

ab102536

BCA Protein Quantification Kit

Instructions for Use

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

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

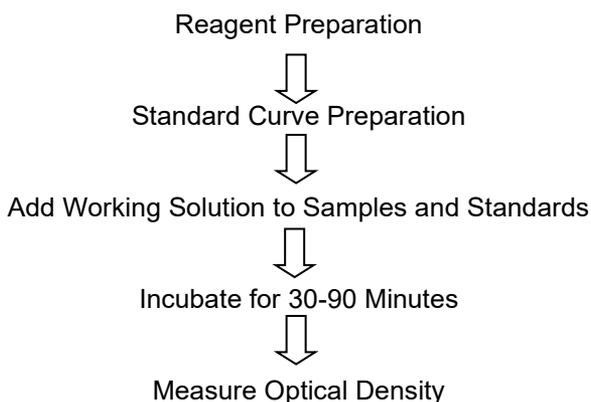
Table of Contents

1. Overview	2
2. Protocol Summary	2
3. Components and Storage	3
4. Assay Protocol	4
5. Data Analysis	7
6. Troubleshooting	8

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

All components in this kit are shipped on blue ice and are suitable for storage at +4°C, unless reconstituted. Upon receipt, immediately store kit at +4°C in the dark. Individual components may be stored at alternative temperatures as show in the table below. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

A. Kit Components

Item	Quantity	Storage Temperature
BCA Reagent	100 mL	+4°C or Ambient
Copper Reagent	2 mL	+4°C or Ambient
BSA Standard III*	1 mL	+4°C

PLEASE NOTE: BSA Standard III was previously labelled as BSA Standard (10 mg/ml). The composition has not changed.

*The BSA Standard III can be aliquoted after the first use and stored at -20°C.

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 μ l 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 to 1 mg/ml Stock Solution (i.e., 40 μ l + 360 μ l buffer). Ideally, use the same buffer contained in your samples.

Transfer 200 μ l 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 μ g
7	Tube 8 (200)	200	16 μ g
6	Tube 7 (200)	200	8 μ g
5	Tube 6 (200)	200	4 μ g
4	Tube 5 (200)	200	2 μ g
3	Tube 4 (200)	200	1 μ g
2	Tube 3 (200)	200	0.5 μ g
1	---	200	0 μ g

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

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

5. Add 100 μ l 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.

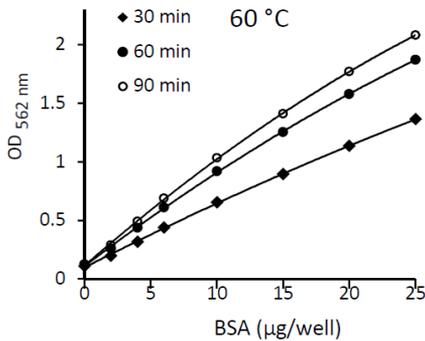
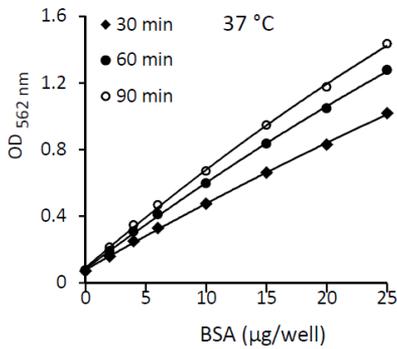
7. Measure OD at 562 nm. The signal is stable for at least 1 hour.

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

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