Bicinchoninic Acid (BCA)

Colorimetric Assay kit

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-44105-96

www.zellx.de

Sample Types Validated for:

Serum, Plasma, Tissue Homogenates and Cell Lysates

!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

Table of Contents

Please read this insert completely prior to using the product.

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Introduction

Background

Bicinchoninic acid (BCA) assay is one of the most common methods of protein determination in biochemical research. The principle of the assay relies on two reactions: first, the reduction of Cu^{2+} to Cu^{1+} in an alkaline medium by cysteine, cystine, tryptophan, tyrosine, and peptide bonds present in the protein, and second, the chelation of two molecules BCA with one Cu^{1+} which forms a water-soluble purple-colored complex. The purple-colored product exhibits a strong absorbance at 562nm that is nearly linear with increasing protein concentrations over a broad working range (6-1,000 µg/mL).

Assay principle

The ZellX® BCA assay is designed to quantitatively measure protein concentration in a variety of samples. ZellX® BCA assay Kit, can measure the amount of protein at both low and normal concentration. Cu^{2+} ion is reduced to Cu^{1+} by protein under alkaline condition which is reacting with bicinchoninic acid to generate a purple-colored product which is read at 560 nm, proportional to the protein amount. A Bovine Serum Albumin standard is provided to create a standard curve for the assay.

General information

Materials supplied in the Kit

Component	*Quantity*
Bovine Serum Albumin Standard (10 mg/mL)	100 µL
BCA Reagent	8 mL
BCA Enhancer	160 µL
Clear Half Area 96 Well Plate	1 plate
Plate sealer	1 sealer

Storage instruction

All reagents should be stored at room temperature until the expiration date of the kit.

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Materials required but not supplied

Deionized water (diH_2O)

Microplate/ELISA reader capable of reading optical absorption at 560 nm

Precision pipettes, multichannel/repeater pipettes and disposable pipettes tips

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The Bovine Serum Albumin (BSA) standard contains low concentrations (≤0.9%) of sodium azide and must be disposed with copious amounts of water.

General remarks

- The instruction must be strictly followed.
- The reading of Microplate/ELISA reader must be set at the appropriate wavelength.
- Pipette tips should not be used more than once to prevent cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Assay protocol

Reagent preparation

BCA Working Solution: Add 150 µL BCA Enhancer to 7.35 mL BCA Reagent. The color of Working Solution changes to light green. **BCA Working Solution should be used within 12 hours of preparation.**

Sample preparation

Samples must be diluted in diH_2O. Dilutions should be made to ensure that protein levels in samples fall within the standard curve range.

- Cell lysate should be diluted ≥ 1:10
- Serum and plasma samples should be diluted ≥ 1:100
- Urine samples should be diluted ≥ 1:2

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The assay will tolerate most common laboratory chemicals. Known incompatibilities include reducing agents such as dithioerythritol (DTE), dithiothreitol (DTT) and ß-mercaptoethanol (BME) at concentrations above 1 mM. Organic solvent content should be below 1%.

