ab207004
BCA protein assay kit reducing agent compatible (test tube)

Instructions for use:

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

This product is for research use only and is not intended for diagnostic use.

Version 5 Last Updated 21 December 2015

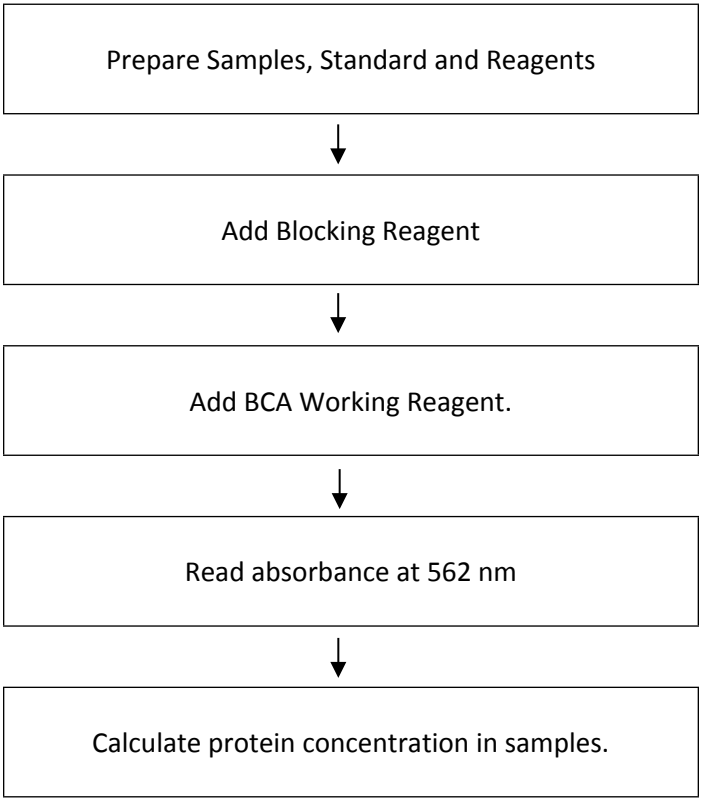
Table of Contents

1. BACKGROUND

Abcam's BCA protein assay kit reducing agent compatible (test tube) (ab207004) measures total protein concentration of pure proteins, extracts or lysates in the presence of reducing agents.

Unlike the majority of kits on the market, ab207004 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 test tube-based 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	250 mL	RT	RT
BCA Reagent B	20 mL	RT	RT
BSA Standard	10 x 1 mL	RT	4°C
Blocking Reagent	20 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
- Incubator
- Parafilm

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single protein reducing assay. 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 1 mL 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 20 samples (50 µL per sample). Keep reagent at RT.

To analyze fewer samples, weigh out necessary amount (1 mg/tube) and dissolve in appropriate volume of Blocking Reagent Buffer (50 µL/tube).

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 using de-ionized water or same diluent used to dilute the protein samples (with or without the reducing agent)*. Other similar dilutions can also be used within the assay range of 25-2000 µg/mL. One tube of BSA Standard is sufficient to make diluted solutions in triplicates.

Vial #	Volume of BSA (µL)	Volume of Diluent (µL)	Final BSA Concentration (µg/mL)
1 (Stock)	200 of 2 mg/mL Stock	0	2000
2	200 of 2 mg/mL Stock	200	1000
3	200 of vial 2	200	500
4	200 of vial 3	200	250
5	200 of vial 4	200	125
6	100 of vial 5	400	25
7 (Blank 1)	0	200**	0
8 (Blank 2)	0	200***	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 50 µL of each BSA Standard, Blank(s) and protein samples into separate clean test tubes.

