Protein Determination (BCA) Kit

Item No. 701780

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Technical Support 888.526.5351
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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a 4°C kit. After opening the kit, remove components and store as stated below.

Item Number	Item	Quantity	Storage
701786	BCA Reagent 1	1 vial/100 ml	RT
701787	BCA Reagent 2	1 vial/5 ml	RT
704003	Protein Determination BSA Standard	3 vials/200 µl	4°C
400014	96-Well Solid Plate (Colorimetric Assay)	5 plates	RT
400012	96-Well Cover Sheet	5 covers	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.

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 capable of measuring absorbance at 562 nm
2. Adjustable pipettes and a multichannel or repeating pipette

INTRODUCTION

About This Assay

Cayman's Protein Determination (BCA) 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: dilute your protein samples, pipette 25 µl of the diluted samples into a well of the 96-well plate, add 200 µl of Working Reagent, incubate for 30 minutes at 37°C, 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 Protein Determination (BCA) 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 25 to 2,000 ug/ml and a lower limit detection (LLOD) of approximately 22 ug/ml.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **BCA Reagent 1 - (Item No. 701786)**

   This vial contains 100 ml of BCA Reagent 1, which is comprised of sodium carbonate, sodium bicarbonate, BCA, and sodium tartrate in 0.1 M sodium hydroxide. It is ready to use as supplied.

2. **BCA Reagent 2 - (Item No. 701787)**

   This vial contains 5 ml of BCA Reagent 2, which is comprised of 4% cupric sulfate. It is ready to use as supplied.

3. **Protein Determination BSA Standard - (Item No. 704003)**

   Each vial contains 200 µl of 10 mg/ml BSA in a 0.9% saline solution with 0.05% sodium azide. Mix 120 µl of Protein Determination BSA Standard with 480 µl of the same diluent as the sample(s) to make a 2 mg/ml BSA Standard Solution. Prepare the BSA Standard dilutions according to Table 1 (see page 7).

Well	Volume of 2 mg/ml BSA Standard (µl)	Volume of Diluent (µl)	Final Concentration (µg/ml)
A	200	0	2,000
B	150	50	1,500
C	100	100	1,000
D	50	150	500
E	25	175	250
F	12.5	187.5	125
G	2.5	197.5	25
H	0	200	0

Table 1. Preparation of the BSA standards

Working Reagent Preparation

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

(# standards + # unknowns) x (# replicates) x (200 µl) =
volume of Working Reagent needed

Example for 4 unknowns and 2 replicates of each sample:

(8 standards + 4 unknowns) x (2 replicates) x (200 µl) =
4.8 ml of Working Reagent needed

Prepare the Working Reagent by mixing 50 parts BCA Reagent 1 (Item No. 701786) with 1 part BCA Reagent 2 (Item No. 701787). For the above example, combine 5 ml of BCA Reagent 1 with 100 µl of BCA Reagent 2.

NOTE: When BCA Reagent 2 is first added to BCA Reagent 1, 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 225 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.
- If the concentration of protein in the sample is not known or if it is expected to be beyond the range of the standard curve, it is prudent to assay the sample at several dilutions.
- It is recommended that the samples and standards be assayed at least in duplicate (triplicate is recommended).

Performing the Assay

1. **Standard Wells:** Add 25 µl of each BSA standard to the designated wells on the plate (see Figure 1, on page 9).
2. **Sample Wells:** Add 25 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 200 µl of the Working Reagent to each well.
4. Cover the plate with the 96-Well Plate Cover and mix thoroughly on an orbital plate shaker for 30 seconds.
5. Incubate at 37°C for 30 minutes.
6. Remove the 96-Well Plate Cover and measure the absorbance at 562 nm using a plate reader.

ANALYSIS

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.

