	Micro BCA Protein Assay Kit, sufficient reagents for 480 tube assays or 3,200 microplate assays

**Kit Contents:****Micro BCA Reagent A (MA)**, 240 ml**Micro BCA Reagent B (MB)**, 240 ml**Micro BCA Reagent C (MC)**, 12 ml**Albumin Standard Ampules, 2 mg/ml**, 10 × 1 ml ampules containing bovine serum albumin (BSA) at 2.0 mg/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**

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**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 Pierce BCA Protein Assay Kit (Product No. 23225), the Micro BCA Kit has been optimized for use with dilute protein samples (0.5-20 µg/ml). The unique, patented method utilizes bicinchoninic acid (BCA) as the detection reagent for Cu<sup>+1</sup>, which is formed when Cu<sup>+2</sup> is reduced by protein in an alkaline environment.<sup>1</sup> A purple-colored reaction product is formed by the chelation of two molecules of BCA with one cuprous ion (Cu<sup>+1</sup>). This water-soluble complex exhibits a strong absorbance at 562 nm 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.<sup>2</sup> 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.<sup>2</sup>

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.

## 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 1 ml ampule of 2.0 mg/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

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

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

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

$$(\# \text{ standards} + \# \text{ unknowns}) \times (\# \text{ replicates}) \times (\text{volume of WR per sample}) = \text{total volume WR required}$$

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

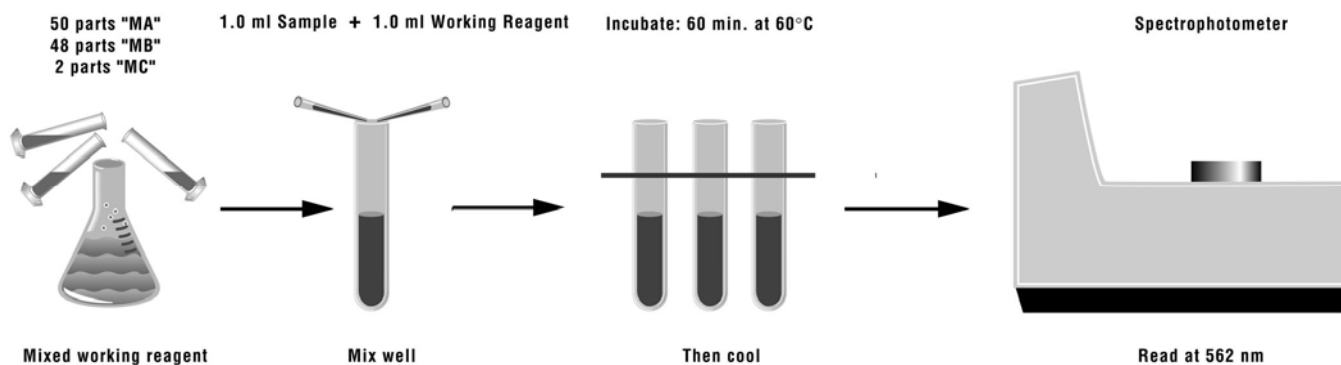
$$(9 \text{ standards} + 3 \text{ unknowns}) \times (2 \text{ replicates}) \times (1 \text{ ml}) = 24 \text{ ml WR required (round up to 25 ml)}$$

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

- 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 12.5 ml of Reagent MA and 12.0 ml Reagent MB with 0.5 ml 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 0.5-20 µg/ml)

1. Pipette 1.0 ml of each standard and unknown sample replicate into appropriately labeled test tubes.
2. Add 1.0 ml 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 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.

6. 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.
7. 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.

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

1. Pipette 150 µl of each standard or unknown sample replicate into a microplate well (Product No. 15041).
2. Add 150 µl 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 562 nm on a plate reader.
6. 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.
7. 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.

