Cyrusbioscience

BCA Protein Assay Kit

Product No.: BCAK

Introduction

BCA Protein Assay Kit is a perfect protein assay method. It based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein in the 5-2,000 μg/mL concentration range. Like the Lowry method, the assay relies on the reduction of Cu2+ ions by protein. The Cu+ thus formed is detected by conversion into a violet-colored substance by reaction with bicinchoninate. BCA Protein Assay Kit is compatible with many detergents but not compatible with reducing agents, such as DTT, DTE, and 2-Mercaptoethanol etc.

Product component

Reagent A: 500 mL
Reagent B: 12 mL
BSA standard (2 mg/mL): 1mL x10

Storage / Stability

BCA Protein Assay Kit could be shipped at room temperature. Reagent A and Reagent B should be stored at room temperature for 24 months, and BSA(bovine serum albumin) Standard should be stored at 4°C.

Materials needed but not provided

1. 96 well plate
2. Test tubes
3. thermo-shaker or water bath
4. Plate Reader capable of measuring absorbance in the region of 562 nm
5. Spectrophotometer capable of measuring absorbance in the region of 562 nm
   (If a 562 nm filter is not available, perform measurement with a 540-570 nm filter. Doing so will have no effect on quantification)

Instruction

A. Preparation of the Working Reagent

1. Prepare Working Reagent by mixing 50 parts of Reagent A and 1 part of Reagent B.
2. The required Working Reagent for each samples of Test Tube Procedure is 2.0 mL and that of the Microplate Procedure is 200 μL.
   Note: *The Working Reagent is a clear, apple green solution and the Working Reagent is stable for several days when stored in a closed container at room temperature.*

B. Preparation of the Protein Standards

1. Preparation of diluted protein standards: prepare a set of protein standard.

Note:

*For "**Test Tube Procedure**", use standard guide of 20-2000 µg/mL in **Table 1** for the standard protocol and 5-250 µg/mL in **Table 2** for the enhanced protocol.*

*For "**Microplate Procedure**", use standard guide of 20-2000 µg/mL in **Table 3** for the standard protocol and 5-250 µg/mL in **Table 4** for the enhanced protocol.*

- Preparation of diluted BSA Standards for Test Tube Procedure

Table 1. Working range: 20-2000 µg/mL

Tube	Volume of Diluent (µL)	Volume and source of protein Standards (µL)	Final BSA Standard Concentration (µg/mL)
A	0	300 of Stock	2000
B	125	375 of Stock	1500
C	325	325 of Stock	1000
D	175	175 of tube B dilution	750
E	325	325 of tube C dilution	500
F	325	325 of tube E dilution	250
G	325	325 of tube F dilution	125
H	400	100 of tube G dilution	25
I	400	0	0

Table 2. Working range: 5-250 µg/mL

Tube	Volume of Diluent (µL)	Volume and source of protein Standards (µL)	Final BSA Standard Concentration (µg/mL)
A	700	100 of Stock	250
B	400	400 of Stock	125
C	450	300 of Stock	50
D	400	400 of tube B dilution	25
E	400	100 of tube C dilution	5
F	400	0	0

- Preparation of diluted BSA Standards for Microplate Procedure

Table 3. Working range: 20-2000 µg/mL

Tube	Volume of Diluent (µL)	Volume and source of protein Standards (µL)	Final BSA Standard Concentration (µg/mL)
A	0	60 of Stock	2000
B	40	80 of Stock	1500
C	60	60 of Stock	1000
D	60	60 of tube B dilution	750
E	60	60 of tube C dilution	500
F	60	60 of tube E dilution	250
G	60	60 of tube F dilution	125
H	240	60 of tube G dilution	25
I	60	0	0

Table 4. Working range: 5-250 µg/mL

Tube	Volume of Diluent (µL)	Volume and source of protein Standards (µL)	Final BSA Standard Concentration (µg/mL)
A	70	10 of Stock	250
B	40	40 of tube A dilution	125
C	45	30 of tube B dilution	50
D	40	40 of tube C dilution	25
E	40	10 of tube D dilution	5
F	40	0	0

C. Test tube Procedure

1. Prepare Working Reagent by mixing 50 parts of Reagent A and 1 part of Reagent B.
   Note: *Certain substances are known to interfere with the BCA assay and it must be avoided in the sample's buffer. The maximum compatible concentrations for these substances are listed in* ***Table 5****.*
2. Add 2.0 mL of the Working Reagent to each tube and mix well.
3. Cover the tubes and incubate at selected temperature and time in a thermo-shaker or water bath.
   Working range: 20- 2,000 µg/mL
   - Standard Protocol: 37°C for 30 minutes
   - RT Protocol: RT for 2 hours

   Working range: 5- 250 µg/mL
   - Enhanced Protocol: 60°C for 30 minutes
4. Cool all tubes to room temperature.
5. Turn on the spectrophotometer and set to 562nm, to measure the absorbance of all the samples and the BSA standard.
   Note: *All the samples and BSA standard must be measured within 10 minutes to avoid significant error of measurements.*
   Note: *If a 562 nm filter is not available, perform measurement with a 540-570 nm filter. Doing so will have no effect on quantification.*
6. Prepare a standard curve by 562 nm BSA measurement and determine the protein concentration of each unknown sample by standard curve.

