ab207003
BCA protein assay kit reducing agent compatible (microplate)

Instructions for use:

For measuring total protein concentration of pure proteins, extracts or lysates in the presence of reducing agents using a microplate reader.

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 6b, Last Updated 15 January 2024

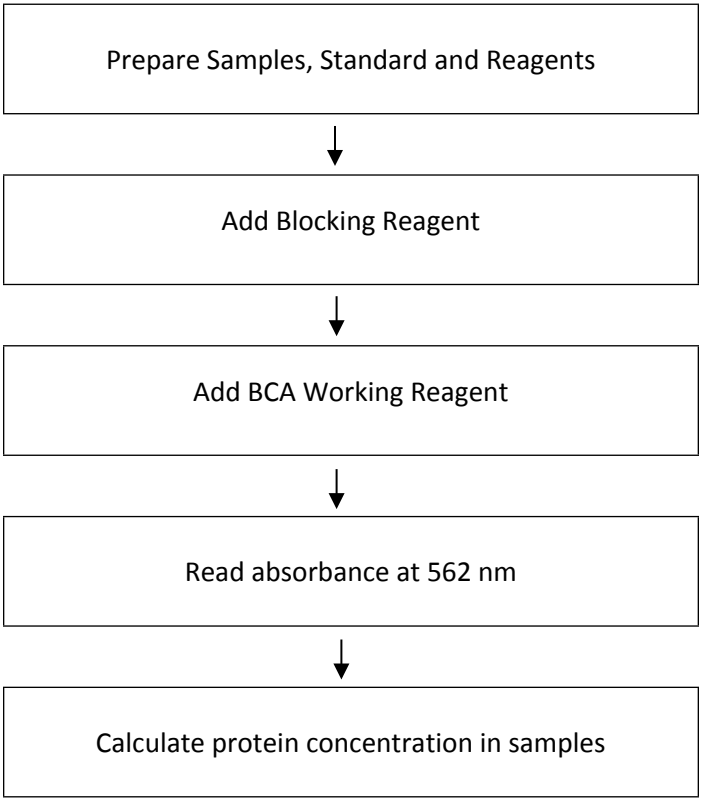
Table of Contents

1. BACKGROUND

Abcam's BCA protein assay kit reducing agent compatible (microplate) (ab207003) measures total protein concentration of pure proteins, extracts or lysates in the presence of reducing agents.

Unlike the majority of protein assay kits, ab207003 is compatible with strong reducing agents such as TCEP up to 20 mM, DTT up to 10 mM and β-mercaptoethanol up to 35 mM. This kit is based on the chelation 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 assay is linear over a wide range of protein concentrations (25-2000 µg/mL). The kit also includes Bovine Serum Albumin (BSA) as a reference protein standard. The kit has been formatted for microplate-based protein assays.

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	200 mL	RT	RT
BCA Reagent B	20 mL	RT	RT
BSA Standard II/BSA	10 x 1 mL	RT	4°C
Blocking Reagent	20 mg x 1 vial	RT	RT
Blocking Reagent Buffer	20 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
- 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** with **BCA Reagent B** in the ratio of 50:1. 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 200 µL of BCA Working Reagent. Prepare sufficient amount of BCA Working Reagent solution needed for samples and all BSA Standards.

9.2. Blocking Reagent:

Dissolve one tube (20 mg) of the **Blocking Reagent** in 1 mL of **Blocking Reagent Buffer**. Vortex for 30 seconds. This amount of solution is enough for the analysis of 50 samples (20 µL per sample). Keep reagent at RT.

To analyze fewer samples, weigh out necessary amount (0.4 mg/well) and dissolve in appropriate volume of Blocking Reagent Buffer (20 µL/well).

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Prepare BSA standards as suggested in the table below by diluting BSA Standard II/BSA Standard using de-ionized water or same diluent used to dilute the protein samples (with or without the reducing agent)*. Other similar concentrations can also be used within the assay range of 25-2000 µg/mL. One tube of BSA Standard II/BSA Standard is sufficient to make diluted solutions in triplicates.

Vial #	Volume of BSA Standard II/BSA (µL)	Volume of Diluent (µL)	Final BSA Concentration (µg/mL)
1 (Stock)	100 of 2 mg/mL Stock	0	2000
2	100 of 2 mg/mL Stock	100	1000
3	100 of vial 2	100	500
4	100 of vial 3	100	250
5	100 of vial 4	100	125
6	100 of vial 5	400	25
7 (Blank 1)	0	100**	0
8 (Blank 2)	0	100***	0

NOTES:* *It is recommended to prepare the BSA Standards using water or protein sample diluent without the reducing agent, and therefore prepare both Blank 1 and Blank 2. However, the BSA Standards can also be made using water or protein sample diluent containing the same amount of reducing agent as that of the protein samples. In that case, only Blank 1 is needed.*

*** Blank 1: Use water or protein sample diluent containing same concentration of reducing agent as that of the protein sample.*

**** Blank 2: Use water or protein sample diluent without reducing agent.*

11. SAMPLE PREPARATION

- Prepare different concentrations of protein samples by diluting with water or an appropriate diluent to a concentration within the assay range (25-2000 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. Add 20 µL of each BSA Standard, Blank(s) and protein samples into separate microtiter plate wells.