Protein (µg/ml) =
[A x (sample absorbance)^2 + B x (sample absorbance) + C] x sample dilution

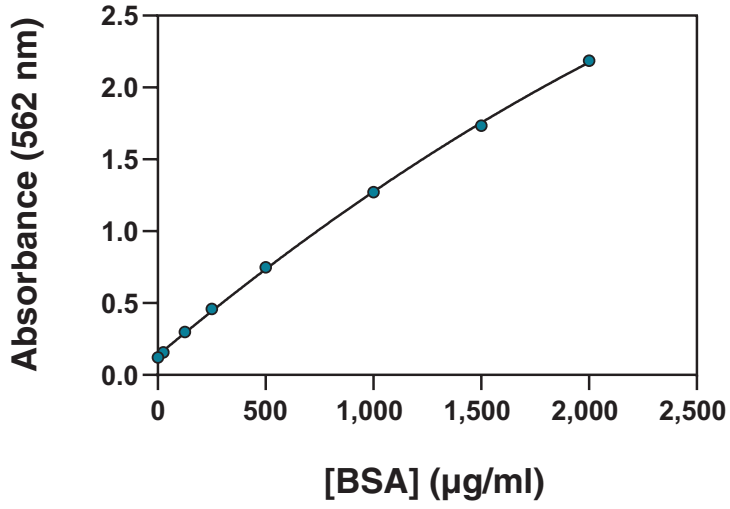


Figure 2. Typical standard curve

Performance Characteristics

Sensitivity:

Under the standardized conditions of the assay described in this booklet, the dynamic range of the kit is 25-2,000 µg/ml.

Precision:

When a series of 80 urine protein measurements were performed on the same day under the same experimental conditions, the intra-assay coefficient of variation was 4.3%. When a series of urine protein measurements were performed using seven replicates of a urine sample in seven separate assays, the inter-assay coefficient of variation was 8.6%.

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.

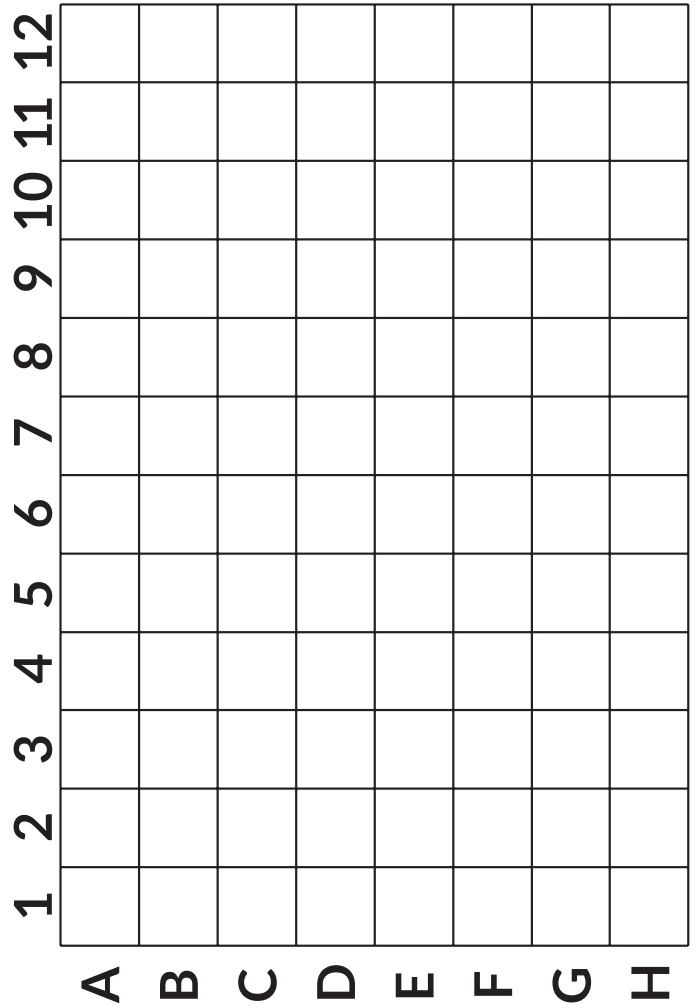
RESOURCES

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

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



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.