D. Microplate Procedure

1. Pipette 25µL of each standard (Table 3. or Table 4.) or unknown sample replicate into a microplate well.

Note: If sample size is limited, 10µL of each unknown sample and standard can be used. However, the working range of the assay in this case will be limited to 125-2000 µg/mL.

Note: *Certain substances are known to interfere with the BCA assay and it must be avoided in the sample's buffer. The maximum compatible concentrations for these substances are listed in Table 5.*

2. Add 200µL of the Working Reagent to each well and mix plate thoroughly on a plate shaker for 30 seconds.

3. Cover plate and incubate at a selected temperature and time in a thermo-shaker or water bath or a thermo-shaker.

   Working range: 20- 2,000 µg/mL

   - Standard Protocol: 37°C for 30 minutes
   - RT Protocol: RT for 2 hours

   Working range: 5- 250 µg/mL

   - Enhanced Protocol: 60°C for 30 minutes

4. Cool plate to room temperature.
5. Measure the absorbance at or near 562nm on a plate reader.

   Note: *If a 562 nm filter is not available, perform measurement with a 540-570 nm filter. Doing so will have no effect on quantification.*

6. Prepare a standard curve by measurement the absorbance of BSA at 562nm and determine the protein concentration of each unknown sample by standard curve.

Appendix

Table 5. Compatible concentration of common substances

Chelating agents	
EDTA	10mM
EGTA	-
Sodium citrate	200mM
Detergents	
Brij-35	5%
Brij-56, Brij -58	1%
CHAPS, CHAPS O	5%
Deoxycholic acid	5%
Octyl β-glucoside	5%
Nonidet P-40 (NP-40)	5%
Octyl β-thioglucopyranoside	5%
SDS	5%
Span 20	1%
Triton X-100	5%
Triton X-114, X-305, X-405	1%
Tween-20, Tween-60, Tween-80	5%
Zwittergent 3-14	1%
Reducing & Thiol-Containing Agents	
N-acetylglucosamine in PBS, pH 7.2	10mM
Ascorbic acid	-
Cysteine	-
Dithioerythritol (DTE)	1mM
Dithiothreitol (DTT)	1mM
Glucose	10mM
Melibiose	-
2-Mercaptoethanol	0.01%
Potassium thiocyanate	3.0M
Thimerosal	0.01%
Misc. Reagents & Solvents	
Acetone	10%
Acetonitrile	10%

Salts or Buffers	
ACES, pH 7.8	25mM
Ammonium sulfate	1.5M
Asparagine	1mM
Bicine, pH 8.4	20mM
Bis-Tris, pH 6.5	33mM
Borate	50mM
Calcium chloride in TBS, pH 7.2	10mM
Na-Carbonate/Na -Bicarbonate, pH 9.4	0.2M
Cesium bicarbonate	100mM
CHES, pH 9.0	100mM
Cobalt chloride in TBS, pH 7.2	0.8mM
EPPS, pH 8.0	100mM
Ferric chloride in TBS, pH 7.2	10mM
Glycine• HCl, pH 2.8	100mM
Guanidine • HCl	4M
HEPES, pH 7.5	100mM
Imidazole, pH 7.0	50mM
MES, pH 6.1	100mM
MOPS, pH 7.2	100mM
Modified Dulbecco 's PBS, pH 7.4	undiluted
Nickel chloride in TBS, pH 7.2	10mM
PBS; Phosphate (0.1 M), NaCl (0.15 M), pH 7.2	undiluted
PIPES, pH 6.8	100mM
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	200mM
Sodium azide	0.2%
Sodium bicarbonate	100mM
Sodium chloride	1M
Sodium citrate, pH 4.8 or pH 6.4	200mM
Sodium phosphate	100mM
Tricine, pH 8.0	25mM

Aprotinin	10mg/L	Triethanolamine, pH 7.8	25mM
DMF	10%	Tris	250mM
DMSO	10%	TBS; Tris (25mM), NaCl (0.15 M), pH 7.6	undiluted
Ethanol	10%	Tris (25mM), Glycine (192mM), pH 8.0	1:3 dilution
Glycerol (Fresh)	10%		
Hydrazides (~continued)	-		
Hydrides (Na2BH4 or NaCNBH3)	-		
Hydrochloric Acid	100mM		
Leupeptin	10mg/L		

Trouble shooting

Problem	Possible cause	Solution
No color development	Chelating agents are present in the sample buffer	Dialyze or desalt the sample. Dilute the sample.
Sample color less intense than expected	pH is altered by strong acid or alkaline buffer	Dialyze or desalt the sample. Dilute the sample.
Sample color is darker than expected	Protein concentration is too high	Dilute the sample
	Sample contains lipids or lipoproteins	Add 2% SDS to the sample to eliminate interference from lipids
All the tubes are dark purple	Reducing agents are present in the sample buffer	Dialyze or desalt the sample
	Thiols are present in the sample buffer	Dialyze or desalt the sample