ab102536

BCA Protein Quantification Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of BCA Proteins in various samples.

View kit datasheet: www.abcam.com/ab102536
(use www.abcam.cn/ab102536 for China, or www.abcam.co.jp/ab102536 for Japan)

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 4a, Last Updated 24 May 2023

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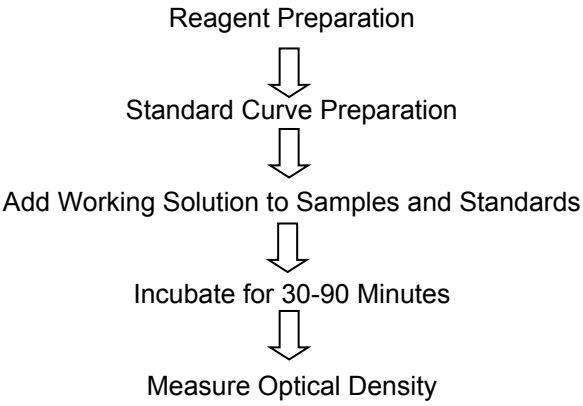
1. Overview

Abcam's BCA Protein Quantification Kit provides a simple, rapid, detergent tolerant (up to 5%) procedure for determining the concentration of proteins in solution. The method utilizes a copper (Cu^{2+}) salt which can be reduced to the cuprous state by protein(s). The generated Cu^{+} ion forms an intensely colored complex with the bicinchoninic acid reagent with a very strong absorbance band centered at 562 nm. The intensity of the blue complex is proportional to the amount of protein in the sample. The BCA Protein Assay is suitable for measuring protein concentration in the range of 0.5-30 µg protein (0.01-0.6 mg/ml).

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
BCA Reagent	100 mL
Copper Reagent	2 mL
BSA Standard III/BSA Standard (10 mg/ml)	1 mL

* Store kit at +4°C. The BCA and Copper Reagents are stable at room temperature. The BSA Standard III/BSA Standard should be aliquoted after the first thaw and stored at -20°C. All reagents are stable for up to 12 months under proper storage conditions.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

Notes:

a) The BCA protocol is very flexible. Both the incubation time and temperature can be varied over a rather wide range. Lower protein samples can be more easily quantified using higher temperatures and longer incubation times.

b) When assaying protein in solutions containing detergent, best results are obtained by adding the same amount of detergent to the wells containing the protein standard.

1. Reagent Preparation:

Prepare Working Solution by adding 1 part of Copper Reagent to 50 parts of BCA Reagent. The total volume made will depend upon the number of samples and standards to be quantitated. Each sample and standard will require 100 µl of working reagent. Once made, the Working Solution is stable for several days.

2. Standard Curve Preparation:

Label 8 tubes 1-8. Dilute the BSA Standard III/BSA Standard to 1 mg/ml Stock Solution (i.e., 40 µl + 360 µl buffer). Ideally, use the same buffer contained in your samples.

Transfer 200 µl from tube 8 to tube 7. Then prepare serial dilutions as in Table 1.

Table 1. Serial Dilutions

Tube	BSA Solution (µl)	Buffer(µl)	50 µl =
8	Stock Solution (256)	144	32 µg
7	Tube 8 (200)	200	16 µg
6	Tube 7 (200)	200	8 µg
5	Tube 6 (200)	200	4 µg
4	Tube 5 (200)	200	2 µg
3	Tube 4 (200)	200	1 µg
2	Tube 3 (200)	200	0.5 µg
1	---	200	0 µg

3. Dilute samples to fall within 0.01-0.6 mg/ml range.

4. Pipette 50 µl Standards or samples into duplicate wells in a clear bottom 96 well plate.

5. Add 100 µl of Working Solution into each well that contains the standard or samples.

6. Shake gently to mix. Incubate for between 30-90 min at 37-60°C. Cool to room temperature.

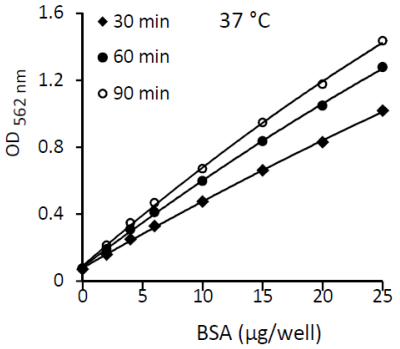
7. Measure OD at 562 nm. The signal is stable for at least 1 hour.

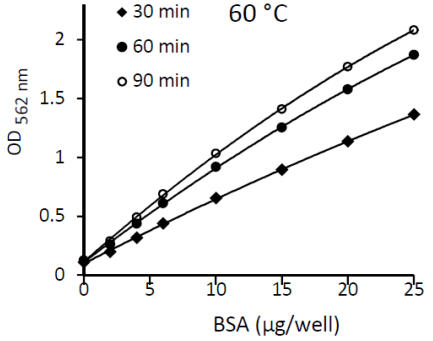
For unknown samples, several dilutions of a sample should be tested to ensure the OD reading is within the standard curve range.

5. Data Analysis

Subtract the blank OD (zero standard) 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.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

ab102536Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)