ab207002
BCA protein assay kit for low concentrations

Instructions for use:

For measuring total protein concentration of pure proteins, extracts or lysates.

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 6a, Last Updated 05 June 2023

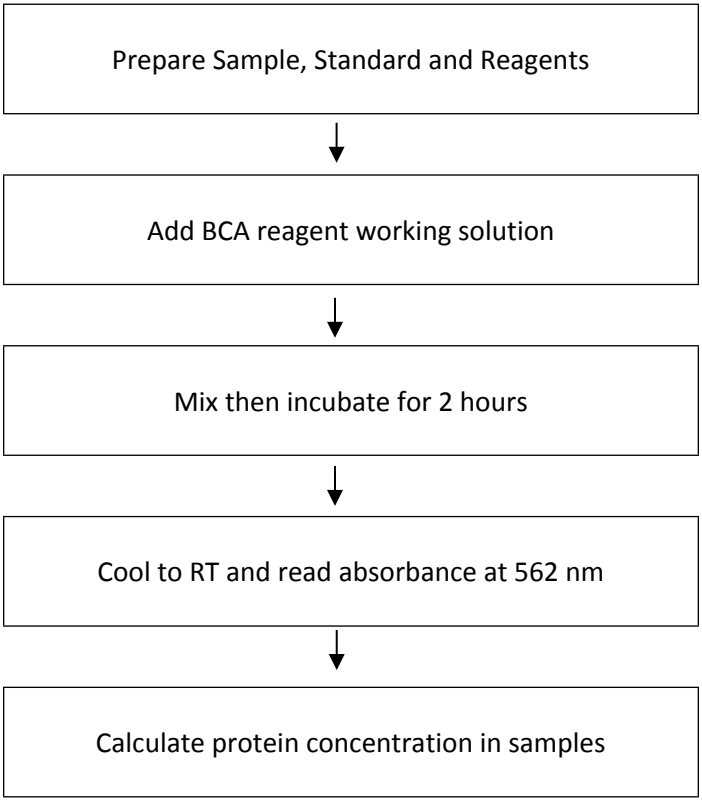
Table of Contents

1. BACKGROUND

Abcam's BCA protein assay kit for low concentrations (ab207002) measures total protein concentration of pure proteins, extracts or lysates. It provides an ultra-sensitive method to detect and quantify total protein concentration. The kit has been optimized for determining total protein content in low protein concentration samples (0.5-40 µg/mL), even in the presence of detergents.

The assay is based on the reaction of bicinchoninic acid (BCA) with the cuprous cation (Cu^{1+}), which is generated by reduction of cupric cation (Cu^{2+}) by the protein in alkaline conditions. The Cu^{1+}-BCA chelate is a water-soluble complex and exhibits a strong absorbance at 562 nm that is linear over a concentration range of 0.5-40 µg/mL. The kit includes Bovine Serum Albumin (BSA) as a reference protein standard.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at room temperature immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 6 and 9.

5. LIMITATIONS

- Kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
BCA Reagent A	190 mL	RT	RT
BCA Reagent B	190 mL	RT	RT
BCA Reagent C	9 mL	RT	RT
BSA Standard II/BSA	5 x 1 mL	RT	RT

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- Sterile microcentrifuge tubes
- Test tubes
- Spectrophotometer
- Microplate
- Microplate reader
- Incubator
- Parafilm
- Microplate adhesive plate sealer

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single protein concentration assessment. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Make sure the spectrophotometer is switched on before starting the experiment.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening

9.1. **BCA Working Reagent:**

Mix **BCA Reagent A**, **BCA Reagent B** and **BCA Reagent C** in the ratio of 25:25:1. Upon mixing, green-colored turbidity will be observed that should disappear upon further mixing to give a green colored solution. Keep reagent at RT.

Each sample replicate requires 150 µL of BCA working reagent for each well in a microplate assay or 1 mL for a test tube procedure. Prepare sufficient amount of BCA Working Reagent solution needed for samples and all BSA Standards.

Note: *It is recommended that BCA reagent working should be prepared just before use.*

9.2. **BSA Standard II/BSA:**

Ready to use as supplied.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- It is recommended that same diluent should be used to make the BSA Standard solutions as that of the protein samples.
- Prepare a 200 µg/mL BSA Standard working solution by diluting 0.5 mL of BSA Standard in 4.5 mL of de-ionized water or the diluent. Using this working solution, prepare BSA Standard solutions as suggested in the table below using the same diluent. Other similar concentrations can also be used within the assay range of 0.5-40 µg/mL. One tube of BSA Standard is sufficient to make diluted solutions in triplicates.

Tube #	Volume of BSA (mL)	Volume of Diluent (mL)	Final BSA Concentration (µg/mL)
1	1 mL of 200 µg/mL Stock	4	40
2	4 mL of tube 1	4	20
3	4 mL of tube 2	4	10
4	4 mL of tube 3	4	5
5	4 mL of tube 4	4	2.5
6	3.2 mL of tube 5	4.8	1
7	4 mL of tube 6	4	0.5
8 (Blank)	0	8	0

11. SAMPLE PREPARATION

- Prepare different concentrations of protein samples by diluting with de-ionized water or an appropriate diluent to a concentration within the assay range (0.5-40 µg/mL).
- For unknown samples, it is recommended to use three different concentrations of samples and perform the assay in duplicates or triplicates.

12. ASSAY PROCEDURE

- It is recommended to assay all standards, controls and samples in duplicate or triplicate.