12.2. Add 20 µL of freshly prepared Blocking Reagent to all wells containing standards, blanks or samples.

12.3. Mix thoroughly for 30 seconds.

12.4. Seal the plate with an adhesive plate sealer and incubate at 37°C for 30 minutes.

12.5. Ensure that there is no liquid on the plate sealer. Add 200 µL of BCA Working Reagent to all wells containing standards, blanks or samples.

12.6. Mix thoroughly for 30 seconds.

12.7. Seal the plate with an adhesive plate sealer and incubate at 37°C for 30 minutes.

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

12.9. Read the absorbance (OD_{562}) of all BSA Standards and 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 2 (0 Standard, # 8) from all BSA Standards.

13.2. For samples, subtract OD_{562} of Blank 1 (0 Standard, # 7) from that of protein samples.

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

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

13.5. 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

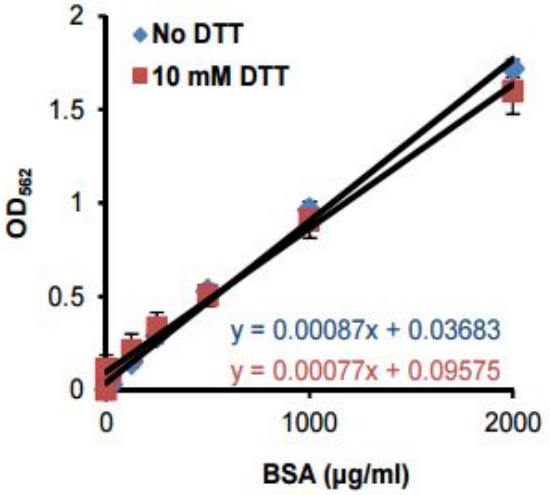


Figure 1: Standard curves for BSA containing blocking reagent in the presence and absence of 10 mM DTT obtained using ab207003.

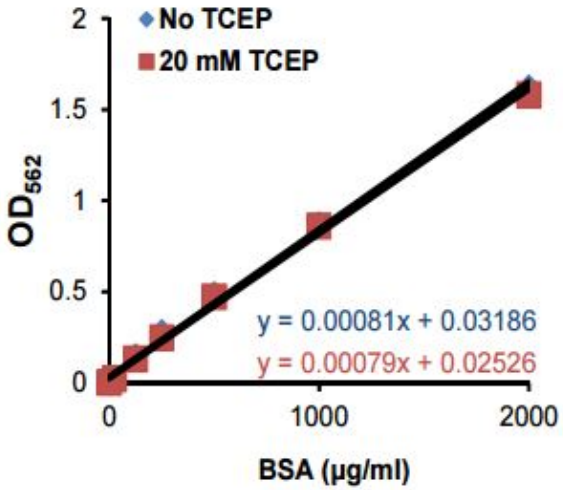


Figure 2: Standard curves for BSA containing blocking reagent in the presence and absence of 20 mM TCEP. Generated using ab207003.

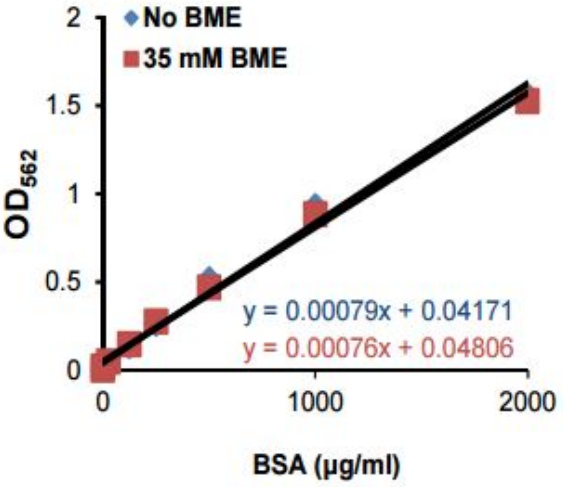


Figure 3: Standard curves for BSA containing blocking reagent in the presence and absence of 35 mM β-mercaptoethanol (BME). Generated using ab207003.

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 with BCA Reagent B in the ratio of 50:1 and Blocking Reagent: dissolve one tube (20 mg) of Blocking Reagent in 1 mL of Blocking Reagent Buffer.
- Prepare standard curve.

Vial #	Volume of BSA Standard II/BSA (µL)	Volume of Diluent (µL)	Final BSA Concentration (µg/mL)
1 (Stock)	100 of 2 mg/mL Stock	0	2000
2	100 of 2 mg/mL Stock	100	1000
3	100 of vial 2	100	500
4	100 of vial 3	100	250
5	100 of vial 4	100	125
6	100 of vial 5	400	25
7 (Blank 1)	0	100**	0
8 (Blank 2)	0	100***	0

- Prepare different concentrations of protein samples in duplicate within the assay range 25-2000 µg/mL.
- Add 20 µL of BSA Blocking Reagent to 20 µL of BSA Standards, Samples and Blanks.
- Mix and incubate for 30 minutes at 37°C.
- Add 200 µL of BCA Working Reagent to each well and mix.
- Incubate at 37°C for 30 minutes.
- Cool to RT and read absorbance at 562 nm.
- Calculate protein concentration in samples.

16. NOTES

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)