Product Information

Bicinchoninic Acid solution

B9643

Product Description

Protein determination is one of the most common procedures in biochemical research. The principle of the bicinchoninic acid (BCA) assay is similar to the Lowry procedure,[1] in that both rely on the formation of a Cu^{2+}-protein complex under alkaline conditions, followed by reduction of the Cu^{2+} to Cu^{1+}. The amount of reduction is proportional to the protein present. It has been shown that Cys, cystine, Trp, Tyr, and the peptide bond[2] are able to reduce Cu^{2+} to Cu^{1+}. BCA forms a purple-blue complex with Cu^{1+} in alkaline environments, and thus provides a basis to monitor the reduction of alkaline Cu^{2+} by proteins.[3]

The BCA assay is more sensitive and applicable than either biuret or Lowry procedures. It also has less variability than the Bradford assay. The BCA assay has many advantages over other protein determination techniques:

- It is easy to use.
- The color complex is stable.
- There is less susceptibility to detergents.
- It is applicable over a broad range of protein concentrations.

In addition to protein determination in solution, the BCA protein assay has other applications, including determination of protein covalently bound to agarose supports and protein adsorbed to multi-well plates.

There are two distinct ways to perform a protein assay. A protein assay can be set up to measure the concentration of the unknown protein sample (mg/mL), or it can be set up to determine the total amount of protein in the unknown protein sample (mg). The BCA assay has a linear concentration range between 200-1,000 µg of protein per mL. In the standard assay, only 0.1 mL protein sample is used, so the assay has a total linear protein range of 20-100 µg.

B9643 is a component of the Bicinchoninic Acid Kit for Protein Determination (Cat. No. BCA1). Several theses[5] and dissertations[6-8] have cited use of product B9643 in their protocols.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Reagent

B9643 is a 1,000 mL solution containing bicinchoninic acid, sodium carbonate, sodium tartrate, and sodium bicarbonate in 0.1 N NaOH (final pH 11.25). B9643 is referred to as "Reagent A" in subsequent sections.

Other BCA1 kit components (Not provided)

- Copper(II) Sulfate Pentahydrate 4% Solution (Cat. No. C2284, "Reagent B")
- Protein Standard (Bovine Serum Albumin - BSA) Solution (Cat. No. P0914)

Materials Required (depending on the assay format used, but not provided)

- Spectrophotometer capable of accurately measuring absorbance in the 560 nm region
- 96-well plates, such as Cat. No. CLS9017
- 96-well plate sealing film: Cat. No. Z369667
- Test tubes, 13 × 100 mm: Cat. No. CLS980013
- 1 mL Disposable Plastic Cuvettes: Cat. No. C5416

Preparation Instructions

- The BCA Working Reagent is prepared by mixing 50 parts of B9643 (Reagent A) with 1 part of C2284 (Reagent B).
- Mix the BCA Working Reagent until it is light green in color.

Storage/Stability

- Store B9643 at Room Temperature.
- B9643 is stable for at least one year at Room Temperature in a closed container.
- The BCA Working Reagent (B9643 mixed with C2284) is stable for one day.

Procedure

In the standard assay, 20 parts of the BCA Working Reagent are then mixed with 1 part of a protein sample. For the 96-well plate assay, 8 parts of the BCA Working Reagent are mixed with 1 part of a protein sample. The sample is either a blank, a BSA protein standard, or an unknown sample. The blank consists of buffer with no protein. The BSA protein standard consists of a known concentration of bovine serum albumin, and the unknown sample is the solution to be assayed.

BCA assays are routinely performed at 37 °C. Color development begins immediately and can be accelerated by incubation at higher temperatures. Higher temperatures and/or longer incubation times can be used for increased sensitivity. Incubation at lower temperatures can slow down color development. The absorbance at 562 nm is recorded and the protein concentration is determined by comparison to a standard curve.

Standard 2.1 mL Assay Protocol

The linear concentration range is 200-1,000 µg/mL or 20-100 µg of total protein.

This is the standard assay that can be performed in a test tube. This procedure uses 0.1 mL of a protein sample and 2 mL of the prepared BCA Working Reagent. The instructions are a step-by-step procedure on how to perform the standard assay. If a nonstandard assay is used (such as a 96-well plate), adjust the volumes accordingly.

Note: It is necessary to create a standard curve during each assay, regardless of the format used.

1. Prepare the required amount of BCA Working Reagent needed for the assays (see Table 1).

   1.1. The final volume used in the assay depends upon the application and the equipment available.

   1.2. Table 1 can be used to determine the volume of BCA Working Reagent to prepare, depending on how many blanks, BSA protein standards, and unknown samples are to be assayed.

