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QuantiEasy™ BCA Protein Assay Kit

QuantiEasy™ BCA Protein Assay is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein concentration in the range of 20-2000 µg/ml in either a standard tube assay or microplate assay configuration. Bicinchoninic acid is a chromogenic reagent that chelates the reduced copper, producing a purple complex with strong absorbance at 562 nm (Smith 1985, Wiechelman 1988). The intensity of the BCA/copper purple complex is proportional to the amount of protein in the sample. Thus measuring the intensity of the absorbance at 562 nm is proportional to protein concentration. QuantiEasy™ BCA protein assay demonstrates higher tolerances towards common interfering substances, such as nonionic detergents and buffer salts, than the Lowry technique. This assay can be used to quantify protein concentration with a wide variety of samples and can be performed in minutes.

Contents

Cat. No.	Product Name	Specifications
QEB500	QuantiEasy™ BCA Protein Assay	QuantiEasy™ BCA Reagent A : 500 ml QuantiEasy™ BCA Reagent B: 12 ml QuantiEasy™ Albumin Standard (2 mg/mL) : 1 ml x 10

Features

High linearity—linear working range for BSA equals 20 to 2000 µg/mL.
Moderately fast—much easier and four times faster than the classical Lowry method.
Compatible—unaffected by typical concentrations of most ionic and nonionic detergents.
Excellent uniformity—exhibits less protein-to-protein variation than dye-binding methods.
Colorimetric—estimate visually or measure with a standard spectrophotometer or plate reader (562nm).

Applications

QuantiEasy™ BCA protein assay can be used to assess yields in whole cell lysates and affinity-column fractions, as well as to monitor protein contamination in industrial applications. It can also be used to study protein-protein interactions, estimate percent recovery of membrane proteins from cell extracts and high-throughput screening of fusion proteins. Compared to most dye-binding methods, QuantiEasy™ BCA protein assay is affected much less by protein compositional differences, providing greater protein-to-protein uniformity.

Shipping and Storage Conditions

QuantiEasy™ BCA protein assay is shipped at room temperature. QuantiEasy™ BCA Reagent A and QuantiEasy™ BCA Reagent B should be stored at room temperature. QuantiEasy™ Albumin Standard should be stored at 2-8℃ upon receipt.

Important Notes

Please read the entire notes prior to starting any of the protocol procedures.

Safety Information:

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

Materials to Be Supplied by User:

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. Doing so will have no effect on quantification)
2. Water bath.
3. Plate Reader.
4. Test tubes.
5. 96 well plate.

Test-Tube Protocol

(Sample to Working Reagent ratio = 1:20)

Please read the entire important notes prior to starting.

Step 1: Preparation of Diluted Albumin (BSA) Standards

Dilution Scheme for **Standard Test Tube Protocol**

(working range: 20-2000 µg/mL)

Tube	Volume of Diluent (µL)	Volume and Source of BSA (µL)	Final BSA 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

Dilution Scheme for **Enhanced Test Tube Protocol**

(working range: 5-250 µg/mL)

Tube	Volume of Diluent (µL)	Volume and Source of BSA (µL)	Final BSA 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

Step 2: Preparation of the BCA Working Reagent

Prepare Working Reagent by mixing 50 parts of QuantiEasy™ BCA Reagent A and 1 part of QuantiEasy™ BCA Reagent B. The required Working Reagent for each samples of Test Tube Procedure is 2.0 mL.

NOTES: 1. 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.

2. 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 Appendix Table 1.

Step 3: Test-Tube Procedure

1. Pipette 0.1mL of each standard and unknown sample replicate into an appropriately labeled test tube.
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.

Standard Protocol: 37℃ for 30 minutes RT Protocol: RT for 2 hours	Enhanced Protocol: 60℃ for 30 minutes
Working range: 20- 2,000 µg/mL	Working range: 5- 250 µg/mL

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.

NOTES: 1. All the samples and BSA standard must be measured within 10 minutes to avoid significant error of measurements.

2. 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.

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Microplate Protocol

(Sample to Working Reagent ratio = 1:8)

Please read the entire important notes prior to starting.

Step 1: Preparation of Diluted Albumin (BSA) Standards

Dilution Scheme for **Standard Microplate Protocol**

(working range: 20-2000 µg/mL)

Tube	Volume of Diluent (µL)	Volume and Source of BSA (µL)	Final BSA 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

Dilution Scheme for **Enhanced Microplate Protocol**

(working range: 5-250 µg/mL)

Tube	Volume of Diluent (µL)	Volume and Source of BSA (µL)	Final BSA 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

Step 2: Preparation of the BCA Working Reagent

Prepare Working Reagent by mixing 50 parts of QuantiEasy™ BCA Reagent A and 1 part of QuantiEasy™ BCA Reagent B. The required Working Reagent for each samples of Mircoplate Procedure is 200 µL.

NOTES: 1. 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.

2. 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 Appendix Table 1.

Step 3: Microplate Procedure

1. Pipette 25 µL of each standard and unknown sample replicate into a microplate well.
   (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.
2. Add 200µL of the Working Reagent to each well and mix plate thoroughly on a plate shaker for 30 seconds.
3. Cover the plate and incubate at selected temperature and time in a water bath or a thermo-shaker.

Standard Protocol: 37℃ for 30 minutes RT Protocol: RT for 2 hours	Enhanced Protocol: 60℃ for 30 minutes
Working range: 20- 2,000 µg/mL	Working range: 5- 250 µg/mL

4. Cool plate to room temperature.

5. Turn on the spectrophotometer. Measure the absorbance at or near 562nm on a plate reader.

NOTES: 1. If a 562 nm filter is not available, perform measurement with a 540-570 nm filter. Doing so will have no effect on quantification.

2. Because plate readers use a shorter light path length than cuvette spectrophotometers, the Microplate Procedure requires a greater sample to Working Reagent ratio to obtain the same sensitivity as the standard Test Tube Procedure. If higher 562nm measurements are desired, increase the incubation time to 2 hours.

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

Troubleshooting

Problem	Possible Reasons / 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

Appendix

Table 1: Compatible concentration of common substances

Salts/Buffers	Compatible Concentration
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	0.20%
Sodium bicarbonate	100mM
Sodium chloride	1M
Sodium citrate, pH 4.8 or pH 6.4	200mM
Sodium phosphate	100mM
Tricine, pH 8.0	25mM
Triethanolamine, pH 7.8	25mM
Tris	250mM
TBS; Tris (25mM), NaCl (0.15 M), pH 7.6	undiluted
Tris (25mM), Glycine (192mM), pH 8.0	1:3 dilution

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

Detergents	Compatible Concentration
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, X-305, X-405	1.00%
Tween-20, Tween-60, Tween-80	5.00%
Zwittergent 3-14	1.00%

Reducing&Thiol-Containing Agents	Compatible Con.
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	Compatible Concentration
Acetone	10%
Acetonitrile	10%
Aprotinin	10mg/L
DMF, DMSO	10%
DMSO	10%
Ethanol	10%
Glycerol (Fresh)	10%
Hydrazides	-
Hydrides (Na2BH4 or NaCNBH3)	-
Hydrochloric Acid	100mM
Leupeptin	10mg/L