**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 562 nm
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 <sup>3</sup>
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 562 nm filter	Wavelengths between 540 nm and 590 nm can be used, but standard curve slope and overall assay sensitivity will be decreased. See Tech Tip on web site

## 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 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

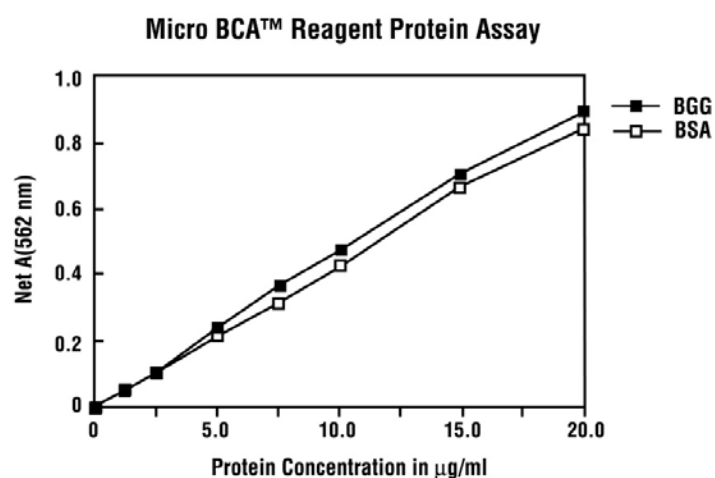
The effects of interfering substances in the Micro BCA Protein Assay may be eliminated or overcome by several methods.

- Remove the interfering substance by dialysis or gel filtration (see Related Pierce 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).<sup>4</sup>
- 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 (562 nm) 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
<b>Average Ratio</b>	<b>1.05</b>
<b>Standard Deviation</b>	<b>0.12</b>
<b>Coefficient of Variation</b>	<b>11.4%</b>

#### 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 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, 200/pkg.
15036	Sealing Tape for 96-Well Plates, 100/pkg.
23209	Albumin Standard Ampules, 2 mg/ml, 10 × 1 ml ampules, containing bovine serum albumin (BSA)
23212	Bovine Gamma Globulin Standard, 2 mg/ml, 10 × 1 ml ampules, containing bovine gamma globulin
23236	Coomassie Plus (Bradford) Protein Assay Kit, working range 1-1,500 µg/ml
89882	Zeba™ Desalt Spin Columns, 0.5 ml, for desalting 20-130 µl samples
89889	Zeba Desalt Spin Columns, 2 ml, for desalting 200-700 µl samples
69576	Slide-A-Lyzer® MINI Dialysis Units, 10 units and float for dialyzing 10-100 µl samples
69576	Slide-A-Lyzer Dialysis Cassettes, 10 units and floats for 100-500 µl samples

#### Cited References

1. Smith, P.K., *et al.* (1985). Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**:76-85.
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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.

#### Product References

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.

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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.

Slide-A-Lyzer® Dialysis Cassette Technology is protected by U.S. Patent # 5,503,741 and 7,056,440.