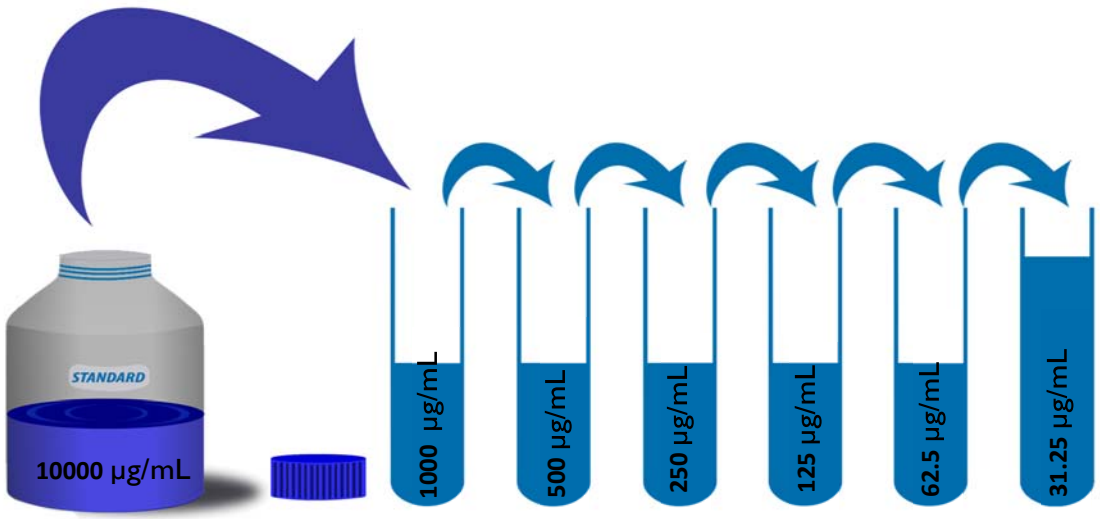
All the samples must be used within 2 hours of dilution

Standard preparation

For Normal protein concentration

- Prepare a 1:10 dilution of BSA Standard with diH_2O (mix 20 µL of standard with 180 µL of diH_2O), and label as the Standard No.6 (1000 µg/mL).
- Make series of lower dilutions as described in the table.
- The diH_2O is used as the 0 µg/mL standard.

No.	*Concentration*	*Material needed*
Standard No.6	1000 µg/mL	20 µL BSA Standard + 180 µL diH_2O
Standard No.5	500 µg/mL	100 µL Standard No.6 + 100 µL diH_2O
Standard No.4	250 µg/mL	100 µL Standard No.5 + 100 µL diH_2O
Standard No.3	125 µg/mL	100 µL Standard No.4 + 100 µL diH_2O
Standard No.2	62.5 µg/mL	100 µL Standard No.3 + 100 µL diH_2O
Standard No.1	31.25 µg/mL	100 µL Standard No.2 + 100 µL diH_2O
Standard No.0	0 µg/mL	100 µL diH_2O



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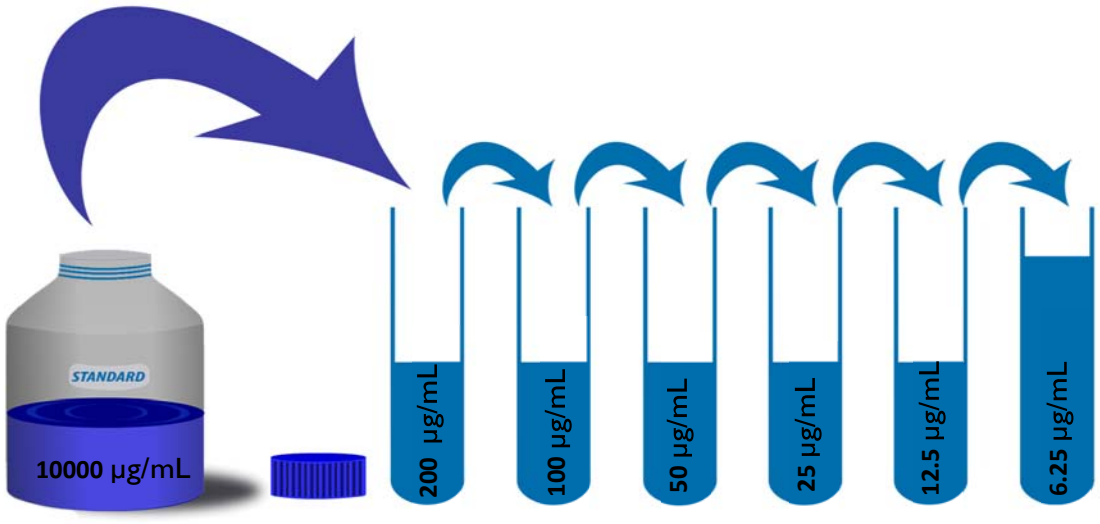
For low protein concentration

- Prepare a 1:50 dilution of BSA Standard with diH_2O (mix 10 µL of standard with 490 µL of diH_2O), and label as the Standard No.6 (200 µg/mL).
- Make series of lower dilutions as described in the table.
- The diH_2O is used as the 0 µg/mL standard.

