Store at RT

#7780

BCA Protein Assay Kit

1 Kit
(960 assays)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 11/26/18

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Component #	Quantity
BCA Protein Assay Reagent A	11621	1 x 250 ml
BCA Protein Assay Reagent B	11622	1 x 25 ml
BCA Protein Assay Albumin Standard	11623	1 x 10 units
Compatibility Reagent	11785	1 x 48 Ea
Reconstitution Buffer	11791	1 x 15 ml

Storage: This kit is stable at room temperature for 12 months past the reference date indicated on the kit box label. Do not freeze and avoid higher than ambient temperatures. Protect from light: It is recommended to keep reagents within the kit box to minimize exposure to light.

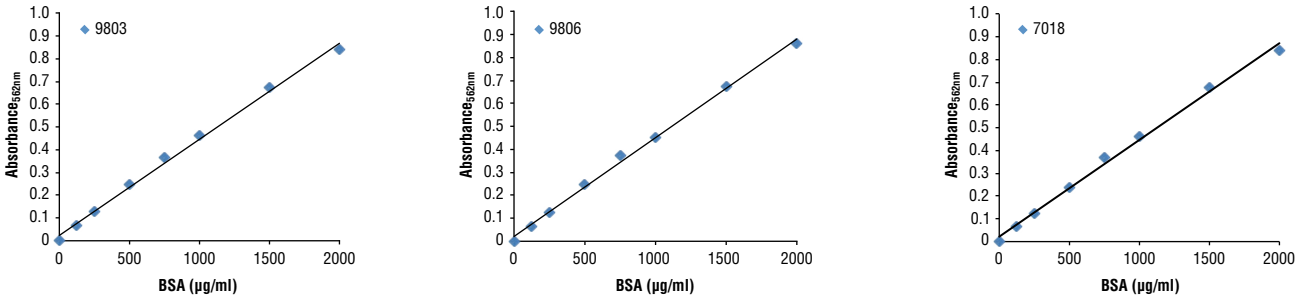
Directions for Use: Please see attached protocol.

Description: The BCA Protein Assay Kit can be used to measure the protein concentration of lysates or homogenates, in microplate format, prepared with the following buffers: Cell Lysis Buffer (10X) #9803, RIPA Buffer (10X) #9806, PathScan® Sandwich ELISA Lysis Buffer (1X) #7018. The dynamic range for this assay is 0.125 - 2 mg/mL. It is recommended that the BCA Compatibility Reagent be used to decrease interference from reducing agents, chelators, detergents, and other common ingredients found in most lysis buffers. Please see the attached protocol for additional details.

Background: Bicinchoninic Acid (BCA) is capable of forming an intense purple complex with cuprous ion, Cu^{1+}, in an alkaline environment. Cu^{1h+} is produced from the reaction of protein with alkaline Cu^{2+}. The resulting reaction and color produced is the basis for a common protein quantification method capable of measuring protein concentration over a wide range. Increasing protein concentrations produce proportionally deeper colors. The BCA protein assay demonstrates higher tolerances towards common interfering substances, such as nonionic detergents and buffer salts, than the Lowry technique (1).

Background References:

(1) Smith, P.K. et al. (1985) *Anal Biochem* 150, 76-85.



Standard curves generated with bovine serum albumin (BSA) ranging from 125 to 2,000 µg/ml using Cell Lysis Buffer #9803, RIPA Buffer #9806, and PathScan® Sandwich ELISA Lysis Buffer #7018.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

BCA Protein Assay Protocol

A Solutions and Reagents

Kit Contents:

1. BCA Reagent A: 250 ml (sodium carbonate, sodium bicarbonate, bicinchoninic acid, and sodium tartarate in 0.1 M sodium hydroxide).
2. BCA Reagent B: 25 ml (4% cupric sulfate).
3. Compatibility Reagent: 9.3 mg × 48 microtubes.
4. Reconstitution Buffer: 15 ml.
5. Albumin Standard (2 mg/ml): 10 × 1 ml ampules of bovine serum albumin (BSA) in 0.9% NaCl and 0.05% sodium azide.