   1.3. Combine the volumes of Reagents A and B specified in the table. Mix until the BCA Working Reagent is a uniform, light green color.

Table 1. Volume of BCA Working Reagent to prepare. This is dependent on how many blanks, BSA protein standards, and unknown samples are to be assayed.

Number of Assays		Amount of Each Reagent Used		
Number of 2.1 mL Standard Test tube assays	Number of wells in a 96-well plate assay	Reagent A (mL)	Reagent B (mL)	Total volume of BCA Working Reagent (mL)
4	40	8	0.16	8.16
8	80	16	0.32	16.32
9	96	19	0.38	19.38
12	127	25	0.5	25.5

2. Prepare standards of different concentrations.

   2.1. These BSA protein standards can range from 200-1,000 µg/mL (20-100 µg of total protein). This is accomplished by making serial dilutions starting from the 1 mg/mL standard, and then using 0.1 mL of each diluted standard in the assay.

   2.2. It is best to make the dilutions in the same buffer as the unknown sample (see Table 2). Deionized water may be used as a substitute for the buffer, but any interference due to the buffer will not be compensated for in the BSA protein standards.

Table 2. Example of Standard Assay Set-Up Table

Tube No.	Sample (mL)	[BSA] Protein Standard (µg/mL)	BCA Working Reagent (mL)
1	0.1	0	2
2	0.1	200	2
3	0.1	400	2
4	0.1	600	2
5	0.1	800	2
6	0.1	1,000	2
7	0.1	(unknown 1)	2
8	0.1	(unknown 2)	2

- For protein samples with unknown concentrations, it may be necessary to prepare a dilution scheme to ensure that the concentration is within the linear range of 200-1,000 µg/mL.
- Two different unknown samples are represented in Table 2 by Tubes 7 and 8.
- Tube 7 is an unknown sample with a 5-fold dilution.

- Tube 8 is a different unknown sample at a 10-fold dilution.

Researchers must determine their own dilution schemes based on their estimation of the concentration of each unknown sample.

3. Add 2 mL of the BCA Working Reagent to 0.1 mL of each BSA protein standard, blank, and unknown sample. Vortex gently for thorough mixing. The total liquid volume in the test tube is 2.1 mL.
4. The following incubation parameters may be used:
   - 60 °C for 15 minutes, **or**:
   - 37 °C for 30 minutes, **or**:
   - 25 °C (Room Temperature) from 2 hours to overnight
5. If required, allow the tubes to cool to room temperature.
6. Transfer the reaction solutions into a cuvette.
7. Measure the absorbance of the solution at 562 nm. Color development continues slowly after cooling to room temperature, but no significant error is seen if all the tubes are read within 10 minutes of each other. Create an assay table as needed and a standard curve based on either the BSA protein standard concentration or on the amount of protein present in the BSA protein standard (Examples are shown in the results).
8. Determine protein concentration by comparison of the absorbance of the unknown samples to the standard curve prepared using the BSA protein standards.

Results Based on the Standard Assay

Create a table with the absorbance results obtained during the assay. A separate standard curve should be generated for each assay performed. The amount of protein for Tubes 1-6 was obtained from the known amount of BSA protein standard added.

Note: The data below should not be used as a replacement for a standard curve. The absorbance of the BSA protein standards (Tubes 1-6) in each assay will differ from those presented here. The amount of protein recorded for Tubes 7 and 8 was obtained from the standard curve.

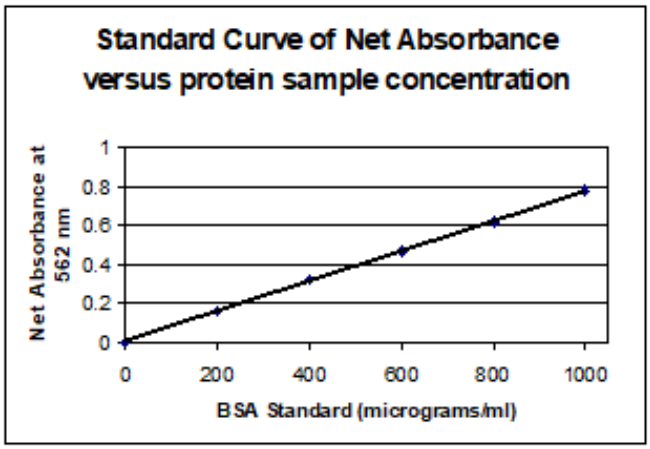
Table 3. Example of Assay Data Table

Tube No.	A_{562}	Net A_{562}	Amount of protein (µg) in sample	[Protein] of protein sample (µg/mL)	Dilution Factor
1	0.045	0	0	0	-
2	0.207	0.162	20	200	-
3	0.364	0.319	40	400	-
4	0.510	0.465	60	600	-
5	0.661	0.616	80	800	-
6	0.823	0.778	100	1,000	-
7	0.587	0.542	70	700	5
8	0.743	0.698	90	900	10

After obtaining the results, create a standard curve to determine the protein concentration in the unknown sample. Plot the Net Absorbance at 562 nm versus the BSA protein standard concentrations (µg/mL, Tubes 1-6).