No.	*Concentration*	*Material needed*
Standard No.6	200 µg/mL	10 µL BSA Standard + 490 µL diH_2O
Standard No.5	100 µg/mL	200 µL Standard No.6 + 200 µL diH_2O
Standard No.4	50 µg/mL	200 µL Standard No.5 + 200 µL diH_2O
Standard No.3	25 µg/mL	200 µL Standard No.4 + 200 µL diH_2O
Standard No.2	12.5 µg/mL	200 µL Standard No.3 + 200 µL diH_2O
Standard No.1	6.25 µg/mL	200 µL Standard No.2 + 200 µL diH_2O
Standard No.0	0 µg/mL	200 µL diH_2O



All standard must be used within 2 hours of preparation

Assay Procedure

For Normal protein concentration

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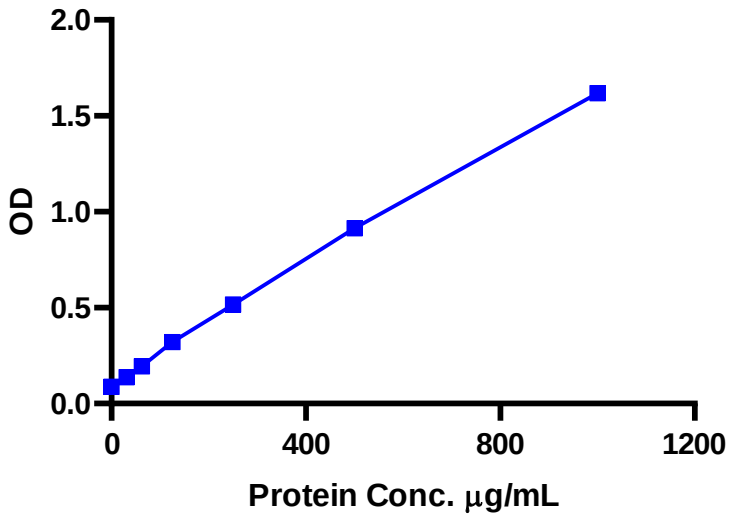
1. Pipette 10 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 10 µL of diH_2O as the Zero standard.
3. Add 75 µL of the BCA Working Solution to each well using a multichannel/repeater pipette.
4. Seal the plate and incubate at 37°C for 2 hours.
5. Read the optical density at 560 nm.

For low protein concentration

1. Pipette 50 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 50 µL of diH_2O as the Zero standard.
3. Add 75 µL of the BCA Working Solution to each well using a multichannel/repeater pipette.
4. Seal the plate and incubate at 37°C for 2 hours.
5. Read the optical density at 560 nm.

Calculation

- Average the duplicate optical density (OD) readings for each standard and sample.
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader using the adjusted OD values
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.



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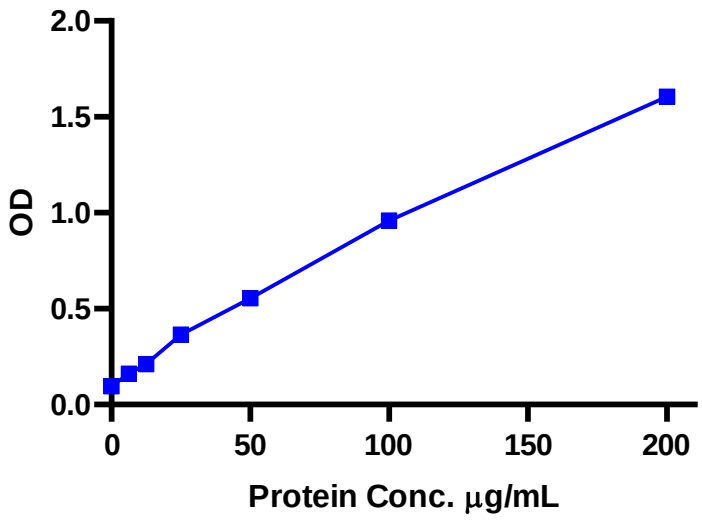
A typical standard curve of ZellX® BCA Assay kit for normal protein concentration

