TECH TIP #8

Eliminate interfering substances from samples for BCA protein assays

TR0008.3

Introduction

Thermo Scientific® Pierce BCA Protein Assays are one of the great workhorses for measuring the protein concentration of a sample. One of the best features of the Pierce® BCA Protein Assay method is its compatibility with many detergents at concentrations up to 1 or 5% (commonly used concentrations for solubilizing proteins from cell membranes). The upper limits of compatibility for a wide variety of substances are listed in the instructions for the BCA Protein Assay Kit (Product No. 23225 or 23227). Some substances, including most reducing agents, interfere at even low concentrations in the BCA Assay. Examples include 5 mM DTT (for the BCA and Micro BCA™ Assays) and 0.1 M Glycine (for the Micro BCA Assay). Interference by individual substances can often be overcome by simple means, but some sample buffers contain several components that interact to produce an interference that is not so easily identified and eliminated.

Several strategies exist for overcoming or eliminating sample incompatibility with protein assays. The simplest method is to assay the sample after diluting it several-fold in a compatible buffer. If the starting concentration of protein is sufficient to remain within the protein assay working range upon its dilution, then this method will often reduce the amount of interfering substance in the sample to the point where it no longer interferes. Another method is to dialyze or desalt samples into a buffer that is compatible with the assay.

When disulfide reducing agents such as dithiothreitol (DTT) and 2-mercaptoethanol (2-ME) are the sole cause of the incompatibility, reagents can be used to chemically block the interfering sulfhydryl groups. The Reducing Agent Compatible BCA Protein Assay Kits (Product No. 23250 and 23252) include an optimized blocking reagent and protocol for samples containing reducing agents.

Finally, precipitation can be used to eliminate interfering substances, a general method for which is given in this Tech Tip. After causing the protein to precipitate with acetone or trichloroacetic acid (TCA), the supernatant containing the interfering substance can be removed. Then the protein pellet is dissolved in the assay working reagent, and the protein assayed performed as usual. Because the assay standards are processed in parallel with the protein samples, the rate of protein recovery caused by the precipitation process is automatically accounted for in the standard curve. Alternatively, use the Thermo Scientific Compat-Able™ Protein Assay Preparation Reagent Set (Product No. 23215, see Related Products), which includes optimized reagents and protocol for performing such precipitations.

Important Notes:

- The choice between acetone and TCA methods depends on which reagent is available in the lab and the properties of the protein and buffer sample being processed. Empirical testing will be necessary to establish which method is most effective for a given protein, buffer and incompatible substance.
- An assumption in these procedures is that the standard protein precipitates and redissolves with similar efficiency to the test protein sample. This assumption may not be valid in all circumstances and underscores the importance of using a protein standard that is as similar as possible in properties to the test protein.
- Single and double precipitation protocols may result in less linear standard curves than non-precipitated standards. Therefore, avoid using these precipitation methods with the Micro BCA Protein Assay if it is necessary to detect slight variations in protein concentrations (e.g.,18 µg/ml vs. 20 µg/ml), especially at the lower end of detection range. Larger differences in protein concentrations (e.g., 15 µg/ml vs. 20 µg/ml) should be detectable after a single precipitation.
- The procedures presented here are written in volumes sufficient to perform BCA or Micro BCA Protein Assays using the Test Tube Protocols. Obviously, an aliquot of the dissolved protein pellet resulting from these procedures could be transferred to microplate wells for absorbance measurement in that format. It may be possible to scale down the entire precipitation methods for direct use in the Microplate Protocols, but this has not been tested. Also, it may be possible to concentrate a dilute large-volume sample by scaling up the precipitation step while maintaining or reducing the volume of working reagent used to dissolve and assay the pellet.

TCA Precipitation Procedure

A. Materials Required

- Protein Standards: prepare according to the instructions for the BCA Protein Assay Reagent Kit (Product No. 23225)
- TCA reagent: 72% (w/v) trichloroacetic acid in ultrapure water
- Sodium deoxycholate reagent: 0.15% (w/v) sodium deoxycholate in ultrapure water
- BCA Working Reagent: (50 parts Reagent A + 1 part Reagent B), prepare according to the instructions for the BCA Protein Assay Reagent Kit (Product No. 23225, 23227)
- SDS reagent: 5% SDS (w/v) in 0.1 N sodium hydroxide (NaOH)
- Microcentrifuge tubes and benchtop microcentrifuge

B. Protocol for BCA Protein Assay (Product No. 23225, 23227)

1. Pipette 50 µl of each protein standard (including a blank) and sample into microcentrifuge tubes in triplicate.
2. Add 450 µl of ultrapure water.
3. Add 100 µl of the sodium deoxycholate reagent.
4. Add 100 µl of the TCA reagent and incubate 10 minutes at room temperature (RT).
5. Vortex, then centrifuge 10 minutes in a microcentrifuge at maximum speed.
6. Remove the supernatant, being careful not to disturb the pellet.