Graph 1. Standard Curve produced from Assay Data

The standard curve indicates the unknown protein sample in Test Tube 7 (Net A_{562} = 0.542) contains 700 µg/mL of protein.



The actual concentration of protein present in the unknown sample is calculated as follows:

(µg/mL of unknown protein sample) times (Dilution Factor)

(700 µg/mL) × (5) = 3,500 µg/mL of protein

96-Well Plate Assay

Note: The linear concentration range is 200-1,000 µg/mL or 5-25 µg of total protein.

The BCA assay can be adapted for use in 96-well plates. 96-well plates can be used, as long as five main points remain unchanged:

1. Read the absorbance at 562 nm. For a plate reader, which does not have the exact wavelength filter, a filter in the range of 540-590 nm can be substituted.
2. The ratio of BCA Working Reagent to protein sample will have to be modified from the Standard Assay. Examples:
   - Standard Assay (Test Tube): 0.10 mL protein sample to 2 mL BCA Working Reagent (1:20)
   - 96-well plate: 25 µL protein sample to 200 µL BCA Working Reagent (1:8). When using multi-well plates, make sure the unknown samples, blanks, or standards are present in the wells prior to adding the BCA Working Reagent to facilitate mixing.
3. Make sure the protein assay containers are sealed (cover the plates with film) and incubate the samples for:
   - 60 °C for 15 minutes. **or**:
   - 37 °C for 30 minutes. **or**:
   - 25 °C (Room Temperature) from 2 hours to overnight
4. Keep the protein sample concentration between 200-1,000 µg/mL (5-25 µg total protein).
5. A separate standard curve will have to be determined for each assay protocol. The pathlength in each assay is dependent on the assay container (cuvettes or multi-well plates) and/or the reaction volume. These and other changes like the BCA Working Reagent-to-protein sample ratio affect the Net Absorbance values.

TCA Concentration-BCA Assay Protocol

By using this procedure, it is possible to remove some of the interfering substances that are described in the compatibility chart. It is also possible to increase the concentration of the unknown sample using this procedure.

1. Add the unknown samples and BSA protein standards to separate microcentrifuge tubes and adjust the final volumes to 1 mL with deionized water. Larger volumes can also be used by adjusting the following volumes accordingly.
2. Add 0.1 mL of a 0.15% (w/v) solution of sodium deoxycholate (Cat. No. D5670) prepared with deionized water.
3. Mix and let stand for 10 minutes at room temperature. It is also acceptable to let stand on ice for 10 minutes.
4. Add 0.1 mL of 6.1 N (~100% w/v) solution of trichloroacetic acid (TCA, Cat. No. T0699).
5. Cap and vortex each sample.
6. Incubate for 5 minutes at room temperature. It is also possible to let stand on ice for 5 minutes.
7. Centrifuge the samples for 15 minutes at room temperature in a microcentrifuge at full speed.
8. Carefully decant or pipette the supernatant of each sample. Do not disturb the pellet.
9. Solubilize each pellet by adding 0.04 mL of a 5% (w/v) solution of sodium dodecyl sulfate (SDS) prepared with a 0.1 N sodium hydroxide solution. Mix well until the pellet is completely dissolved.
10. Pipette 0.06 mL of deionized water into the tube to bring the sample volume to 0.10 mL, which can then be used in the standard 2.1 mL assay procedure. It is possible to add less water if a smaller volume assay is to be performed.
11. Vortex each sample and proceed onto the 2.1 mL standard assay protocol or a custom assay.

Compatibility Chart

The amount listed in the chart is the maximum amount of material allowed in the protein sample without causing a noticeable interference.

