Bicinchoninic Acid (BCA) Protein Assay Kit

P8100

Storage

Store at Room Temperature.

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

Contents

- Product Manual
- Bicinchoninic Acid Reagent A, 450ml,
  contains sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartarte in 0.1M sodium hydroxide
- Bichnchoninic Acid Reagent B, 12ml,
  contains 4% cupric sulfate
- Albumin Standard, 2mg/ml, 5X1ml,
  Contains bovine serum albumin (BSA) at 2.0 mg/ml in 0.9% saline and 0.05% sodium azide

Shipping Condition

Ship at ambient.

Introduction

The Bichinchoninic Acid (BCA) Protein Assay is a detergent-compatible formu -lation based on bicinchoninic acid (BCA) for the colorimeteric detection and quantitiation of total protein. This mothod combines the well-known reduction of Cu+2 to Cu-1 by protein in an alkaline medium (the biuret reaction) with the higly sensitive and selective colormetric detection of the cuprous cation (Cu+1) using a unique reagent containing bicinchoninic acid. The purple-colored reac -tion product of this assay is formed by the chelation of two molecules of bichi -nchoninic acid with one cuprous ion. This water-soluble complex exhibits a strong absorbance at 562 that is nearly linear with incresing protein concentration over a broad working range (20-2,000 ug/ml). The Bicinchoninic Acid method is not a true end-point method; that is, the final color continues to develop. However, following incubation, the rate of continued color development is su -fficiently slow to allow large numbers of samples to be assayed together.

The macromolecular structure of protein, the number of peptide bonds and the presence of gour particular amino acids (cysteine, cystine, tryptophan and tyrosine) are reported to be responsible for color formation caused by more than the mere sum of individual color-producing functional groups. Accordingly, protein concentrations generally are determined and reported with reference to standards of a common protein such as bovine serum albumin (BSA). A series of dilution of known concentration are basd on the standard curve. If precise quantitation of an unknown protein is required, it is advisable to select a protein standard that is similar in quality to the unknown; for example, a bovine gamma globulin (BGG) standard may be used when assaying immunoglobulin samples.

Two assay procedures are presented. Of these, the Test Tube Procedure requires a larger volume (0.1 ml) of protein sample; however, because it uses a sample to working reagent ratio of 1:20 (v/v), the effect of interfering substances is minimized. The Microplate Procedure affords the sample handling ease of a microplate and requires a smaller volume (10-25ul) of protein sample; however, because the sample to working reagent ratio is 1:8 (v/v), it offers less flexibility in overcoming interfering substance concentrations and obtaining low levels of detection.

Procedure

Preparation of Standards and working Reagent
(required for both assay procedures)

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 Standard (BSA) into several clean vials, prepderably using the same dilutent as the sample(s). Each 1ml ampule of 2.0 mg/ml Albumin Standard is sufficient to prepare a set of diluted standards for either working range su -ggested in Table 1. There will be sufficient volume for three replications of each dilted standards.

Table 1: Preparation of Diluted Albumin (BSA) Standards

Dilution Scheme for Standard Test tube Protocol and Microplate Procedure (Working Range = 20-2,000 ug/ml)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	0	300 ul of Stock	2,000 ug/ml125 ul
B	125 ul	375 ul of Stock	1,500 ug/ml125 ul
C	325 ul	325 ul of Stock	1,000 ug/ml125 ul
D	175 ul	175 ul of vial B dilution	750 ug/ml125 ul
E	325 ul	325 ul of vial C dilution	500 ug/ml125 ul
F	325 ul	325 ul of vial E dilution	250 ug/ml125 ul
G	325 ul	325 ul of vial F dilution	125ug/ml125 ul
H	400 ul	100 ul of vial F dilution	25 ug/ml125 ul
I	400 ul	0	0 ug/ml = Blank

Dilution Scheme for Enhanced Test Tube Protocol
(Working Range = 5-250 ug/ml)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	700 ul	100 ul of Stock	250ug/ml
B	400 ul	400 ul of vial A dilution	125ug/ml
C	450 ul	300 ul of vial B dilution	50ug/ml
D	400 ul	400 ul of vial C dilution	25ug/ml
E	400 ul	100 ul of vial D dilution	5ug/ml
F	400 ul	0	0 ug/ml = Blank

B. Preparation of the Bichinchoninic Acid (BCA) Working Solution (WR)

1. Use the following formula to determine the total volume of WR required: (#standards+#unknowns) x (#replicates) x (volume of WR per sample) = total volume WR required.

