



Micro BCA Protein Assay Kit

Item No. 760200

www.caymanchem.com

Customer Service 800.364.9897

Technical Support 888.526.5351

1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity	Storage
760201	Micro BCA Reagent 1	1 vial/15 ml	RT
760202	Micro BCA Reagent 2	1 vial/15 ml	RT
760203	Micro BCA Reagent 3	1 vial/1 ml	RT
704003	Protein Determination BSA Standard	1 vial/200 µl	4°C
700020	Half-Volume 96-Well Clear Plate	5 plates	RT
400012	96-Well Cover Sheet	5 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent via email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader with the ability to measure absorbance at 562 nm
2. An orbital microplate shaker
3. Adjustable pipettes; multichannel or repeating pipettor recommended
4. Microcentrifuge tubes

INTRODUCTION

About This Assay

Cayman's Micro BCA Protein Assay Kit is a microplate-based, colorimetric method for rapid total protein quantification. Based on the well-known bicinchoninic acid (BCA) method, the reduction of Cu^{2+} to Cu^{1+} by protein in an alkaline solution (the biuret reaction) is combined with the highly sensitive colorimetric detection of the cuprous cation (Cu^{1+}) by BCA. As two molecules of BCA are chelated by one cuprous ion, a purple-colored product is formed, with strong absorbance at 562 nm.

Performing the assay is simple: pipette 50 μl of the diluted protein samples into a well of the half-volume 96-well plate, add 50 μl of Working Reagent, incubate for 2 hours with shaking at room temperature, and measure the absorbance at 562 nm. Protein concentrations are calculated from a second-order polynomial curve fit to a series of standard protein dilutions assayed on the same plate as the unknown samples. While the BCA method is not a true end-point, the rate of continued color development is sufficiently slow to assay a large number of samples together.

Cayman's Micro BCA Protein Assay Kit is a quantitative assay that can be used with various buffers, water, plasma, serum, urine, and cell lysates. The assay has a dynamic range of 4 to 40 $\mu\text{g}/\text{ml}$, a lower limit of detection (LLOD) of approximately 2 $\mu\text{g}/\text{ml}$, and a lower limit of quantification (LLOQ) of 4 $\mu\text{g}/\text{ml}$.

PRE-ASSAY PREPARATION

Reagent Preparation

1. Micro BCA Reagent 1 - (Item No. 760201)

This vial contains 15 ml of Micro BCA Reagent 1, which includes sodium carbonate, sodium bicarbonate, and sodium tartrate. It is ready to use as supplied.

2. Micro BCA Reagent 2 - (Item No. 760202)

This vial contains 15 ml of Micro BCA Reagent 2, which includes BCA. It is ready to use as supplied.

3. Micro BCA Reagent 3 - (Item No. 760203)

This vial contains 1 ml of Micro BCA Reagent 3, which includes 4% cupric sulfate. It is ready to use as supplied.

4. Protein Determination BSA Standard - (Item No. 704003)

Each vial contains 200 μ l of 10 mg/ml BSA in a 0.9% saline solution with 0.05% sodium azide. Mix 10 μ l of Protein Determination BSA Standard with 990 μ l of the same diluent as the sample(s) to make a 100 μ g/ml solution. Prepare the BSA Standard dilutions according to Table 1 (see page 7).

Well	Volume of 100 μ g/ml BSA Standard (μ l)	Volume of Diluent (μ l)	Final Concentration (μ g/ml)
A	200	300	40
B	150	350	30
C	100	400	20
D	75	425	15
E	50	450	10
F	40	460	8
G	20	480	4
H	0	500	0

Table 1. Preparation of the BSA standards

Working Reagent Preparation

Use the following formula to determine the total volume of Working Reagent required:

$$(\# \text{ standards} + \# \text{ unknowns}) \times (\# \text{ replicates}) \times (50 \mu\text{l}) = \text{volume of Working Reagent needed}$$

Example for 4 unknowns and 2 replicates of each sample:

$$(8 \text{ standards} + 4 \text{ unknowns}) \times (2 \text{ replicates}) \times (50 \mu\text{l}) = 1.2 \text{ ml of Working Reagent needed}$$

Prepare the Working Reagent by mixing 25 parts Micro BCA Reagent 1 with 24 parts Micro BCA Reagent 2 and 1 part Micro BCA Reagent 3. For the above example, combine 0.75 ml of Micro BCA Reagent 1 with 0.72 ml of Micro BCA Reagent 2 and 30 μl of Micro BCA Reagent 3.

NOTE: When Micro BCA Reagent 3 is first added to Micro BCA Reagents 1 and 2, turbidity will be observed but will quickly disappear upon mixing to yield a clear, green Working Reagent. Once mixed, the Working Reagent is stable for several days when stored in a closed container at room temperature.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout of standards and samples to be measured in duplicate is given below in Figure 1. The user may vary the location of wells as needed for the number of samples being assayed. We suggest you record the contents of each well on the template sheet provided (see page 17).

