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A Geno Technology, Inc. (USA) brand name

Bicinchoninic Acid (BCA) Reducing Agent Compatible Protein Assay

INTRODUCTION

The Bicinchoninic Acid (BCA) Protein Assay is a highly sensitive colorimetric assay that is compatible with detergent solubilized protein solutions, however is dramatically affected by the presence of reducing agents, including DTT, ß-mercaptoethanol and TCEP. 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 Bicinchoninic Acid (BCA) Reducing Agent Compatible Protein Assay is supplied with the Reducing Agent Compatibility Agent (RACA) that modifies reducing agents to limit their effect on the reduction of the assay's copper ions, preventing inhibition of the assay. The use of RACA allows for samples containing up to 5mM DTT, 10mM TCEP or 35mM ß-mercaptoethanol.

The Bicinchoninic Acid (BCA) Reducing Agent Compatible Protein Assay is suitable for quantifying protein solutions in 1ml assays or in micro-wells. This kit is suitable for is for 250 x 1ml assays.

ITEM(S) SUPPLIED	**786-573**
BCA Solution	250ml
Copper Solution	10ml
Reducing Agent Compatibility Agent (RACA)	16 x 15mg
RACA Reconstitution Buffer	15ml
Bovine Serum Albumin Standard (2mg/ml)	2 x 5ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the Bovine Serum Albumin Standard at 4°C and the Reducing Agent Compatibility Agent (RACA) at -20°C. The remaining kit components should be stored at room temperature. When stored properly, the kit is stable for 1 year.

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.*

Ideally, the protein standards used in the assay should contain the same reducing agents, and other agents, as the samples being quantified, however this is labor intensive and typically yields <5% errors, compared to normal standards without reducing agents. We recommend creating a single blank that has the same amount of reducing agents as the proteins to be assayed.

A. **Standard Preparation for Standard Assay:** Label 8 clean tubes with A-H 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	Diluent (µl)	Final Concentration (µg/ml)
A	100µl from Stock	0	2,000
B	150µl from Stock	50	1,500
C	100µl from Stock	100	1,000
D	100µl from Tube B	100	750
E	100µl from Tube C	100	500
F	100µl from Tube E	100	250
G	150µl from Tube F	100	125
H	-	100	0

B. **Prepare Reducing Agent Blank (RA Blank):** Prepare 200µl of the same buffer as your samples with the same concentration and type of reducing agent.

C. **Prepare Working RACA Reconstitution Buffer:** Dilute the RACA Reconstitution Buffer 1:1 with deionized water. Use undiluted RACA Reconstitution Buffer for samples with a pH<5.

D. **Prepare Working Reducing Agent Compatibility Agent (RACA):** Add 150µl diluted RACA Reconstitution Buffer to a vial of RACE and pipette up and down to completely dissolve. Add 100µl RACA solution to 400µl RACA Reconstitution Buffer to dilute 1:5. Use immediately.

E. **Preparation for Working Solution:** To determine the amount of working solution required, use the following formula. The standard assays require 1ml:
(Total number of samples (blanks, 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 25µl of each standard and protein samples into an appropriately labeled tube.

2. Add 25µl Working RACA Solution and vortex to mix. Incubate in a 37°C waterbath for 15 minutes.

3. Add 1ml Working Solution to each tube, seal and vortex to mix.

4. Incubate the assays at 37°C for 30 minutes or room temperature for 2 hours. We recommend a waterbath for even heat transfer.

5. Cool the tubes to room temperature and transfer 1ml sample to a cuvette.

6. Set a spectrophotometer to 562nm and blank with water. Read all the samples.

7. Subtract the average absorbance of Tube H standard from the other standards and subtract the average absorbance of the RA Blank from the samples and then prepare a standard curve to determine protein concentrations.

Bicinchoninic Acid (BCA) Protein Assay TOLERANCE GUIDE

- 2-Mercaptoethanol, 35mM
- 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, 5mM
- 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%
- TCEP, 10mM
- 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%

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	Reducing agent higher than upper limit	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

1. ***Tube-O-DIALYZER™ (Cat. # 786-610 to 786-624):*** *Tube-format dialysis devices suitable for the rapid dialysis of small sample volumes (20-250µl and 200µl-2.5ml). Supplied with molecular weight cut offs (MWCO) of 1, 4, 8, 15 and 50kDa.*
2. ***NI™ (Non-Interfering™) Protein Assay (Cat. # 786-005):*** *A highly sensitive, colorimetric protein assay that overcomes interference by common agents, including detergents and reducing agents*
3. ***CB-X™ Protein Assay (Cat. # 786-12X):*** *A simple, fast, sensitive single-tube protein assay that is supplied with optional reagents to remove interfering agents for improved assay reproducibility.*
4. ***Protein Standards****: We supply Bovine Gamma Globulin (2mg/ml) [Cat. #786-007] and Bovine Serum Albumin (2mg/ml) [Cat #786-006] as protein standards.*

NOTE: *For other related products, visit our web site at www.GBiosciences.com or contact us.*

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