12.2. Add 50 µL of freshly prepared Blocking Reagent to each tube.

12.3. Mix thoroughly for 30 seconds.

12.4. Seal the tubes with parafilm and incubate at 37°C for 30 minutes.

12.5. Ensure that there is no liquid on the parafilm. Add 1 mL of BCA Working Reagent to each tube.

12.6. Mix thoroughly for 30 seconds.

12.7. Seal the tubes with parafilm 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 parafilm.

12.9. Read the absorbance (OD562) 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 reanalyzed, then multiplying the concentration found 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 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 = concentration of protein sample

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

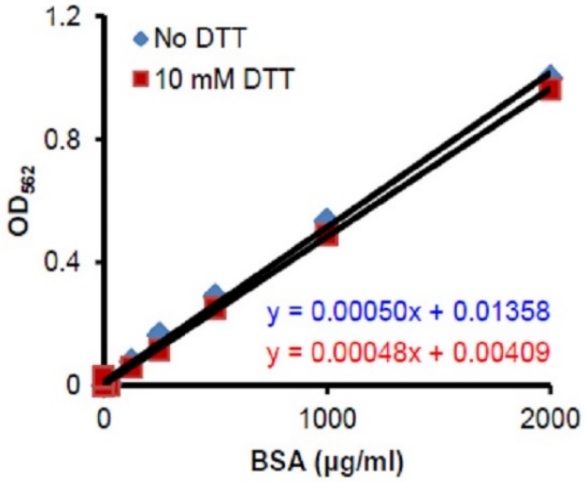


Figure 1: Standard curves for BSA containing blocking reagent in the presence and absence of 10 mM DTT obtained using ab207004.

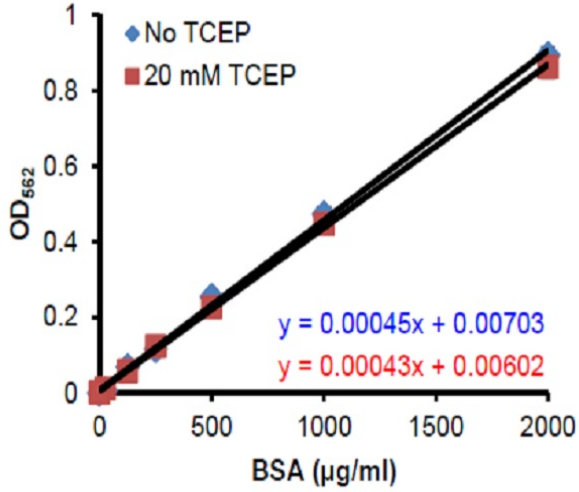


Figure 2: Standard curves for BSA containing blocking reagent in the presence and absence of 20 mM TCEP. Generated using ab207004.

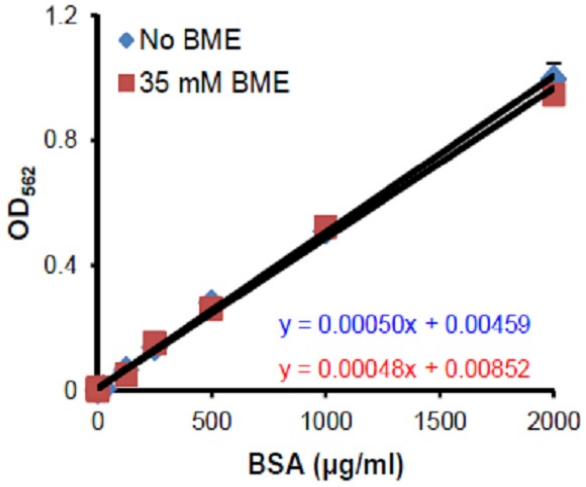


Figure 3: Standard curves for BSA containing blocking reagent in the presence and absence of 35 mM β-mercaptoethanol (BME). Generated using ab207004.

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 (µL)	Volume of Diluent (µL)	Final BSA Concentration (µg/mL)
1 (Stock)	200 of 2 mg/mL Stock	0	2000
2	200 of 2 mg/mL Stock	200	1000
3	200 of vial 2	200	500
4	200 of vial 3	200	250
5	200 of vial 4	200	125
6	100 of vial 5	400	25
7 (Blank 1)	0	200**	0
8 (Blank 2)	0	200***	0

- Prepare different concentrations of protein samples in duplicate within the assay range 25-2000 µg/mL.
- Add 50 µL of BSA Blocking Reagent to 50 µL of BSA Standards, samples and Blanks to.
- Mix and incubate for 30 minutes at 37°C.
- Add 1 mL of BCA Working Reagent to each tube 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

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 108008523689 (中國聯通)

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940