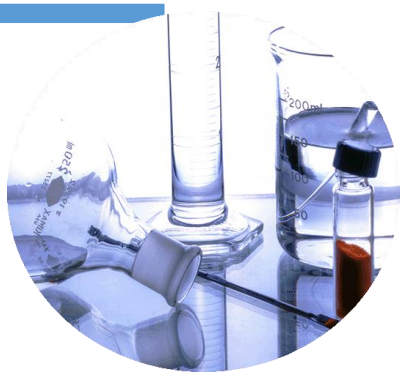
# BCA – Protein Assay Kit

# 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)<br>Copper Solution B (15mL)<br>BSA 5x1mL - 1500µg/mL | BCA2500 | 2500 | 500 |

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

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# 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 µg/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 µg/mL to 50 µg/mL by serial dilutions according to the protocol below.

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

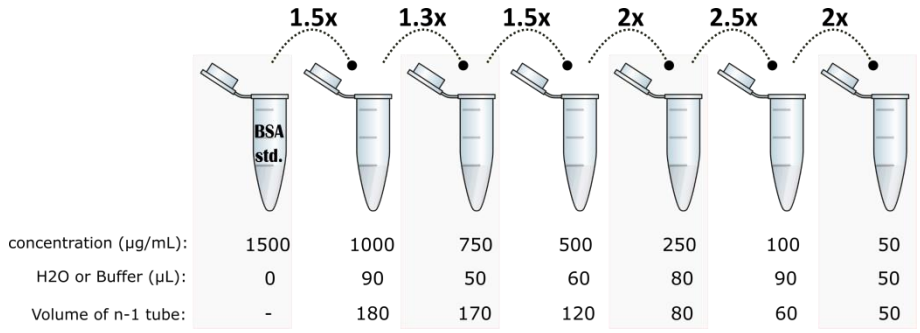


**Figure 1:** Recommendations for performing serial dilutions using standard BSA.

| 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).

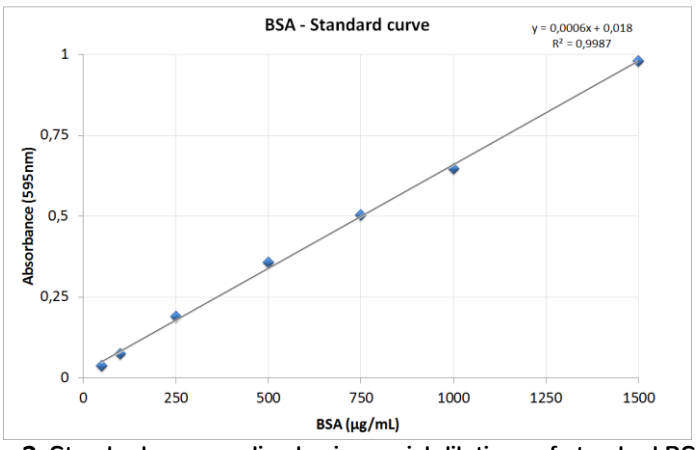


**Figure 2:** Standard curve realized using serial dilutions of standard BSA

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.

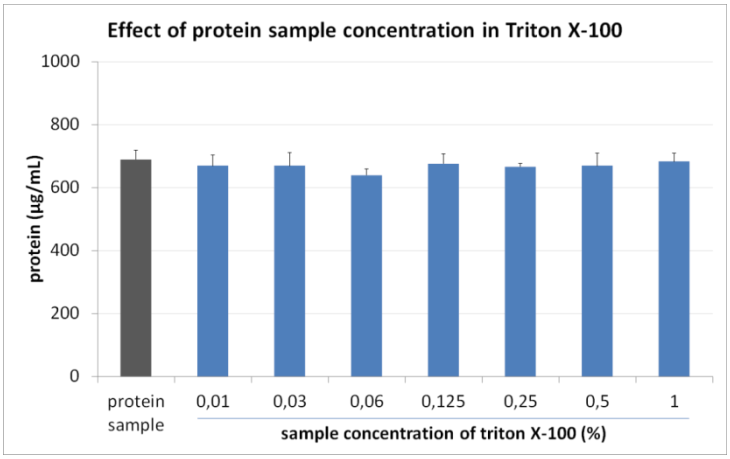


**Figure 3:** Triton X-100 concentration effect on BCA quantification

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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