   Note: If two precipitations are necessary to remove the interfering substance, dissolve the pellet in 100 µl ultrapure water and then repeat steps 3-6 one time.
7. Add 50 µl of SDS reagent to completely dissolve the protein pellet.

   Note: As used in this protocol, 5% SDS is compatible with the BCA Protein Assay.
8. Add 1 ml of BCA Working Reagent.
9. Vortex, then incubate for 30 minutes at 37° C.
10. Measure the absorbance at 562 nm.
11. Plot a standard curve based on absorbance of the protein standards and determine the protein concentration of each unknown sample according to the BCA Protein Assay Kit instructions.

Acetone Precipitation Procedures

A. Materials Required

- Protein Standards: prepare according to the instructions for the BCA Protein Assay Reagent Kit (Product No. 23225)
- Cold acetone, -20°C
- BCA or Micro BCA Working Reagent: prepare according to the product instructions for the BCA Protein Assay Reagent Kit (Product No. 23225, 23227) or Micro BCA Protein Assay Reagent Kit (Product No. 23235)
- Microcentrifuge tubes and benchtop microcentrifuge

B. Protocol for BCA Protein Assay

1. Pipette 50 µl of each protein standard (including a blank) and sample into 1.5 ml microcentrifuge tubes in triplicate.
2. Add 200 µl of cold (-20°C) acetone to each tube.
3. Vortex and incubate 30 minutes at -20°C.
4. Centrifuge 10 minutes at maximum speed in a microcentrifuge.
5. Pour off the supernatants and allow the acetone to evaporate from the tubes at room temperature (RT) for 30 minutes.

   Note: If two precipitations are necessary to remove the interfering substance, repeat Steps 2-5 before proceeding.

6. Add 50 µl of ultrapure water to the protein pellets and vortex.
7. Add 1 ml of BCA Working Solution (50 parts Reagent A and 1 part Reagent B) to each tube and vortex. Incubate 30 minutes at 37°C.
8. Cool samples to RT and measure absorbance at 562 nm.
9. Plot a standard curve based on absorbance of the protein standards and determine the protein concentration of each unknown sample according to the BCA Protein Assay Kit instructions.

C. Protocol for Micro BCA Protein Assay

1. Pipette 500 µl of each standard (including a blank) and sample into 1.5 ml microcentrifuge tubes in triplicate.
2. Add 1 ml of cold acetone (-20°C) to each tube.
3. Vortex and incubate 30 minutes at -20°C.
4. Centrifuge 10 minutes at maximum speed in a microcentrifuge.
5. Pour off the supernatants and allow the acetone to evaporate from the tubes at RT for 30 minutes.

   Note: If two precipitations are necessary to remove the interfering substance, repeat Steps 2-5 before proceeding.
6. Add 500 µl of ultrapure water to the protein pellets and vortex.
7. Add 500 µl of Micro BCA Working Solution (50 parts Reagent A: 48 parts Reagent B: 2 parts Reagent C) to each tube and vortex. Incubate 60 minutes at 60°C.
8. Cool samples to RT and measure absorbance at 562 nm.
9. Plot a standard curve based on absorbance of the protein standards and determine the protein concentration of each unknown sample according to the Micro BCA Protein Assay Kit instructions.

Related Thermo Scientific Products

23215	**Compat-Able Protein Assay Preparation Reagent Set**, sufficient reagents to pre-treat 500 samples to remove interfering substances before total protein quantitation
23209	**Bovine Serum Albumin Standard Ampules, 2 mg/ml**, 10 × 1 ml ampules containing bovine serum albumin (BSA) at a concentration of 2.0 mg/ml in 0.9% saline and 0.05% sodium azide.
23208	**Bovine Serum Albumin Standard Pre-Diluted Set**, 7 × 3.5 ml aliquots in the range of 125-2000 µg/ml
23212	**Bovine Gamma Globulin Standard Ampules, 2 mg/ml,** 10 × 1 ml
23213	**Bovine Gamma Globulin Standard Pre-Diluted Set**, 7 × 3.5 ml aliquots in the range of 125-2000 µg/ml
23250	**Pierce BCA Protein Assay Kit – Reducing Agent Compatible**
23252	**Pierce Microplate BCA Protein Assay Kit – Reducing Agent Compatible**
23225, 23227	**Pierce BCA Protein Assay Kits**, working range 20-2000 µg/ml
23235	**Pierce Micro BCA Protein Assay Kit**, working range of 0.5-20 µg/ml
23236	**Pierce Coomassie Plus (Bradford) Protein Assay Kit**, working range of 1-1500 µg/ml

Current versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.