	1	2	3	4	5	6	7	8	9	10	11	12
A	(A)	(A)	(S1)	(S1)	(S9)	(S9)	(S17)	(S17)	(S25)	(S25)	(S33)	(S33)
B	(B)	(B)	(S2)	(S2)	(S10)	(S10)	(S18)	(S18)	(S26)	(S26)	(S34)	(S34)
C	(C)	(C)	(S3)	(S3)	(S11)	(S11)	(S19)	(S19)	(S27)	(S27)	(S35)	(S35)
D	(D)	(D)	(S4)	(S4)	(S12)	(S12)	(S20)	(S20)	(S28)	(S28)	(S36)	(S36)
E	(E)	(E)	(S5)	(S5)	(S13)	(S13)	(S21)	(S21)	(S29)	(S29)	(S37)	(S37)
F	(F)	(F)	(S6)	(S6)	(S14)	(S14)	(S22)	(S22)	(S30)	(S30)	(S38)	(S38)
G	(G)	(G)	(S7)	(S7)	(S15)	(S15)	(S23)	(S23)	(S31)	(S31)	(S39)	(S39)
H	(H)	(H)	(S8)	(S8)	(S16)	(S16)	(S24)	(S24)	(S32)	(S32)	(S40)	(S40)

A-H = Standards

S1-S40 = Sample wells

Figure 1. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 100 μl in all wells.
- All reagents except samples must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the samples and standards be assayed at least in duplicate (triplicate is recommended).

Performing the Assay

1. **Standard Wells:** Add 50 μl of each BSA standard to the designated wells on the plate (see Figure 1, on page 9).
2. **Sample Wells:** Add 50 μl of sample to the designated wells. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed at least in duplicate (triplicate recommended).
3. Add 50 μl of the Working Reagent to each well.
4. Cover the plate with the 96-Well Plate Cover (Item No. 400012).
5. Incubate at room temperature for two hours on an orbital plate shaker.
6. Remove the 96-Well Plate Cover and measure the absorbance at 562 nm using a plate reader.

Calculations

Many plate readers come with data reduction software that plot data automatically. Alternatively, a spreadsheet program can be used. We recommend using a second-order polynomial curve fit (see Figure 2 on page 13).

1. Calculate the average absorbance of each standard and sample.
2. Subtract the average absorbance of standard H from itself and all other values (standards and samples). This is the corrected absorbance.
3. Plot the corrected absorbance of the standards (from step 2 above) versus the final concentration of BSA (see Table 1 on page 7). See Figure 2, on page 13, for a typical standard curve.
4. Using the standard curve, calculate the protein concentration of each unknown sample.

Second-order Polynomial Fit: $(y = Ax^2 + Bx + C)$

For ease of calculation, the plot could be constructed with concentration on the y-axis and absorbance on the x-axis. The equation below can then be used to calculate the protein concentration of each unknown sample:

$$\text{Protein } (\mu\text{g/ml}) = [A \times (\text{sample absorbance})^2 + B \times (\text{sample absorbance}) + C] \times \text{sample dilution}$$

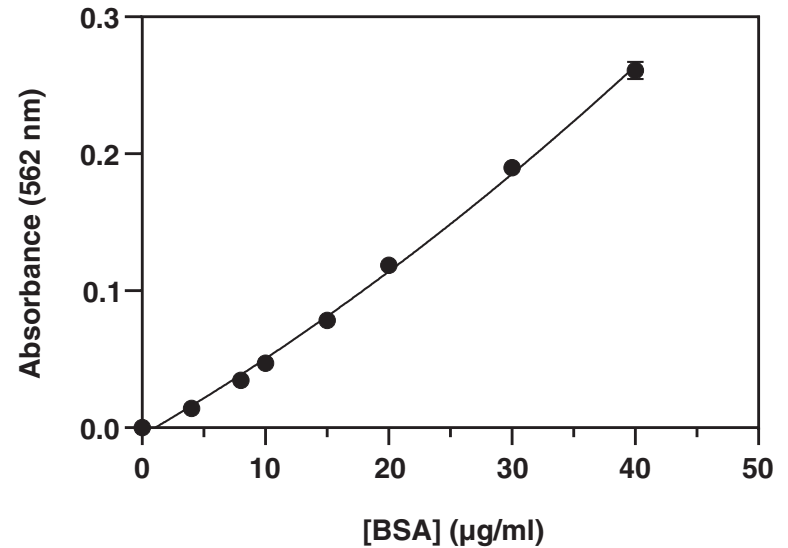


Figure 2. Typical standard curve

Linearity

Samples were serially diluted with appropriate buffers or water, and evaluated for linearity using the Micro BCA Protein Assay Kit. The results are shown in Table 2, below.

Dilution	Concentration (µg/ml)	Dilutional Linearity (%)
Purified Protein		
10	369.3	100
20	383.0	104
40	385.6	104
Urine		
5	2,336.6	100
10	2,589.9	111
20	2,749.3	118
40	2,904.3	124
80	3,002.9	129
Plasma		
4,000	86,558.6	100
8,000	88,792.8	103

Table 2. Linearity in purified protein, urine, and plasma

Interferences

Certain common laboratory solutions and solvents interfere with this assay. For example, buffers containing chelating agents, strong acids, strong bases, or substances with reducing potential are known to interfere with this assay.¹ Avoid the following substances:

Ascorbic acid, catecholamines, creatinine, cysteine, EGTA, impure glycerol, hydrogen peroxide, hydrazides, iron, lipids, melibiose, phenol red, impure sucrose, tryptophan, tyrosine, uric acid

To overcome any interference due to a particular solvent or solution:

1. Prepare the BSA standard curve using the same diluent as the samples.
2. Dialyze, desalt, or dilute the sample.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No color in any tubes	Sample contains a substance that chelates copper	Dialyze, desalt, or dilute the sample
Standards and samples show less color than expected	A. Strong acid or base will alter the pH of the working reagent B. Color is measured at the wrong wavelength	A. Dialyze, desalt, or dilute the sample B. Measure the absorbance at 562 nm
All tubes (including blank) are dark purple	Buffer contains a reducing agent, thiol, or biogenic amine	Dialyze or dilute the sample

Reference

- Smith, P.K., Krohn, R.I., Hermanson, G.T., *et al.* Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150(1)**, 76-85 (1985).

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H

Warranty and Limitation of Remedy

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