Easy II Protein Quantitative Kit (BCA)

Cat. No. DQ111

Storage: BSA Standard Solution at -20°C for two years; others at room temperature for one year

Description

The BCA protein assay is one of the most commonly used methods for protein quantification. Under alkaline condition, the reduction of Cu^{2+} to Cu^{+} is realized by peptide bonds in proteins (biuret reaction). The amount of reduced copper is directly proportional to the amount of total proteins.

Measurement Range

20-2000 μg/ml

Kit Contents

Components	DQ111-01
Solution A	100 ml
Solution B	3 ml
BSA Standard Solution (2 mg/ml)	2×1 ml

(Applicable for detection by enzyme-labeled instrument (for 500 rxns) or by spectrophotometer (for 50 rxns))

Interfering Substances

Certain substances are known to interfere with the BCA assay including those substances with reducing potential, chelating agents, and strong acids/bases. The following table shows the highest concentration of these substances in the protein sample buffer without interfering the BCA assay.

Interfering Substances	Tolerant Concentration	Interfering Substances	Tolerant Concentration
Salts/Buffer		Detergents	
HEPES (pH 7.9)	100 mM	NP 40	5%
PIPES (pH 6.8)	100 mM	Triton X-100	5%
Ammonium sulfate	1.5 M	CHAPS, CHAPSO	5%
Sodium chloride	1 M	SDS	5%
Sodium bicarbonate	100 mM	Tween 20	5%
MPOS (pH 7.2)	100 mM	Tween 60	5%
Sodium citrate	200 mM	Tween 80	5%
TRICINE (pH 8.0)	25 mM	Mixture/Polar compounds	
Sodium Acetate	200 mM	PMSF	1 mM
Guanidine.HCl	4 M	Acetone	10%
Tris	250 mM	Ethanol	10%
Chelating Agents		Glycerol	10%
EDTA	10 mM	Urea	3 M
Reducing Agents		DMSO	10%
DTT	1 mM	Sucrose	40%
2-Mercaptoethanol	0.01%		

Procedures

A. Detection by Enzyme-Labeled Instrument

1. Dilute BSA Standard Solution to the concentration at 500 μg/ml using 1×PBS (supplied by user).
2. Make the proper volume of BCA working solution by mix solution A with solution B at 50:1 (vol/vol). The working solution can be used within 24 hours after prepared.
3. Transfer 0, 1, 2, 4, 8, 12, 16, 20 μl of diluted standard solution into a 96-well plate, add PBS to each well to make the final volume of 20 μl.
4. Dilute the sample with 1×PBS (supplied by user), and add 20 μl of diluted sample into another well of the 96-well plate.
5. Pippette 200 μl BCA working solution into each well, incubate at 37°C for 30-90 minutes (or incubate at room temperature for 2 hours, or at 60°C for 30 minutes).
6. Place the plate into enzyme-labeled instrument for detection with wavelength at 562 nm. Alternatively, wavelength at 540-595 nm can also be used.
7. Plot the standard curve and calculate the unknown protein concentration.

B. Detection by Spectrophotometer

1. Dilute BSA Standard Solution to the concentration at 500 μg/ml using 1×PBS (supplied by user).
2. Make the proper volume of BCA working solution by mix solution A with solution B at 50:1 (vol/vol). The working solution can be used within 24 hours after prepared.
3. Label eight 1.5 ml microcentrifuge tubes from A to H, transfer 0, 2.5, 5, 10, 20, 30, 40, 50 μl of diluted standard solution into each labeled tube. Add PBS to each tube to make the final volume of 50 μl.
4. Dilute the sample with 1×PBS (supplied by user), add 50 μl of the diluted sample into a new 1.5 ml microcentrifuge tube.
5. Pippette 500 μl of BCA working solution into each tube (tubes from in step 3 and step 4). Mix thoroughly by vortexing, and incubate at 37°C for 30-90 minutes (or incubate at room temperature for 2 hours, or at 60°C for 30 minutes)
6. Transfer 200 μl of reaction product from step 5 respectively into the cuvettes. Measure the absorbance of the sample by spectrophotometer with wavelength at 562 nm. Alternatively, wavelength at 540-595 nm can also be used.
7. Plot the standard curve and calculate prot ein concentration in samples.

Notes

- Wavelength for detection is 540 to 595 nm, and the optimal wavelength is 562 nm.
- If there is no enzyme-labeled instrument, spectrophotometer can also be used for measurement. Samples detected by spectrophotometer should be equilibrated to room temperature, and the measurement and record should be completed within 10 minutes after adding BCA working solution.
- To ensure the accuracy of measurement, please be assure that the concentration of diluted protein sample falls into the range of the standard curve.
- BCA protein assay is not suitable for protein samples in some buffers (e.g. DTT concentration at over 1 mM, β-mercaptoethanol concentration at over 0.01%, EDTA concentration at over 10 mM, or presence of EGTA in samples). Please use TransGen *Easy* Protein Quantitative Kit (Bradford), Cat.No. DQ101 for these samples.
- Use duplicate or triplicate for accurate detection.

Service telephone +86-10-57815020
Service email complaints@transgen.com