Incompatible Substances	Amount Compatible
Buffer Systems	
N-Acetylglucosamine (10 mM) in PBS, pH 7.2	10 mM
ACES, pH 7.8	25 mM
Bicine, pH 8.4	20 mM
Bis-Tris, pH 6.5	33 mM
CelLytic™ B Reagent	Undiluted, no interference
Calcium chloride in TBS, pH 7.2	10 mM
CHES, pH 9.0	100 mM
Cobalt chloride in TBS, pH 7.2	0.8 M
EPPS, pH 8.0	100 mM
Ferric chloride in TBS, pH 7.2	10 mM
HEPES	100 mM
MOPS, pH 7.2	100 mM

Incompatible Substances	Amount Compatible
Nickel chloride in TBS	10 mM
PBS; Phosphate (0.1 M), NaCl (0.15 M), pH 7.2	Undiluted, no interference
PIPES, pH 6.8	100 mM
Sodium acetate, pH 4.8	200 mM
Sodium citrate, pH 4.8 or pH 6.4	200 mM
Tricine, pH 8.0	25 mM
Triethanolamine, pH 7.8	25 mM
Tris	250 mM
TBS; Tris (25 mM), NaCl (0.15 M), pH 7.6	Undiluted, no interference
Tris (25 mM), Glycine (1.92 M), SDS (0.1%), pH 8.3	Undiluted, no interference
Zinc chloride (10 mM) in TBS, pH 7.2	10 mM
Buffer Additives	
Ammonium sulfate	1.5 M
Aprotinin	10 µg/mL
Cesium bicarbonate	100 mM
Glucose	10 mM
Glycerol	10%
Guanidine hydrochloride	4 M
Hydrochloric acid	100 mM
Imidazole	50 mM
Leupeptin	10 µg/mL
PMSF	1 mM
Sodium azide	0.20%
Sodium bicarbonate	100 mM
Sodium chloride	1 M
Sodium hydroxide	100 mM
Sodium phosphate	25 mM
Sucrose	40%
TLCK	0.1 µg/mL
TPCK	0.1 µg/mL
Sodium orthovanadate in PBS, pH 7.2	1 mM
Thimerosal	0.01%
Urea	3 M
Chelating agents	
EDTA	10 mM
EGTA	not compatible
Sodium citrate	200 mM
Detergents	
Brij™ 35	5%
Brij™ 52	1%
CHAPS	5%
CHAPSO	5%
Deoxycholic acid	5%
Nonidet P-40 (IGEPAL® CA-630)	5%
Octyl β-glucoside	5%
Octyl β-thioglucopyranoside	5%
SDS	5%
Span® 20	1%
TRITON® X-100	5%
TRITON® X-114	1%
TRITON® X-305	1%
TRITON® X-405	1%
TWEEN® 20	5%
TWEEN® 60	5%
TWEEN® 80	5%
Zwittergents®	1%
Reducing & Thiol Containing Agents	
Dithioerythritol (DTE)	1 mM
Dithiothreitol (DTT)	1 mM
2-Mercaptoethanol	1 mM
Tributyl Phosphine	0.01%
Solvents	
Acetone	10%
Acetonitrile	10%
DMF	10%
DMSO	10%
Ethanol	10%
Methanol	10%

Note: This is not a complete compatibility chart. Many substances can affect different proteins in different ways. Researchers may assay the protein of interest in deionized water alone, then in the buffer with possible interfering substances. Comparison of the readings will indicate if an interference exists. Refer to References 1-4 for additional information on interfering substances.[1-4]

Reagents that chelate metal ions, change the pH of the assay, or reduce copper will interfere with the BCA assay. Examples are shown below:

- Metal chelators such as EDTA (>10 mM) and EGTA (any level).
- Thiol-containing reagents such as cysteine (any level), DTT (>1 mM), dithioerythritol (>1 mM), and 2-mercaptoethanol (>0.01%).
- High salt or buffers concentrations such as ammonium sulfate (>1.5 M), Tris (>0.25 M), and sodium phosphate (>0.1 M).

Troubleshooting Guide

For example, the protein sample may contain incompatible reagents or substance:

1. If the starting concentration of the protein is high, try diluting the sample so that the substance no longer interferes.
2. Use the TCA Concentration-BCA procedure. Discard the incompatible liquid after the pellet is spun down.
3. The interference caused by chelating reagents decreases when the relative amount of the copper(II) sulfate solution is increased in the prepared BCA Working Reagent. The standard preparation has 50 parts of bicinchoninic acid solution to 1 part copper(II) sulfate solution. The amount of copper(II) sulfate solution may be increased to 3 parts.

Technical Tips

1. Make sure the glassware being used has been cleaned well.
2. Consider a different protein assay procedure. If certain incompatible reagents cannot be removed from the assay, consider the use of the Bradford Reagent (Cat. No. B6916).
3. If the protein levels are too low, try using the QuantiPro BCA Kit (Cat. No. QPBCA).

References

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