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

Substance	Compatible Concentration	Substance	Compatible Concentration
<b>Salts/Buffers</b>		<b>Detergents**</b>	
ACES, pH 7.8	10 mM	Brij <sup>®</sup> -35	5.0%
Ammonium sulfate	-----	Brij-56, Brij-58	1.0%
Bicine, pH 8.4	2 mM	CHAPS (CHAPSO)	1.0% (5.0%)
Bis-Tris, pH 6.5	0.2 mM	Deoxycholic acid	5.0%
Borate (50 mM), pH 8.5 (#28384)	1:4 dilution*	Nonidet P-40 (NP-40)	5.0%
B-PER <sup>®</sup> Reagent (#78248)	1:10 dilution*	Octyl β-glucoside	0.1%
Calcium chloride in TBS, pH 7.2	10 mM	Octyl β-thioglucopyranoside	5.0%
Na-Carbonate/Na-Bicarbonate (0.2 M), pH 9.4 (#28382)	undiluted	SDS	5.0%
Cesium bicarbonate	100 mM	Span <sup>®</sup> 20	1.0%
CHES, pH 9.0	100 mM	Triton <sup>®</sup> X-100	5.0%
Na-Citrate (0.6 M), Na-Carbonate (0.1 M), pH 9.0 (#28388)	1:600 dilution*	Triton X-114	0.05%
Na-Citrate (0.6 M), MOPS (0.1 M), pH 7.5 (#28386)	1:600 dilution*	Triton X-305, X-405	1.0%
Cobalt chloride in TBS, pH 7.2	-----	Tween <sup>®</sup> -20, Tween-80	5.0%
EPPS, pH 8.0	100 mM	Tween-60	0.5%
Ferric chloride in TBS, pH 7.2	0.5 mM	Zwittergent <sup>®</sup> 3-14	-----
Glycine	n/a	<b>Chelating agents</b>	
Guanidine•HCl	4 M	EDTA	0.5 mM
HEPES, pH 7.5	100 mM	EGTA	-----
Imidazole, pH 7.0	12.5 mM	Sodium citrate, pH 4.8 (or pH 6.4)	5 mM (16.7 mM)
MES, pH 6.1	100 mM	<b>Reducing &amp; Thiol-Containing Agents</b>	
MES (0.1 M), NaCl (0.9%), pH 4.7 (#28390)	1:4 dilution*	<i>N</i> -acetylglucosamine in PBS, pH 7.2	-----
MOPS, pH 7.2	100 mM	Ascorbic acid	-----
Modified Dulbecco's PBS, pH 7.4 (#28374)	undiluted	Cysteine	-----
Nickel chloride in TBS, pH 7.2	0.2 mM	Dithioerythritol (DTE)	-----
PBS; Phosphate (0.1 M), NaCl (0.15 M), pH 7.2 (#28372)	undiluted	Dithiothreitol (DTT)	-----
PIPES, pH 6.8	100 mM	Glucose	1 mM
RIPA lysis buffer; 50 mM Tris, 150 mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0	1:10 dilution*	2-Mercaptoethanol	1 mM
Sodium acetate, pH 4.8	200 mM	Thimerosal	-----
Sodium azide	0.2%	<b>Misc. Reagents &amp; Solvents</b>	
Sodium bicarbonate	100 mM	Acetone	1.0%
Sodium chloride	1 M	Acetonitrile	1.0%
Sodium citrate, pH 4.8 (or pH 6.4)	5 mM (16.7 mM)	Aprotinin	1 mg/L
Sodium phosphate	100 mM	DMF, DMSO	1.0%
Tricine, pH 8.0	2.5 mM	Ethanol	1.0%
Triethanolamine, pH 7.8	0.5 mM	Glycerol (Fresh)	1.0%
Tris	50 mM	Hydrazide	-----
TBS; Tris (25 mM), NaCl (0.15 M), pH 7.6 (#28376)	1:10 dilution*	Hydrides (Na <sub>2</sub> BH <sub>4</sub> or NaCNBH <sub>3</sub> )	-----
Tris (25 mM), Glycine (192 mM), pH 8.0 (#28380)	1:10 dilution*	Hydrochloric Acid	10 mM
Tris (25 mM), Glycine (192 mM), SDS (0.1%), pH 8.3 (#28378)	undiluted	Leupeptin	10 mg/L
Zinc chloride in TBS, pH 7.2	0.5 mM	Methanol	1.0%
		Phenol Red	-----
		PMSF	1 mM
		Sodium Hydroxide	50 mM
		Sucrose	4%
		TLCK	0.1 mg/L
		TPCK	0.1 mg/L
		Urea	3 M
		<i>o</i> -Vanadate (sodium salt), in PBS, pH 7.2	1 mM

\* Diluted with ultrapure water

\*\* Detergents were tested using Pierce high-purity Surfact-Amps<sup>®</sup> Products, which have low peroxide content

--- Dashed-line entry indicates that the material is incompatible with the assay