Run your own standard curves for calculation of results



A typical standard curve of ZellX® BCA Assay kit for low protein concentration

Run your own standard curves for calculation of results

Assay range

The detection limit of ZellX® BCA assay was determined as 6.98 µg/mL for normal protein concentration and 2.68 µg/mL for low protein concentration.

Sensitivity

The sensitivity of the ZellX® BCA assay was determined as 6.65 µg/mL for normal protein concentration and 1.68 µg/mL for low protein concentration.

Precision

For Normal protein concentration

Intra-Assay Precision (Precision within an assay): 3 samples were tested 20 times in an assay.

Inter-Assay Precision (Precision between assays): 3 samples were tested in duplicate on 13 different assays over multiple days.

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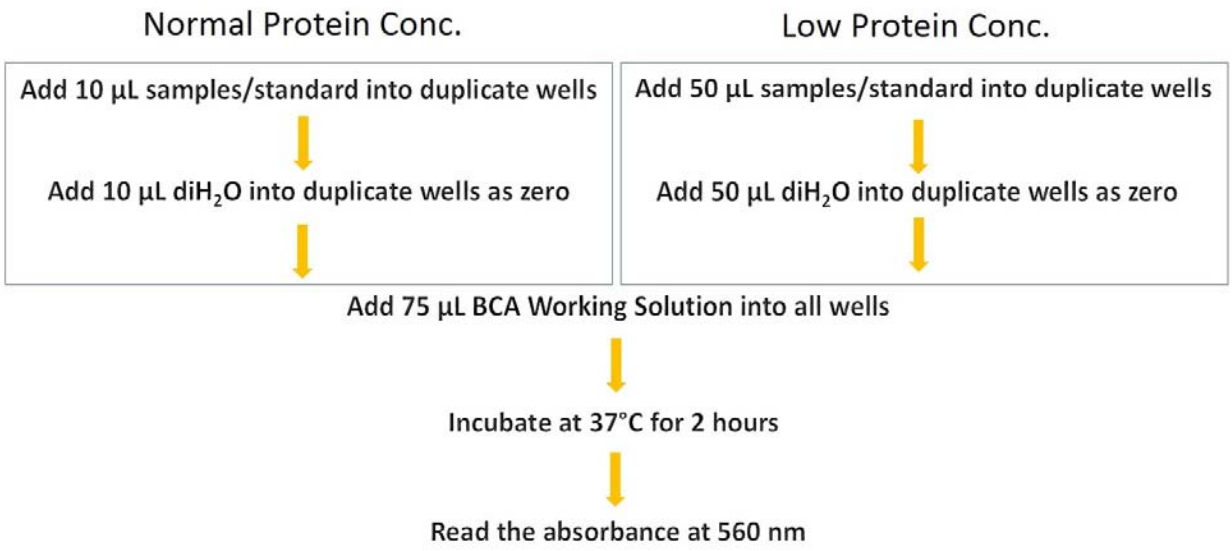
Item	*%CV*
Intra assay	3.5, 5.5, 6.9
Inter assay	6.3, 3.8, 10.4

Protocol summary



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References

1. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ., “Protein measurement with the Folin phenol reagent”. J. Biol. Chem. 1951, 193 (1): 265–75.
2. Smith, PK, et. al., “Measurement of protein using bicinchoninic acid.”, Anal. Biochem., 1985, 150: (1), 76-85

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