Example: for the Standard Test Tube Protocol with 3 unknowns and 2 replicates of each sample:

(9 standards + 3 unknowns) x (2 replicates) x (2ml) = 48 ml WR required

GenDEPOT PO Box 454 Barker, Tx 77413 tech@gendepot.com

Note: 2.0ml of the WR is required for each sample in the Test Tube Procedure, while only 200ul of WR reagent is required for each sample in the Microplate Procedure.

2. Prepare WR by mixing 50 parts of Bicinchoninin Acid (BCA) Reagent A with 1 part of Bichinchoninin Acid (BCA) Reagnet B.

Note: When Reagent B is first added to Reagent A, a turbidigy is observed that quickly disappears upon mixing to yield a clear, green WR. Prepare sufficient vol -ume of WR based on the number of samples to be assayed. The WR is stable for several days when stored in a closed container at room temperature (RT).

Testing Tube Procedure (Sample to WR ratio = 1:20)

1. Pipette 0.1 ml of each standard and unknown sample replicate into an appro -priately labeled test tube.
2. Add 2.0 ml of the WR to each tube and mix well.
3. Cover and incubate tubes at selected termperature and time:
 * Standard Protocol: 37°C for 30 minutes (working range = 20-2,000 ug/ml)
 * RT Protocol: RT for 2 hours (working range = 20-2,000 ug/ml)
 * Enhanced Protocol: 60°C for 30 minutes (working range = 5-250 ug/ml)

Note:* Increasing the incubation time or temperature increase the net 562 nm absorbance for each test and decreases both the minimum detection level of the reagent and the working range of protocol.

* Use a water bath to heat tubes for either Standard (37°C incubation) or Enhan -ced (60°C incubation) Protocol. Using a forced-air incubation can introduce sig -nificant error in color development because of uneven heat transfer.

4. Cool all tubes to 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: Because the Bichinchoninin Acid (BCA) Assay does not reach a true end point, color development will continue even after cooling to RT. However, because the rate of color development is low at RT, no significant error will be introduced if the 562 nm absorbance measurement of all tubes are made within 10 minutes of each other.

6. Subtract the average 562nm absorbance measurements of the Blank standard replicates from the 562 nm absorbance measurement of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 562 nm measurement for each BSA standard vs. its concentration in ug/ml. Use the standard curve to determine the protein concentration of each unknown sample.

Microplate Procedure (Sample to WR ratio=1:8)

1. Pipette 25ul of each standard or unkown sample replicate into a microplate well (working range = 20-2,000 un/ml).

Note: If sample size is limited, 10ul of each unknown sample and standard can be used (sample to WR ratio = 1:20). However, the working range of the assay in this case will be limited to 125-2,000 ug/ml.

2. Add 200 ul of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds.
3. Cover plate and incubate at 37oC for 30 minutes.
4. Cool plate to RT.
5. Measure the absorbance at or near 562 nm on a plate reader.

Note:

* Wavelengths from 540-590 m, have been used sucessfully with this method.
* Because plate readers use a shorter light path length than cuvette spectropho -tometers, the Microplate Procedure requires a greater sample to WR ratio to obtain the same sensitivity as the standard Test Tube Procedure. If higher 562 nm measurements are desired, increase the incubation time to 2 hours.
* Increasing the incubation time or ratio of sample volume to WR increase the net 562 nm measurement for wach well and lowers both the minumum detection level of the reagent and the working range of the assays. As long as all standards and unknowns are treated identically, such modifications may be useful.

6. Subtract the average 562 nm absorbance measurement of the Blank standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank - corrected 562 nm measurement for each BSA standard vs. its concentration in ug/ml. Use the standard curve to determine the protein concentration of each unknown sample.

Note: If using curve-filtering algorithms associatd with a microplate reader, a four-parameter (quadratic) or best-fit curve will provide more accurate results than a purely linear fit. It plotting results by hand, a point-to-point curve is pre -ferable to a linear fit to the standard points.

