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A Geno Technology, Inc. (USA) brand name

Bicinchoninic Acid (BCA) Protein Assay

(Cat. # 786-570, 786-571, 786-890, 786-891)

INTRODUCTION

The Bicinchoninic Acid (BCA) Protein Assay is a highly sensitive colorimetric assay that is compatible with detergent solubilized protein solutions. The 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 that is linear for increasing protein concentrations between the range of 0.02-2mg/ml. 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 Bicinchoninic Acid (BCA) Protein Assay is suitable for quantifying protein solutions in 1ml assays or in micro-wells. Cat. # 786-570 is for 500 x 1ml assays or 2,500 x Micro-well assays; Cat. # 786-571 is for 1,000 x 1ml assays or 5,000 x Micro-well assays.

ITEM(S) SUPPLIED

Description	**Cat. # 786-570**	**Cat. # 786-571**	**Cat. # 786-890**	**Cat. # 786-891**
BCA Solution, 500ml	1	2	1	2
Copper Solution, 10ml	1	2	1	2
Bovine Serum Albumin Standard [2mg/ml], 5ml	2	2	-	-
Non-Animal Protein Standard [2mg/ml], 5ml	-	-	2	2

STORAGE CONDITIONS

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

TOLERANCE GUIDE

- 2-Mercaptoethanol, 0.01%
- Ammonium sulfate, 1.5M
- Ascorbic acid, *Not Compatible*
- Brij® 35, 5%
- Catecholamines, *Not Compatible*
- CHAPS, 5%
- CHAPSO, 5%
- Creatinine, *Not Compatible*
- Cysteine, *Not Compatible*
- Deoxycholic acid, 5%
- DTT, 1mM
- EDTA, 10mM
- EGTA, *Not Compatible*
- Glycerol, 10%
- Guanidine.HCl, 4M
- HEPES, 0.1M
- Hydrogen peroxide, *Not Compatible*
- hydrazides, *Not Compatible*
- Imidazole, 0.05M
- Iron, *Not Compatible*
- Lipids, *Not Compatible*
- N-Octyl Glucosidase, 5%
- Phenol red, *Not Compatible*
- Phosphate buffer, 0.1M
- SDS, 5%
- Sodium azide, 0.2%
- Sodium Chloride, 1M
- Sucrose, 40%
- Tris.HCl , 0.25M
- Triton® X-100, 5%
- Triton® X-114, 1%
- Tryptophan, *Not Compatible*
- Tyrosine, *Not Compatible*
- Tween® 20, 5%
- Urea, 3M
- Uric acid, *Not Compatible*
- Zwittergent® 3-12, 1.0%

PREPARATION BEFORE USE

NOTE: *The BCA and Copper Solutions may precipitate in cold weather or after long term storage, simply warm and stir to re-dissolve.*

Standard Preparation for Standard Assay

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 (µl)	Final Concentration (µg/ml)
A	300µl from Stock	0	2,000
B	150µl from Tube A	150	1,000
C	150µl from Tube B	150	500
D	150µl from Tube C	150	250
E	150µl from Tube D	150	125
F	150µl from Tube E	150	62.5
G	150µl from Tube F	150	31.25
H	150µl from Tube G	150	15.625
I	-	150	0 (Blank)

Standard Preparation for Enhanced Assay:

Label 7 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 assays.

Tube	Bovine Serum Albumin or Non Animal Protein Standard	Diluent (µl)	Final Concentration (µg/ml)
A	100µl from Stock	700µl	250
B	400µl from Tube A	400	125
C	400µl from Tube B	400	62.5
D	400µl from Tube C	400	31.25
E	400µl from Tube D	400	15.625
F	400µl from Tube E	400	7.825
G	-	400	0 (Blank)

Preparation for Working Solution:

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:

NOTE: *For the test-tube methods, use 2ml working solution and 0.1ml sample. This will allow for 250 test tube assays per BCA Solution bottle*

(Total number of samples (standards and test samples) x (Number of replicates) x (Volume of WS/ sample)

Combine 50 parts BCA solution with 1 part Copper solution, for example, for 10ml working solution combine 10ml BCA solution with 0.2ml Copper Solution. The mixed Working Solution should be a clear, green solution.

STANDARD PROTOCOL

1. Pipette 50µl 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 37°C for 30 minutes or room temperature for 2 hours. For the enhanced protocol, incubate at 60°C for 60 minutes. We recommend a waterbath 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 25µl of each standard and protein samples into a microplate well.
2. Add 200µl Working Solution to each tube, seal and vortex to mix.
3. Cover the plate and incubate the assays at 37°C for 30 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.

TROUBLESHOOTING:

Problem	POSSIBLE Reason	Solution
No color visualized in tube	A metal (copper) chelator is present	Dialyze the sample (Use Tube-O-DIALYZER™) Prepare Working Solution at a 50:2 ratio of BCA Solution to Copper Solution 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™) 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™) 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 Assays and Bioassays Handbooks.
http://info.gbiosciences.com/complete-protein-assay-guide
http://info.gbiosciences.com/complete-bioassay-handbook/
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