

INSTRUCTION MANUAL

Assay kits



Bicinchoninic Acid Protein Assay Kit (BCA)

The BCA protein assay kit provides a robust and detergent-tolerant colorimetric detection and quantification of total protein.

Description	Content	Catalog Number	Number of assays (96-well plate)	Number of assays (1mL cuvettes)
BCA Protein Assay Kit	BCA Reagent A (500mL) Copper Solution B (15mL) BSA 5x1mL - 1500µg/mL	BCA2500	2500	500

For any technical questions, contact us at tech@ozbiosciences.com

1. Technology

1.1. Description

BCA assay kit offers a fast colorimetric detection and quantification method of total protein content even in the presence of detergents. This kit is based on the reduction of Cu^{2+} to Cu^{1+} by protein in alkaline solution; monovalent copper ions produced are detected in a concentration-dependent manner. Bicinchoninic acid (BCA) chelates with the reduced copper Cu^{1+} and form a water-soluble purple reaction complex that exhibits a strong absorbance at 562 nm. Absorbance is linear over a wide range of protein concentrations between 25-2000 $\mu\text{g/mL}$.

In general, protein concentrations are estimated with reference to a commonly used protein standard; the kit also includes Bovine Serum Albumin (BSA) at 1.5 mg/mL as a protein standard for a convenient preparation of protein concentration standard curves.

1.2. Storage and shipping condition

Storage: Store at 4°C.

Shipping condition: The kit is shipped at RT.

NOTE: During long term storage or upon shipping in cold weather, Reagent A or B may precipitate; we recommend to gently warming and stirring the solution to dissolve precipitates.

2. Applications and Protocols

2.1. General Considerations

- We recommend using a 50:1 ratio between Reagent A and B. Different ratios were demonstrated to impair protein detection.
- Detection of small protein amount can be performed by increasing time of incubation at 37°C.

2.2. Solution preparation

Standard Solution.

Prepare a range of concentrations from 1500 $\mu\text{g/mL}$ to 50 $\mu\text{g/mL}$ by serial dilutions according to the protocol below.

Prepare 7 tubes containing H₂O or sample buffer, refer to the illustration and table below:

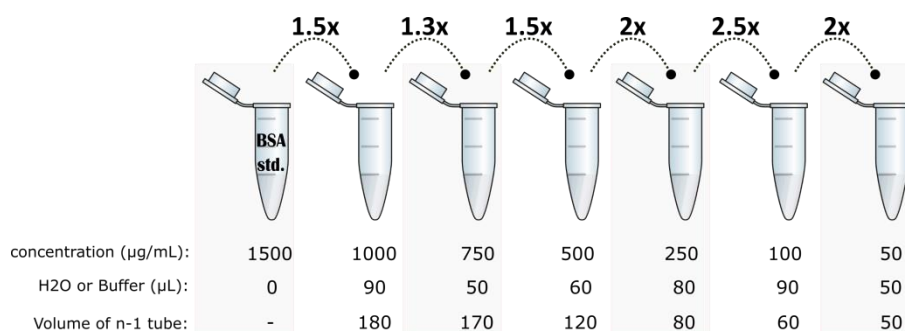


Figure 1: Recommendations for performing serial dilutions using standard BSA.

Vial	Volume of BSA (µL)	Volume of Diluent (µL)	Final BSA concentration (µg/mL)
1	280 of 1500 µg/mL stock	0	1500
2	180 of vial 1	90	1000
3	170 of vial 2	50	750
4	120 of vial 3	60	500
5	80 of vial 4	80	250
6	60 of vial 5	90	100
7	50 of vial 6	50	50
8 (blank)	0		0

Table 1: Volumes to consider for preparing BSA standards:

Working Solution (WR).

Prepare a working solution by mixing 50 parts of BCA reagent A with 1 part of copper solution B. Refer to the table 1 below to prepare solution for 1 mL cuvette or 96-well plate (200µL).

Number of assays		Volumes		
96-well plate (200 µl)	Cuvette (1mL)	BCA Reagent A	Copper solution B	Total Working solution (WR)
1	-	196 µL	4 µL	0.2 mL
5	1	980 µL	20 µL	1.0 mL
25	5	4.9 mL	0.1 mL	5.0 mL
50	10	9.8 mL	0.2 mL	10.0 mL
100	20	19.6 mL	0.4 mL	20.0 mL

Table 2: Volumes to consider for preparing Working Solution

2.3. Microplate procedure – General protocol for 96-well plate

1. Add **25 µL** of each standard point to a 96-well plate
2. Add **25 µL** of sample to 96-well plate.

NOTE: We recommend performing at least duplicate; for concentrated samples, dilute 5x or 10x your sample in sample buffer or in H₂O.

3. Prepare a blank with **25 µL** of sample buffer or H₂O.
4. Add **200 µL** of Working Solution to each sample, standard and blank wells
5. Incubate **1 H** at **37°C**.
6. Read absorbance at **562 nm**

NOTE: Wavelengths from 540-595 nm can also be used.

