



DNAbiotech Biotechnology is our expertise

BCA Protein Quantification Kit

Catalog no.: DB9684 Unit Size: 25,50 and 100 ml Related products:

Bradford protein assay kit

Intended for Research Use Only

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Customer and technical support

If you have any question, do not hesitate to ask! DNAbiotech would be highly appreciated for any comment(s).

Contact us at

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Quality Control

In accordance with DNAbiotech Co. Management System, each part of the product tested against predetermined specifications to ensure consistent product quality.

Safety Notes

Always wear gloves and eye protector and a lab coat. Contact with skin may change it color.

Note:	



General description

DNAbiotech BCA Protein Assay Kit is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein. This method was based on the reduction of Cu^{+2} to Cu^{+1} by protein in an alkaline medium with the highly sensitive and selective colorimetric detection. The purple-colored reaction product of this assay is formed and exhibits a strong absorbance at 562 nm that is nearly linear with increasing protein concentrations over a broad working range (20-2,000 µg/ml). The reaction occurred in DNAbiotech BCA Protein Assay Kit is not a true end-point method; that is, the final color continues to develop. However, following incubation, the rate of continued color development is sufficiently slow to allow large numbers of samples to be assayed together. Thus, protein concentrations generally are determined and reported with reference to standards of bovine serum albumin (BSA). A series of dilutions of known concentration are prepared from the protein and assayed alongside the unknown(s) before the concentration of each unknown is determined based on the standard curve.

Product Information

Cat #: DB9684 (25, 25, 50 and 100 ml) Form: Liquid Featured industry: For Research Use Only Shipped in: RT, for long distance wet ICE Min shelf time: 24 months Storage condition: 4°C

Description

BCA Protein Quantification Kit provides a simple, procedure for determining the concentration of proteins in solution. The BCA Protein Assay is suitable for measuring protein concentration in the range of 5-800 ug/ml. This product is for research use only and is not intended for diagnostic use.



Kit contents

No.	Item	Quantity	Storage
1	BCA Reagent A	25, 50 or 100 mL	2-8 °C
2	BCA Reagent B	1-2 vial	2-8 °C
3	BSA Standard vial (10 mgr)	1ml	2-8 °C
4	96 well ELISA plate	1 pcs.	RT

Note: The BCA and Copper Reagents are stable at room temperature. The BSA Standard should be first reconstitute in DW or PBS and aliquoted. After this the BSA standard should be stored at -20°C. All reagents are stable for up to 24 months under proper storage conditions.

Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- Shaker

Advantages

For the rapid, sensitive and accurate measurement of protein in various

samples

General Protocol

Reagent Preparation:

Prepare Working Solution by adding 1 part of copper reagent to 49 parts of BCA Reagent. The total volume made will depend upon the number of samples and standards to be quantified. Each sample and standard will require 75 μ l or 250 μ l of working reagent depending on the protocol. Once made, the working solution is stable for a week at +4°C.



Standard Curve Preparation:

- 1. Label 10 vials (microtubes) from 1 to 10.
- 2. In the first vial, dilute the stock solution of BSA standard to 1 mg/ml (i.e., $100 \mu l + 900 \mu l$ buffer). This would be your vial number 1.
- Prepare below dilutions (note: this dilution is general, user can change it according to own method)
 Vial 2: 300 ul of vila 1 + 200 ul DW or PBS
 Val 3: 200 ul of vial 1 : 300 ul DW or PBS
 Add 250 μl 1 X PBS buffer or distilled water to the rest of the tubes (vial 4-10) and prepare the serial dilution by transferring 250 μl from vial 3 to vial 4. Continue the series of two-fold dilutions until vial 9. Throwaway 250 ul from vial 9.

Vial no.	1	2	3	4	5	6	7	8	9	10
Final Concent.	1000	600	400	200	100	50	25	12.5	6.25	DW
Ug/ml										
Final vol. ul	500	500	250	250	250	250	250	250	250	250

Note: Mix completely each well by pipetting and if your sample(s) has a high content of total protein, dilute it (them) to fall within 0.015-1 mg/ml range.

5. Pipette 25 μ l of standards or samples into duplicate wells in a clear bottom 96 well plate and according to the concentration of the protein in your sample choose one of the method:

A) **Micro-assay,** range: $5-250\mu g$ (1:3 sample to working reagent ratio): Add 75 μ l of working reagent to each standards and sample tube/well.

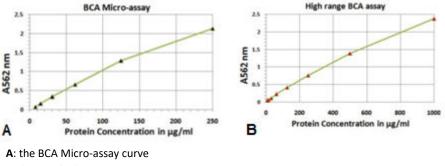
B) High range assay, range: 15-1000 μ g (1:9 sample to working reagent ratio): Add 225 μ l of sample tube/well.

6. Shake gently to mix. Incubate for 60 min at 60° C. Cool to room temperature.

7. Measure OD at 562 nm (or 545 nm, if your spectrophotometer does not support 562 nm). The signal is stable for at least 1 hour. For unknown samples, several dilutions of a sample should be tested to ensure the OD reading is within the standard curve range.



Figure A and B show representative curves for the BCA Micro-assay and High range BCA assay, respectively.



B: the BCA high range assay curve

Data Analysis

Subtract the blank OD (zero standards) from all standard and sample OD values. Plot the corrected OD against standard protein concentrations. Use the standard curve to determine the sample protein concentration. Alternatively, the equation for the best line fitting the standards can be used to determine the protein concentration of your samples. Standard curves carried out according to assay protocol.

References: Book Molecular & Cellular Proteomics

Troubleshooting

Problem	Comments			
	Standard solution of BSA do not prepare			
All of the wells have a similar color in standard	correctly.			
curve preparation	Check the procedure and make the standard			
	solutions again.			