Additional Required Materials:

1. 96-well plate compatible with absorbance measuring plate readers (such as Costar #3797)
2. Adjustable volume pipettes and disposable pipette tips
3. An incubator set at 37°C
4. Plate reader capable of measuring absorbance at 562 nm

B Standard Preparation

Dilute the contents of one Albumin Standard (BSA) ampule into several microcentrifuge tubes, using the same buffer as the unknown sample(s). Use the following table as a guide to prepare a set of standards (assay range = 125-2,000ug/ml).

Vial	Diluent/Sample Buffer Volume (µl)	BSA Source and Volume (µl)	Concentration (µg/ml)
A	0	200 of stock	2,000
B	66	200 of stock	1,500
C	100	100 of vial A	1,000
D	100	100 of vial B	750
E	100	100 of vial C	500
F	100	100 of vial E	250
G	100	100 of vial F	125

Note: Do not discard any unused, undiluted BSA standard (2 mg/ml). BSA standard can be stored in a microcentrifuge tube at 4°C for up to 2 weeks. Do not freeze.

C Reagent Preparation

Working Reconstitution Buffer:

Dilute the Reconstitution Buffer 1:1 with dH_2O. Do not dilute the Reconstitution Buffer if the protein sample has a pH < 6.0 or if it contains EDTA or imidazole.

Compatibility Reagent Solution:

Puncture the foil covering on the Compatibility Reagent tube with a clean pipette tip. Add 100 µl of Working Reconstitution Buffer into the tube and dissolve by stirring the bottom of the tube and pipetting up and down 15-20 times. Store this solution for up to 8 hr at 4°C protected from light. Used microtubes may be cut and discarded from the unused microtubes. Return the unused microtubes to pouch containing the desiccant pack.

Note: 4 µl Compatibility Reagent Solution will be required for each sample assayed.

BCA Working Reagent (WR):

Use the following formula to determine the total volume of WR required:
(# controls + # standards + # unknowns) × (# replicates) × (volume of WR per sample) = total volume WR required.
Example: For three unknowns and two replicates of each sample:
(2 controls + 7 standards + 3 unknowns) × (2 replicates) × (0.26 ml) = 6.24 ml WR required.

To prepare the WR, mix 50 parts BCA Reagent A with 1 part of BCA Reagent B (50:1, Reagent A:B). *6.24 ml WR = 6.12 ml Reagent A + 0.12 ml Reagent B*

Note: When Reagent B is added to Reagent A, the solution appears turbid but yields a clear, green WR upon mixing.

D Assay

Notes:

Precision pipetting is essential. For best results, use 1-10 µl pipettes. Completely evacuate the tip of any fluid. Small errors when pipetting account for large errors when measuring the absorbance.
Add samples directly to the center of the well and avoid touching the sides of the well. The same tip may be used within the same group of samples. Use a different tip for each sample when adding the Compatibility Reagent Solution.
If the protein sample has a pH < 5.0, dilute the sample 1:1 with Reconstitution Buffer.

Protocol:

1. Add 9 µl of Standard Control, Sample Control, Protein Standard, or unknown sample to individual wells on a microplate.
2. Add 4 µl Compatibility Reagent Solution to the sample in each well.
3. Cover plate and mix on a plate shaker at medium speed for 1 min and then incubate at 37°C for 15 min.
4. Add 260 µl WR to each well. Cover plate and mix on a plate shaker for 1 min. If the samples contain detergents, cover the plate after mixing. Incubate plate at 37°C for 30 min.
5. Cool plate at room temperature (RT) for 5 min.
6. Use the Standard Control as the blank. Measure the absorbance of the standards, unknown samples, and Sample Controls at 562 nm on a plate reader.

E Data Analysis

Because the BCA Assay does not reach a true end point, color development will continue even after cooling to RT. The absorbance increases at a rate of ~0.25% per minute at RT.

1. Subtract the average 562 nm absorbance value of the Sample Control replicates from the 562 nm absorbance value of all unknown sample replicates.
2. Prepare a standard curve by plotting the average blank-corrected 562 nm value for each BSA standard against its concentration (µg/ml). Use the standard curve to determine the protein concentration of each unknown sample.

Note: If using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) curve produces more accurate fit than a linear curve.

Important: This BCA Protein Assay Kit is compatible and has been thoroughly tested and approved to work with Cell Lysis Buffer (10X) #9803, RIPA Buffer (10X) #9806, and PathScan® Sandwich ELISA Lysis Buffer (1X) #7018 when each is used at 1X. The compatibility of this assay has not been evaluated on any other buffers.