7. Subtract background fluorescence of the blank from all other values.
8. Calculate the amount of protein present in samples.

2.4. General protocol for 1mL Test Tubes

1. Add **100 µL** of each standard point into 1.5 mL tube
2. Add **100 µL** of sample into 1.5 mL tube
3. Prepare a blank with **100 µL** of sample buffer or H₂O.
4. Add **1 mL** of Working Solution to each sample, standard and blank wells
5. Incubate **1 H** at **37°C**.
6. Read absorbance at **562 nm**

NOTE: Wavelengths from 540-595 nm can also be used.

7. Subtract background fluorescence of the blank from all other values.
8. Calculate the amount of protein present in samples.

2.5. Results

Standard curve

Create a standard curve by plotting Absorbance over protein standard amount (µg/mL).

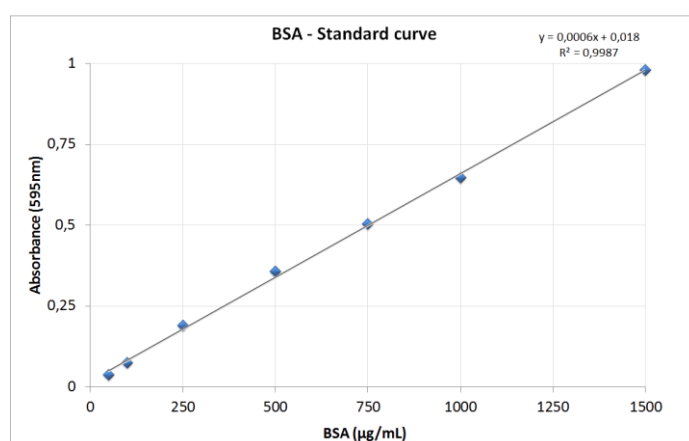


Figure 2: Standard curve realized using serial dilutions of standard BSA

Use the standard curve to determine the sample protein concentration.

2.5. Interfering compounds and compatible substances

The BCA protein assay kit is detergent-tolerant as demonstrated by the experiment below.

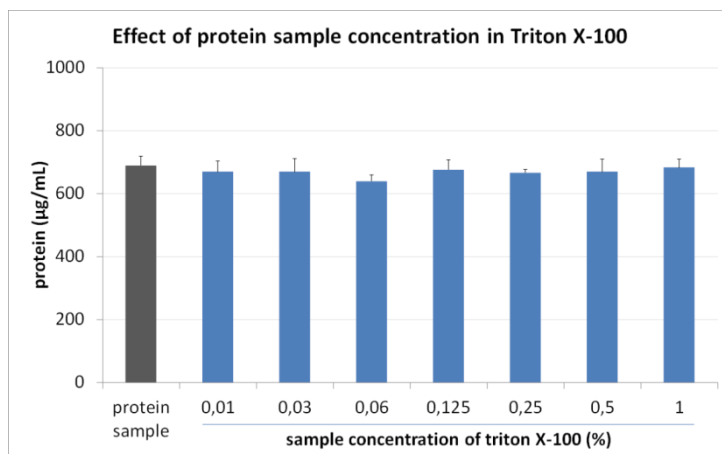


Figure 3: Triton X-100 concentration effect on BCA quantification

Results demonstrated that, as opposed to other quantification methods (Bradford), up to 1% triton X-100 in sample does not interfere with protein quantification using BCA assay kit

The following table list compounds that are compatible with BCA – Protein Assay Kit and their maximal non-interfering concentrations:

Compound	Maximal non interfering concentration
2-Mercaptoethanol	0.01 %
Acteone	10 %
CaCl	10 mM
CHAPS	5 %
DMF	10 %
DMSO	10 %
EDTA	50 mM
Ethanol	10 %
Glycerol	10 %
HCl	100 mM
Imidazole, pH 7.0	50 mM
NaCl	10 mM
MOPS	100 mM
NP40	5 %
SDS	5 %
Sucrose	40 %
TBP	10 mM
TCEP	2 mM
TFA	0.005 %
Thiourea	500 mM
Tris	0.5 M
Triton™ X-100	5 %
TWEEN®	5 %
Urea	3 M

Table 3: Compatible compounds and their maximal non-interfering concentrations.

Substances such as Asorbic acid, EGTA, Iron, Hydrogen Peroxide, Tyrosine, Uric Acid, Phenol Red, Creatinin are known to interfere with BCA – Protein Assay Kit, even when used at small concentrations.

These lists are not exhaustive. For an optimal reading, we recommend assaying the protein of interest in ultrapure water alone; dialysis or protein precipitation may also be used to remove interfering substance.

3. Related Products

ASSAY KITS
Luciferase Assay kit OZBlue Cell viability kit Bradford – Protein Assay Kit FluoProdige – Fluorescent Protein Assay Kit MTT cell proliferation kit SEAP Assay Kit X-Gal Staining Kit Senescence Kit for Stem Cells β -Galactosidase assay kits (CPRG/ONPG)

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