12.1. **Microplate Procedure**

12.1.1. Add 150 µL of each BSA Standard and protein samples into separate microtiter plate wells.

12.1.2. Add 150 µL of BCA reagent working solution to each well containing BSA Standards and samples.

12.1.3. Mix thoroughly for 30 seconds.

12.1.4. Seal the plate with an adhesive plate sealer and incubate at 37°C for 2 hours.

12.1.5. After incubation, cool the plate to room temperature and ensure that there is no liquid on the plate sealer.

12.1.6. Set the absorption wavelength of a microplate reader to 562 nm and record the absorbance (OD_{562}) of all BSA Standards and samples.

12.2. **Test Tube Procedure**

12.2.1. Add 1 mL of each BSA Standard and protein samples into a separate 4 mL test tube.

12.2.2. Add 1 mL of BCA reagent working to each tube and mix well.

12.2.3. Seal the tubes with parafilm and incubate at 60°C for 1 hour.

12.2.4. After incubation, cool the tubes to room temperature and ensure that there is no liquid on the parafilm seal.

12.2.5. Set the wavelength of spectrophotometer at OD 562 nm. Calibrate the instrument to zero by using water or the diluent only.

12.2.6. Measure the absorbance (OD_{562}) of all the BSA Standards (Sample 1-7) and protein samples.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalysed. The actual concentration should be calculated by multiplying the measured concentration by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1. Subtract OD_{562} of Blank (0 Standard, # 8) from all readings.

13.2. Plot the BSA standard curve: OD_{562} (on Y axis) vs BSA Standard concentration (on X axis).

13.3. Obtain the equation from the plot in the form of Y = aX + b

13.4. Use the obtained value of slope (a) to calculate protein concentration in samples.

Protein concentration in sample:

$$C = DX = Dilution\ Factor\ x\ \frac{(Y - b)}{a} = \mu g/mL$$

Where:

C = protein concentration of sample

Y = OD_{562} of protein sample

X = measured concentration of protein sample (measured after dilution)

a = Slope of BSA standard curve

b = Y axis intercept of the standard Curve

D = Dilution factor of protein sample

Alternatively, get the sample concentration from the standard curve. Then calculate protein concentration in sample as:

$$C = DX$$

14. TYPICAL DATA

TYPICAL STANDARD CURVE – data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed

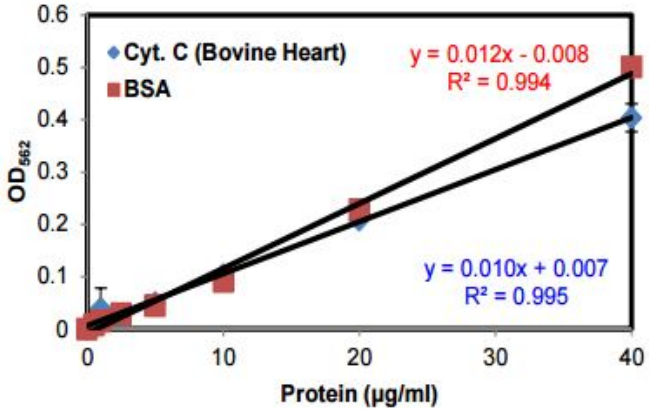


Figure 1: Typical standard curve obtained for BSA and Cytochrome C from bovine heart using ab207002 and the microplate procedure (37°C for 2 hours).

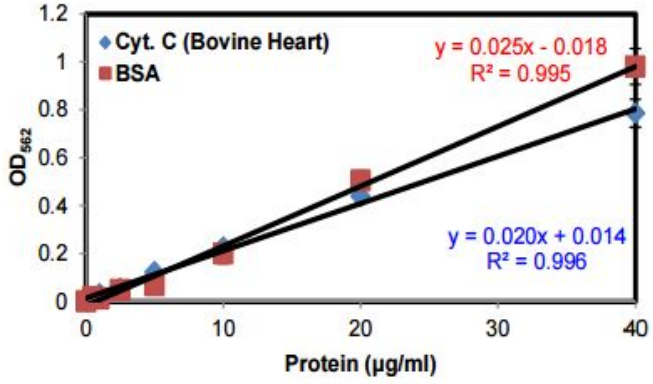


Figure 2: Typical standard curve obtained for BSA and Cytochrome C from bovine heart by using ab207002 and the test tube procedure (60°C for 1 hour).

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare BCA Working Reagent: mix BCA Reagent A, BCA Reagent B and BCA Reagent C in the ratio of 25:25:1.
- Prepare Standard curve:

Tube #	Volume of BSA (mL)	Volume of Diluent (mL)	Final BSA Concentration (µg/mL)
1	1 mL of 200 µg/mL Stock	4	40
2	4 mL of tube 1	4	20
3	4 mL of tube 2	4	10
4	4 mL of tube 3	4	5
5	4 mL of tube 4	4	2.5
6	3.2 mL of tube 5	4.8	1
7	4 mL of tube 6	4	0.5
8 (Blank)	0	8	0

- Prepare different concentrations of protein samples in duplicate within the assay range 0.5-40 µg/mL.
- Add 150 µL of BCA reagent working solution to 150 µL of BSA Standards and samples for microplate procedure. Add 1 mL of BCA reagent working solution to 1 mL of BSA Standards and samples for test tube procedure.
- Mix and incubate for 2 hours at 37°C (microplate procedure) or 1 hour at 60°C (test tube procedure).
- Cool to RT and read absorbance at 562 nm.
- Calculate protein concentration in samples.

16. NOTES

RESOURCES

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)