Troubleshooting

Problem	Possible Caus	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 50:2, Reagent A:B)
Blank absorbance is OK, but standards and samples show color than expected	Strong acid or alkine buffer, alters working reagent pH	Dialyze, desalt, or dilute sample
	Color measured at the wrong wavelenth	Measure the absornace at 562nm
Color of samples appears darker than expected	Protein concentraiton is too high	Dilute sample
	Sample contains lipids or lipoproteins	Add 2% SDS to the sample to elimicate interference from lipids.
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	Color may be measure at any wavelenth bewteen 650nm and 590nm, although the slope of standard curve and overall assay sensitivity will be reduced

GenDEPOT PO Box 454 Barker, Tx 77413 tech@gendepot.com

A. Interfering Substances

Certain substances are knwon to interfere with the Bichinchoninic Acid (BCA) Assay including those with erducing potential, chelating agents, and strong acids or bases. Because they are knwon to interfere with protein estimating at even minute concentration, avoid the following substances as components of the sample buffer:

Ascorbic Acid	EGTA	Iron	Impure Sucrose
Catecholamines	Impure Glycerol	Lipids	Tryptophan
Creatinine	Hyrogen Peroxide	Melibiose	Tyrosine
Cysteine	Hydrazides	Phenol Red	Uric Acid

Other substances interfere to a lesser extent with protein estimation using the BCA assay, and these have only minor (tolerable) effects below a certain concen -tration in the original sample. Maximum conpatible concentration for many substances in the Standard Test Tube Protocol Test Tube Protocol if the error in protein concentration estimation caused by the presence of the substance was less or equal to 10%. The substances were tested using WR prepared immediately before each expermient. Blank-corrected 562nm absorbance measurements (for a 1000ug/mL BSA standard + substance) were compared to the net 562nm measurements of the same standard prepared in 0.9% sailne. Maximum compa -tible concentrations will be lower in the Microplate Procedure where the sample to WR ratio is 1:8 (v/v).

Furthermore, it si possible to have a substance additive affect such that even though a single component is present at a concentration below its compatibility, a sample buffer containing a combination of substances could interfere with the assay.

B. Strategies for eliminating or minimizing the effects of interfering substances

The effects of interfering substances in the Bicinchoninic Acid (BSA) protein Assay may be eliminated or overcome by one of several methods.

* Remove the interfering substance by dialysis or get filteration.

* Dulute the sample until the substance no longer interferes. This strategy is effective only if the starting protein.

* Precipitate the proteins in the sample with acetone or trichloroacetic acid (TCA). The liquid containing the substance that interdered is discared and the protein pellet is easily solubilized in ultrapure water or directly in the alkaline Bicincho -ninic Acid (BSA) WR.

* Increase the amount of copper in the WR (prepare WR as 50:2 or 50:3, Reagent A:B), which may eliminate interference by copper chelating agents.

Note: For greatest accuracy, the protein standards must be treated identically to the sample(s).

Table 2. Compatible Substance Concentrations in the BCA Protein Assay

Substance Salts and Buffers	Compatible Concentration
ACES, pH 7.8	25 mM
Ammonium sulfate	1.5 M
Asparagine	1 mM
Bicine, pH 8.4	20 mM
Bis-Tris, pH 6.5	33 mM
Borate (50mM), pH8.5	undiluted
B-PER® Reagent	undiluted
Calcium choloride in TBS, pH 7.2	10 mM
Na-Carbonate/Na-Bicarbonate (0.2M), pH 9.4	undiluted
Cesium bicarbonate	100 mM
CHES, pH 9.0	100 mM
Na-Citrate (0.6M), Na-Carbonate (0.1M), pH 9.0	1:8 dulution *
Na-Citrate (0.6M), MOPS (0.1M), pH 7.5	1:8 dulution *
Cobalt chloride in TBS, pH 7.2	0.8 mM
EPPS, pH 8.0	100 mM
Ferric cholride in TBS, pH 7.2	10 mM
Glycine	100 mM
Guanidine, HCl	4 M
HEPES, pH 7.5	100 mM
Imidazole, pH 7.0	50 mM
MES, pH 7.0	100 mM
MES (0.1M), NaCl (0.9%), pH 7.4	undiluted
MOPS, pH 7.2	100 mM
Modified Culbecco's PBS, pH 7.4	undiluted
Nickel cholride in TBS, pH 7.2	10 mM
PBS;Phosphate(0.1M), NaCl(0.15M), pH 7.2	undiluted
PIPES, pH 6.8	100 mM
RIPA lysis buffer, 50mM Tris, 150mM NaCl, 0.5% DOC,1% NP-40, 0.1% SDS, pH 8.0	undiluted
Sodium Acetate, pH 4.8	200 mM
Sodium azide	0.2%
Sodium bicarbonate	100 mM
Sodium chloride	1 mM
Sodium citrate, pH 4.8 or pH 6.4	200 mM
Sodium Phosphate	100 mM
Tricine, pH 8.0	25 mM
Triethanolamine, pH 7.8	25 mM
Tris	250 mM
TBS, Tris (25mM), NaCl (0.15M), pH 7.6	undiluted
Tris (25mM), Glycine(192mM), pH 8.0	1:8 diluted*

