Cat. No. 296

CounPro®

BCA Protein Quantitation Assay Instructions

Quick Facts

1. Storage conditions before opening:
   Package A：4°C / Protect from light for a year；
   Package B：≦-20℃/ Protect from light for a year；
2. Absorption spectrometer detection wavelength (nm): 562。
3. The volume ratio of regent A / ragent B: 50 / 1.。
4. Sample / Working solution volume (ratio): 10 µL/200 µL (1/20).
5. Reaction temperature and time: 120 minutes at room temperature.
6. Working range of protein concentration：20~2000 µg/mL。

Introduction

The CounPro® BCA Protein Assay Kit is a formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein. This method combines the well-known reduction of Cu^{2+} to Cu^{+} by protein in an alkaline medium (the biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{+}) using a unique reagent containing BCA (**Fig. 1**). The purple-colored reaction product of this assay is formed by the chelation of two molecules of BCA with one cuprous ion. This water-soluble complex exhibits a strong absorbance at 562 nm that is nearly linear with increasing protein concentrations over a broad working range (20–2000 µg/mL). The BCA method is not a true end-point method; that is, the final color continues to develop. However, following incubation, the rate of continued color development is sufficiently slow to allow large numbers of samples to be assayed together. If the volume of the protein sample is large (0.1 mL), a cuvette can be used; if the sample volume is small (~10 µL), a 96-well plate can be used. Or you can use a 0.5 mL thin-walled PCR tube with the recommended spectrometer for measurement. Please contact us for more detail.

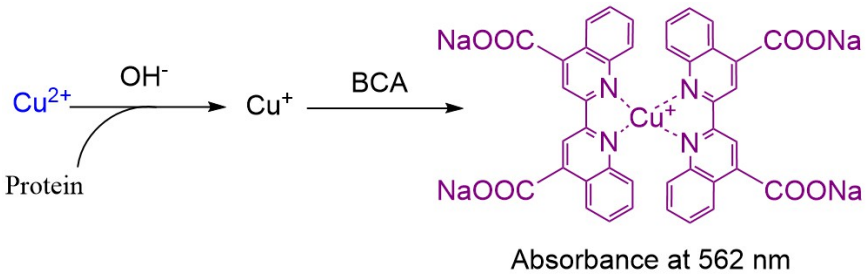

Fig. 1 Introduction of BCA Protein Assay

Kit Contents

Package A

1. CounPro® BCA-A reagent (black bottle)：500 ml X 1
2. CounPro® BCA-B reagent (transparent glass bottle)：11 ml X 1

Package B

3. Bovine serum albumin (BSA) standards：

A vial (2000 µg/mL)：5.0 mL X 1 瓶 (**Blue cap red label plastic bottle**)

B vial (1000 μg/mL)：5.0 mL X 1 瓶 (**Blue cap orange label plastic bottle**)
C vial (500 μg/mL)：5.0 mL X 1 瓶 (**Blue cap yellow label plastic bottle**)
D vial (250 μg/mL)：5.0 mL X 1 瓶 (**Blue cap green label plastic bottle**)
E vial (125 μg/mL)：5.0 mL X 1 瓶 (**Blue cap blue label plastic bottle**)
F vial (50 μg/mL)：5.0 mL X 1 瓶 (**Blue cap indigo label plastic bottle**)
G vial (25 μg/mL)：5.0 mL X 1 瓶 (**Blue cap purple label plastic bottle**)
H vial (0 μg/mL)：5.0 mL X 1 瓶 (**translucent plastic bottle**)

Shipping and Storage Information

Package	Contents	Shipping	Storage
A	Reagent A (black bottle)	4~8 °C	12 months under room temperature with protection from light.
	Reagent B (transparent glass bottle)		
B	BSA standards (5 ml plastic bottles)		12 months under -20 ℃ with protection from light.

Protocol

I、Preparation of the BCA working solution (WS)：

1. Use the following formula to determine the total volume of WS required:
   2.0 mL of WS is required for each sample in cuvette method；
   200 μl of WS is required for each sample in 96-well microplate method；
   400 μl of WS is required for each sample in 0.5 mL thin-walled PCR tube；
   A. total volume WS required：

(# standards + # unknowns) X (# replicates) X (volume of WS per sample) = total volume WR required

   B. example：For the standard cuvette procedure with 3 unknowns and 2 replicates of each sample:：

(8 standards + 3 unknowns) X (2 replicates) X (2 mL) = 44 mL WS

2. Prepare WS by mixing 50 parts of Reagent A (black bottle) with 1 part of Reagent B (transparent glass bottle). For the above example, combine 50 mL of Reagent A with 1mL of Reagent B.
   Note：When Reagent B is first added to Reagent A, turbidity is observed that quickly disappears upon mixing to yield a clear, green WS. Prepare sufficient volume of WS based on the number of samples to be assayed. Although the WS is stable for several days when stored in a closed container at room temperature, it is **NOT** recommended to store the WS overnight.

