EZ-BCA Protein Quantification Kit

Cat. No. DG-BCA500

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

DoGenBio Co., Ltd.

▪ Product Description

The EZ-BCA Protein Quantification Kit is a colormetric detection and quantification kit for total protein using bicinchoninic acid(BCA). In an alkaline solution, the peptide bonds of the protein to be quantified reduce Cu^{2+} to Cu^{+} (biuret reaction). Then, two molecules of BCA coordinate with the reduced Cu^{+}, forming a purple-colored complex. This complex is measured at 562nm using a spectrophotometer.

Protein quantification using BCA is less affected by protein type differences and can be applied over wide range of concentrations (0.02 ~ 2 ㎎/㎖). Additionally, it is resistant to interference from surfactants.

▪ Contents

Component	Volume	Storage
BCA Reagent A	500 ㎖	RT
BCA Reagent B	25 ㎖	RT
BCA Standard Solution (2 ㎎/㎖)	10 X 1 ㎖	4℃

* This product is intended for research use only and should not be used for human applications or diagnostic purposes.

▪ General Protocol

1. Preparation of diluted albumin (BSA) standards.

Vial No.	Vol. of Diluent (㎕)	Vol. and Source of BSA (㎕)	Final BSA Conc. (㎍/㎖)
1	0	300 of Stock	2000
2	125	375 of Stock	1500
3	325	325 of Stock	1000
4	175	175 of vial 2 dilution	750
5	325	325 of vial 3 dilution	500
6	325	325 of vial 5 dilution	250
7	325	325 of vial 6 dilution	125
8	400	100 of vial 7 dilution	25
9	400	0	0

① Use the table to prepare diluted BSA standard (enough for three measurements).

② Use the same buffer for both the BSA standard and the sample being measured.

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2. Preparation of the BCA working reagent (WR)

① Working Reagent (WR) = BCA reagent A (50) : BCA reagent B(1)

② Mixing BSA Reagent A and B creates a clear green solution. Prepare enough for the number of samples you plan to analyze.

3-1. Measurement using Test Tubes (Sample : WR = 1: 20)

① Add 100 ㎕ each of the standard and sample into separate test tubes.

② Add 2 ㎖ of WR to each test tube and mix well.

③ Allow the reaction to proceed in the test tubes under one of the following conditions:

a. 37 ℃ for 30 minutes: Measurement range = 20 ~ 2,000 ㎍/㎖

b. Room temperature (RT) for 2 hours: Measurement range = 20 ~ 2,000 ㎍/㎖

c. 60 ℃ for 30 minutes: Measurement range = 5 ~ 250 ㎍/㎖

④ Cool the test tubes to room temperature (RT).

⑤ Measure the absorbance at 562 nm.

3-2. Measurement using a Microplate (Sample : WR = 1 : 8)

① Add 25 ㎕ each of the standard and sample into each well of the micropate.

② Add 200 ㎕ of WR to each well and mix thoroughly.

③ Cover the plate and incubate at 37 ℃ for 30 minutes.

④ Cool the microplate to room temperature (RT).

⑤ Measure the absorbance at 562 nm.

4. Data Processing

4-1. When Using Test Tubes

① Set the baseline of the measuring device using a tube filled with D.W.

② Measure the absorbance (O.D 562 nm) of the samples and standards withing 10 minutes.

③ Subtract the absorbance value of standard vial No. 9 from the absorbance values of both the samples and standards.

④ Use the corrected absorbance values of the standards to create a standard curve. Determine the concentration (㎍/㎖) of the samples by referencing their absorbance values against the standard curve.

4-2. When using a Microplate

① Subtract the absorbance value of standard vial No. 9 from the absorbance values of both the standards and samples measured at O.D 562 nm.

② Use the corrected absorbance values of the standards to create a standard curve. Determine the concentration (㎍/㎖) of the samples by referencing their

absorbance values against the standard curve.

▪ Notice

Bicinchoninic acid (BCA) can be interfered with by reducing agents, chelating agents, strong acids, or strong bases. Even trace amounts of certain substances can make analysis difficult, so please exercise caution.

Examples) Ascorbic Acid, EGTA, Iron, Impure Sucrose, Catecholamines, Impure Glycerol, Lipid, Tryptophan, Creatinine, Hydrogen Peroxide, Melibiose, Tyrosine, Cysteine, Hydrazides, Phenol Red, Uric Acid

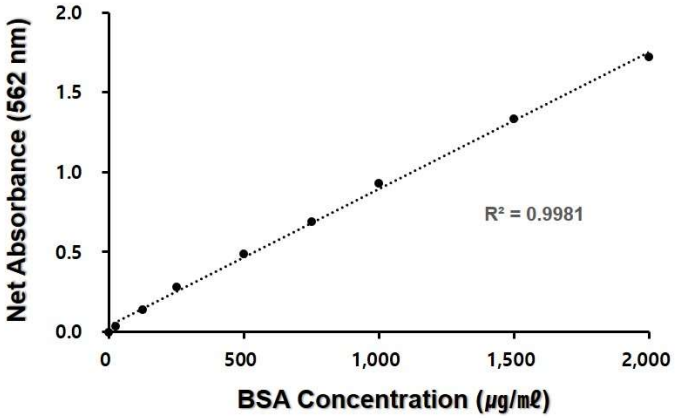

Fig 1. Standard Curve for BSA(0~2,000 μg/mL):
Measured after a 30 minute reaction at 37 ℃ using a microplate.

■ Related Product

I. EZ-Western series: Western blot detection kit (ECL solution) and others

Product	Catalog No.	size
EZ-Western (Nano~mid picogram)	DG-W100	100 ml (A: 50 ml + B: 50 ml)
	DG-W250	250 ml (A: 125 ml + B: 125 ml)
	DG-W500	500 ml (A: 250 ml + B: 250 ml)
EZ-Western Lumi Pico (Low picogram)	DG-WP100	100 ml (A: 50 ml + B: 50 ml)
	DG-WP250	250 ml (A: 125 ml + B: 125 ml)
	DG-WP500	500 ml (A: 250 ml + B: 250 ml)
EZ-Western Lumi Pico Alpha (Fast Detection)	DG-WPAL120	120 ml (A: 60 ml + B: 60 ml)
	DG-WPAL250	250 ml (A: 125 ml + B: 125 ml)
EZ-Western Lumi La (Mid femtogram, Long duration)	DG-WD100	100 ml (A: 50 ml + B: 50 ml)
	DG-WD200	200 ml (A: 100 ml + B: 100 ml)
EZ-Western Lumi Femto (Low femtogram)	DG-WF100	100 ml (A: 50 ml + B: 50 ml)
	DG-WF200	200 ml (A: 100 ml + B: 100 ml)
EZ-Gel Staining Solution	DG-GS1000	1000 ml (Without De-stain)
EZ-Western Stripping Buffer	DG-WSB500	500 ml

II. Protein marker

Product	Catalog No.	size
3-Color Regular Range Protein Marker, 10-245kDa	DG-PMC245	250㎕ x 2
3-Color Broad Range Protein Marker PLUS, 5-245kDa	DG-PMP245	250㎕ x 2