Substance Detergnets **	Compatible Concentration
Brij® -35	0.5%
Brij® -56, Brij® -58	1.0%
CHAPS, SHAPSO	5.0%
Deoxycholic acid	5.0%
Octyl-glucoside	5.0%
Octyl β-thioglucopyranoside	5.0%
SDS	5.0%
Span®20	1.0%
Triton®X-100	5.0%
Triton®X-114	1.0%
Triton®X-305	1.0%
Triton®X-405	1.0%
Tween®-20	5.0%
Tween®-60	5.0%
Tween®-80	5.0%
Zwittergent®-3-14	1.0%

* Dilyted with ultrapure water.

** Detergent Detergents were tested using high-purity GenDEPOT Surfact-Amps products, which have low peroxide content.

- - Dashed-line entry indicated that the material is incompatible with the assay.

Table 2. Compatible Substance Concentrations in the BCA Protein Assay

Chelating agents	Compatible Concentration
EDTA	10 mM
EGTA	----------
Sodium Citrate	200 mM

Reducing & Thiol-Containing Agents	Compatible Concentration
N-acetylglucosamine in PBS, pH 7.2	10 mM
Ascorbic acid	----------
Cysteine	----------
Dithioerythritol (DTE)	1 mM
Dithiothreitol (DTT)	1 mM
Glucose	10 mM
Melbiose	---------
2-Mercaptoethanol	0.01%
Potassium thiocyanate	3.0 M
Thimerosal	0.01%

Mis. Reagents & Solvents	Compatible Concentration
Acetone	10 %
Acetonitrile	10 %
Aprotinin	10 mg/L
DMF, DMSO	10 %
Ethanol	10 %
Glycerol (Fresh)	10%
Hyrochloric Acid	100 mM
Leupeptin	100 mg/mL
Hydrazides	---------
Hyrides (Na_2BH_4 or $NaCNBH_4$)	----------

* Dilyted with ultrapure water.

** Detergent Detergents were tested using high-purity GenDEPOT Surfact-Amps products, which have low peroxide content.

- - Dashed-line entry indicated that the material is incompatible with the assay.

Related GenDEPOT Products

Product Name	Cat No
Albumin, Ultra pure Bovine Serum Albumin, IgG free	A0100
West-EZier Super Blocking Buffer	W3700
West-Ez Blocking Buffer, 3% BSA-IgG Free	W3710
West-EZier Rapid Blocking Buffer , 5min	W3871
TBS Buffer, 20X , pH 7.4	T8054
TBS Buffer, 20X, pH 7.6	T8055
TBST Buffer , 10X , pH 7.4	T8056
West-EZ Stripping Buffer	S2100
West-EZier Wash Buffer(10X)	W3683
Goat anti-Mouse IgG(H+L)-HRP, 1mg/ml, HSA	SA001
Goat anti-Rabbit IgG(H+L)-HRP, 1mg/ml, HSA	SA002
Rabbit anti-Goat IgG(H+L)-HRP, 1mg/ml, HSA	SA007
West-EZier 2nd anti-Goat IgG,HRP conjugated, 0.2mg/ml	W3901
West-EZier 2nd anti-Rabbit IgG, HRP conjugated, 0.2mg/ml	W3902
West-EZier 2nd anti-Mouse IgG, HRP conjugated, 0.2mg/ml	W3903
West-Q Pico ECL Solution	W3652
West-Q Pico Dura ECL Solution	W3653
West-Q Femto Clean ECL Soution	W3680
NP-40 Cell Lysis Buffer(2X)	N1200
RIPA Cell Lysis Buffer(1X) with EDTA	R4100
RIPA Cell Lysis Buffer(1X) without EDTA	R4200
Xpert 2 Prestained Protein Marker	P8503
Xpert Protease Inhibitor Cocktail Solution(100X)	P3100
Xpert Phosphatase Inhibitor Cocktail Solution(100X)	P3200
Laemmli Sample Buffer(4X), Reducing	L1100
Native Sample Buffer(4x) , Non-Reducing	L1200
10X Tris-Glycine Native Buffer(Transfer Buffer)	T8052
10X Tris-Glycine SDS Buffer(Running Buffer)	T8053