II、Cuvette (sample to WS ratio = 1:20)

1. Pipette 0.1 mL of each standard and unknown sample replicate into an appropriately labeled cuvette.
2. Add 2.0 mL of the WS to each cuvette and mix well.
3. Cover and incubate tubes at selected temperature and time: 37°C for 30 minutes or RT for 2 hours (working range = 20–2000 μg/mL)；60°C for 30 minutes (working range = 5–250 μg/mL).
4. Cool all tubes to RT.

5. With the spectrophotometer set to 562 nm, zero the instrument on a cuveĵe filled only with water, then measure the absorbance of all the samples within 10 minutes.
   Note：Because the BCA assay does not reach a true end point, color development will continue even after cooling to RT. However, because the rate of color development is low at RT, no significant error will be introduced if the 562 nm absorbance measurements of all tubes are made within 10 minutes of each other.
   Note：The detection is successful in the wavelength range of 540-590 nm.
6. Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562nm absorbance measurement of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 562 nm measurement for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

III、Microplate (sample to WR ratio = 1:8)

1. Pipette 25 µL of each standard or unknown sample replicate into a microplate well (working range = 20–2000 µg/mL).
2. Add 200 µL of the WS to each well and mix plate thoroughly on a plate shaker for 30 seconds.
3. Cover plate and incubate at 37°C for 30 minutes or RT for 2 hours (working range = 20–2000 µg/mL)；60°C for 30 minutes (working range = 5–250 µg/mL).
4. Cool plate to RT. Measure the absorbance at or near 562 nm on a plate reader.
   Note：Wavelengths from 540–590 nm have been used successfully with this method.
5. Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562 nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank–corrected 562 nm measurement for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

IV、0.5 mL thin-walled PCR tube（sample to WS ratio = 1:20）

1. Pipette 20 µL of each standard or unknown sample replicate into tubes.
2. Add 400 µL of the WS to each tube and mix well.
3. Cover tubes and incubate at 37°C for 30 minutes or RT for 2 hours (working range = 20–2000 µg/mL)；60°C for 30 minutes (working range = 5–250 µg/mL).
4. Cool tubes to RT. Measure the absorbance at or near 562 nm on the suggested reader.
   Note：Wavelengths from 540–590 nm have been used successfully with this method.
5. Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562 nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank–corrected 562 nm measurement for each BSA standard vs. its concentration in µg/mL. Use the

standard curve to determine the protein concentration of each unknown sample.

Additional information

1. Please protect all solutions from light.。
2. **Increasing the incubation time or temperature increases the net 562 nm absorbance for each test and decreases both the minimum detection level of the reagent and the working range of the protocol.**
   If prolonging the incubation time or increasing the temperature still CANNOT meet your detection needs at low protein concentration, you can adjust the ratio of agent A/agent B to **25/2**, and adjust the ratio of sample to working solution to **1/10**, incubate at room temperature for **15-30** minutes, in our experience, this adjustment can improve the detection sensitivity of the kit.
3. **Using a forced-air incubator can introduce significant error in color development because of uneven heat transfer.**

Troubleshooting

Symptom	Possible cause	Solution
No color development in any of the reactions	Sample contains a copper chelating agent	1. Dialyze, desalt, or dilute protein sample. 2. Increase copper concentration in BCA working reagent (i.e., use a 50:2 ratio of reagent A to 4% reagent B.
Blank absorbance is OK, but standards and samples absorbance measurements are lower than expected	Sample is in a strong acid or alkaline buffer	1. Dialyze, desalt, or dilute sample.
Absorbance measurements of protein samples higher than expected	Protein concentration is too high	1. Dilute sample.
	Sample contains lipids or lipoproteins	1. Add SDS to the protein sample (final concentration 2%) to reduce interference from lipids.
All reactions, including blank are dark purple	Buffer contains a reducing agent	1. Dialyze or dilute sample.
	Buffer contains a thiol	1. Dialyze or dilute sample.
	Buffer contains biogenic amines (catecholamines)	1. Dialyze or dilute sample.

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