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# Micro Bicinchoninic Acid (BCA) Protein Assay

## A BCA Protein Assay for Dilute Protein Samples

## (Cat. # 786-572, 786-895)

## INTRODUCTION

The Micro Bicinchoninic Acid (BCA) Protein Assay is a highly sensitive colorimetric assay that is compatible with detergent solubilized protein solutions and is a modification of the Bicinchoninic Acid (BCA) Protein Assay for dilute protein samples (0.5-20µg/ml). The Micro Bicinchoninic Acid (BCA) Protein Assay primarily relies on two reactions. Firstly, the peptide bonds in the protein sample reduce $Cu^{2+}$ ions, in a temperature dependent reaction, from the copper solution to $Cu^{+}$. The amount of $Cu^{2+}$ reduced is proportional to the amount of protein present in the solution. Next, two molecules of bicinchoninic acid (BCA) chelate with each $Cu^{+}$ ion, forming a purple-colored product that strongly absorbs light at a wavelength of 562 nm. The Micro BCA Assay uses concentrated solutions and extended incubation times for the detection of dilute protein samples. The amount of protein present in a solution can be quantified by measuring the absorption spectra and comparing with protein solutions with known concentrations.

The Micro Bicinchoninic Acid (BCA) Protein Assay is suitable for quantifying protein solutions in 1ml assays or in micro-wells and is for 500 x 1ml assays or >3,300 x Micro-well assays.

## ITEM(S) SUPPLIED

| **Description** | **Cat. # 786-572** | **Cat. # 786-895** |
|---|---|---|
| Assay Buffer | 250ml | 250ml |
| Micro BCA Solution | 240ml | 240ml |
| Copper Solution | 10ml | 10ml |
| Bovine Serum Albumin Standard [2mg/ml] | 5ml | - |
| Non-Animal Protein Standard [2mg/ml] | - | 5ml |

## STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the Protein Standard at 4°C. The remaining kit components should be stored at room temperature. When stored properly, the kit is stable for 1 year.

## BICINCHONINIC ACID (BCA) PROTEIN ASSAY TOLERANCE GUIDE

| | |
|---|---|
| 2-Mercaptoethanol, 0.01% | Imidazole, 12mM |
| Ammonium sulfate, *Not Compatible* | Iron, *Not Compatible* |
| Ascorbic acid, *Not Compatible* | Lipids, *Not Compatible* |
| Brij® 35, 5% | N-Octyl Glucosidase, 0.1% |
| Catecholamines, *Not Compatible* | Phenol red, *Not Compatible* |
| CHAPS, 1% | Phosphate buffer, 0.1M |
| CHAPSO, 5% | SDS, 5% |
| Creatinine, *Not Compatible* | Sodium azide, 0.2% |
| Cysteine, *Not Compatible* | Sodium Chloride, 1M |
| Deoxycholic acid, 5% | Sucrose, 4% |
| DTT, *Not Compatible* | Tris.HCl , 0.05M |
| EDTA, 0.5mM | Triton® X-100, 5% |
| EGTA, *Not Compatible* | Triton® X-114, 0.05% |
| Glycerol, 10% | Tryptophan, *Not Compatible* |
| Guanidine.HCl, 4M | Tyrosine, *Not Compatible* |
| HEPES, 0.1M | Tween® 20, 5% |
| Hydrogen peroxide, *Not Compatible* | Urea, 3M |
| Hydrazides, *Not Compatible* | Uric acid, *Not Compatible* |

## PREPARATION BEFORE USE

***Note:*** *The solutions may precipitate in cold weather or after long term storage, simply warm and stir to re-dissolve.*

1. **Dilute Standard Preparation:** Label 9 tubes with A-I and prepare the standards as indicated below. The diluent used should be the same as used for the protein samples. The following dilutions are suitable for triplicate Standard 1ml assays.

