VISUAL PROTEIN

Manual

Dual-Range™ BCA Protein Assay Kit

BC03-500

V4.2

For Research Use Only

Introduction

Dual-Range™ 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 Cu^{2+} ions by protein. The Cu+ thus formed is detected by conversion into a violet-colored substance by reaction with bicinchoninate. **Dual-Range™ BCA Protein Assay Kit** is compatible with many detergents but not compatible with reducing agents, such as DTT, DTE, and 2-Mercaptoethanol etc.

Product Components

Dual-Range™ BCA Protein Assay Kit (BC03-500)

Reagent A		500 mL	1 bottle
Reagent B		12 mL	1 bottle
Bovine Serum Albumin Standard (2 mg/mL)	AS02-2S	2 mL	5 tubes
User's manual			

Safety Information

Please wear gloves, lab coat and goggles while operating. Prevent contact product directly. In case of contacting, wash with large amount of water.

Storage

Dual-Range™ 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 Albumin Standard should be stored at 4 °C. Expiration date is labeled on the bottle or box.

VISUAL PROTEIN

Materials needed but not provided

1. 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)
2. Water bath
3. Plate Reader
4. Test tubes
5. 96 well plate

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 protein standards.
2. For **"Test Tube Procedure"**, use standard guide of 20-2,000 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 20-2,000 µg/mL in **Table 3** for the standard protocol and 5-250 µg/mL in **Table 4** for the enhanced protocol.

Table 1. Preparation of Diluted Albumin (BSA) Standards for Test tube Procedure (working range: 20-2,000 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	2,000
B	125	375 of Stock	1,500
C	325	325 of Stock	1,000
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. Preparation of Diluted Albumin (BSA) Standards for Test tube Procedure (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 tube A dilution	125
C	450	300 of tube B dilution	50
D	400	400 of tube C dilution	25
E	400	100 of tube D dilution	5
F	400	0	0

Table 3. Preparation of Diluted Albumin (BSA) Standards for Microplate Procedure (working range: 20-2,000 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	2,000
B	40	80 of Stock	1,500
C	60	60 of Stock	1,000
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. Preparation of Diluted Albumin (BSA) Standards for Microplate Procedure (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. Pipette 100 µL of each standard (Table 1. or Table 2.) and unknown sample replicate into an appropriately test tube.

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.**

C. Test tube Procedure (~continued)

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 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 562 nm, 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 25 µL of each standard (Table 3. or Table 4.) and unknown sample replicate into a microplate well.

NOTE: If sample size is limited, 10 µL of each unknown sample and standard can be used. However, the working range of the assay in this case will be limited to 125-2,000 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 200 µL 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 water bath or a thermo-shaker.

VISUAL PROTEIN

D. Microplate Procedure (~continued)

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 562 nm 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 562 nm and determine the protein concentration of each unknown sample by standard curve.

Troubleshooting

Problem	Possible cause	Remedy
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

Appendix

Table 5. Compatible concentration of common substances

Salts/Buffers		Salts/Buffers	
ACES, pH 7.8	25 mM	CHES, pH 9.0	100 mM
Ammonium sulfate	1.5 M	Cobalt chloride in TBS, pH 7.2	0.8 mM
Asparagine	1 mM	EPPS, pH 8.0	100 mM
Bicine, pH 8.4	20 mM	Ferric chloride in TBS, pH 7.2	10 mM
Bis-Tris, pH 6.5	33 mM	Glycine• HCl, pH 2.8	100 mM
Borate, pH 9.5	50 mM	Guanidine•HCl	4 M
Calcium chloride in TBS, pH 7.2	10 mM	HEPES, pH 7.5	100 mM
Na-Carbonate/Na-Bicarbonate, pH 9.4	0.2 M	Imidazole, pH 7.0	50 mM
Cesium bicarbonate	100 mM	MES, pH 6.1	100 mM

VISUAL PROTEIN

Appendix

Table 5. Compatible concentration of common substances (~continued)

Salts/Buffers	
MOPS, pH 7.2	100 mM
Modified Dulbecco's PBS, pH 7.4	undiluted
Nickel chloride in TBS, pH 7.2	10 mM
PBS; Phosphate (0.1 M), NaCl (0.15 M), 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	0.20%
Sodium bicarbonate	100 mM
Sodium chloride	1 M
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 (0.15 M), pH 7.6	undiluted
Tris (25mM), Glycine (192mM), pH 8.0	1:3 diluted

Misc. Reagents & Solvents	
Acetone	10%
Acetonitrile	10%
Aprotinin	10 mg/L
DMF, DMSO	10%
Ethanol	10%
Glycerol (Fresh)	10%
Hydrazides	-
Hydrides (Na_2BH_4 or $NaCNBH_3$)	-
Hydrochloric Acid	100 mM
Leupeptin	10 mg/L

Chelating agents	
EDTA	10 mM
EGTA	-
Sodium citrate	200 mM

Detergents	
Brij-35	5.00%
Brij-56, Brij-58	1.00%
CHAPS, CHAPSO	5.00%
Deoxycholic acid	5.00%
Octyl β-glucoside	5.00%
Nonidet P-40 (NP-40)	5.00%
Octyl β-thioglucopyranoside	5.00%
SDS	5.00%
Span 20	1.00%
Triton X-100	5.00%
Triton X-114, Triton X-305, X-405	1.00%
Tween-20, Tween-60, Tween-80	5.00%
Zwittergent 3-14	1.00%

Reducing &Thiol-Containing Agents	
N-acetylglucosamine in PBS, pH 7.2	100 mM
Ascorbic acid	-
Cysteine	-
Dithioerythritol (DTE)	1 mM
Dithiothreitol (DTT)	5 mM
Glucose	10 mM
Melibiose	-
2-Mercaptoethanol	0.01%
Potassium thiocyanate	3 M
Thimerosal	0.01%