| Tube | Bovine Serum Albumin or Non-Animal Protein Standard | Diluent (ml) | Final Concentration (µg/ml) |
|---|---|---|---|
| A | 300µl from Stock | 5.7 | 100 |
| B | 3ml from Tube A | 3 | 50 |
| C | 3ml from Tube B | 3 | 25 |
| D | 3ml from Tube C | 3 | 12.5 |
| E | 3ml from Tube D | 3 | 6.25 |
| F | 3ml from Tube E | 3 | 3.125 |
| G | 3ml from Tube F | 3 | 1.563 |
| H | 3ml from Tube G | 3 | 0.781 |
| I | - | 3 | 0 (Blank) |

2. **Preparation for Working Solution:**
   a. To determine the amount of working solution required, use the following formula. The standard assays require 1ml and the micro-well assays require 200µl working solution:
   (Total number of samples (standards and test samples) x (Number of replicates) x (Volume of WS/ sample)
   b. Combine 25 parts Assay Buffer and 24 parts Micro BCA solution with 1 part Copper solution, for example, for 10ml working solution combine 5ml Assay Buffer, 4.8ml BCA solution with 0.2ml Copper Solution. The mixed Working Solution should be a clear, green solution.

## STANDARD PROTOCOL (0.5-20µg/ml)

1. Pipette 1ml of each standard and protein samples into an appropriately labeled tube.
2. Add 1ml Working Solution to each tube, seal and vortex to mix.
3. Incubate the assays at 60°C for 60 minutes. We recommend a water bath for even heat transfer.
4. Cool the tubes to room temperature and transfer 1ml sample to a cuvette.
5. Set a spectrophotometer to 562nm and blank with water. Read all the samples.
6. Subtract the average absorbance of the Blank standard from the samples and then prepare a standard curve to determine protein concentrations.

## MICRO-WELL PROTOCOL

1. Pipette 150µl of each standard and protein samples into a microplate well.
2. Add 150µl Working Solution to each tube, seal and mix on a plate shaker for 20-30 seconds.
3. Cover the plate and incubate the assays at 37°C for 120 minutes.
4. Cool the plate to room temperature.
5. Measure the absorbance at 562nm, or between 540-590nm.
6. Subtract the average absorbance of the Blank standard from the samples and then prepare a standard curve to determine protein concentrations. We recommend using a best-fit polynomial equation if using graphing software. If not, a point-to-point fit is preferable to a linear fit of the standards.

## TROUBLESHOOTING

| PROBLEM | POSSIBLE REASON | SOLUTION |
|---|---|---|
| No color visualized in tube | A metal (copper) chelator is present | Dialyze the sample (Use Tube-O-DIALYZER™)<br>Prepare Working Solution at a 50:2 ratio of BCA Solution to Copper Solution<br>Use a protein assay that is unaffected by interfering agents (NI™ Protein Assay (Cat # 786-005) or CB-X™ Protein Assay (Cat. # 786-12X) |
| All tubes turn a very dark purple | A reducing agent is present | Dialyze the sample (Use Tube-O-DIALYZER™)<br>Use a protein assay that is unaffected by interfering agents (NI™ Protein Assay (Cat # 786-005) or CB-X™ Protein Assay (Cat. # 786-12X) |
| | Thiol containing agents are present | |
| | Catecholamines are present | |
| Low or Limited Color development compared to blank | Sample has a acid or alkaline buffer that interferes with assay | Dialyze the sample (Use Tube-O-DIALYZER™)<br>Use a protein assay that is unaffected by interfering agents (NI™ Protein Assay (Cat# 786-005) or CB-X™ Protein Assay (Cat. # 786-12X) |
| | Incorrect wavelength | Ensure wavelength is 562nm, or between 540-590nm |
| Assayed samples appear darker compared to standards | Protein concentration too high | Dilute samples |
| | Lipids or lipoproteins are present | Use a protein assay that is unaffected by interfering agents (NI™ Protein Assay (Cat# 786-005) or CB-X™ Protein Assay (Cat. # 786-12X) |

## RELATED PRODUCTS

Download our Protein Assay Handbook or Bioassays Handbooks
http://info.gbiosciences.com/complete-protein-assay-guide
http://info.gbiosciences.com/complete-bioassay-handbook
*For other related products, visit our web site at www.GBiosciences.com or contact us.*

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