INSTRUCTIONS

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Compat-Able™ Protein Assay Preparation Reagent Set

23215

1308w

Number	Description
23215	**Compat-Able™ Protein Assay Preparation Reagent Set** Contains sufficient reagents to quickly and easily pre-treat up to 500 samples to remove interfering substances prior to total protein quantitation. **Compat-Able™ Protein Assay Preparation Reagent 1**, 250 ml **Compat-Able™ Protein Assay Preparation Reagent 2**, 250 ml **Storage**: Store the Compat-Able™ Protein Assay Preparation Reagents at room temperature. *This product is guaranteed for one year from the date of purchase when handled and stored properly.*

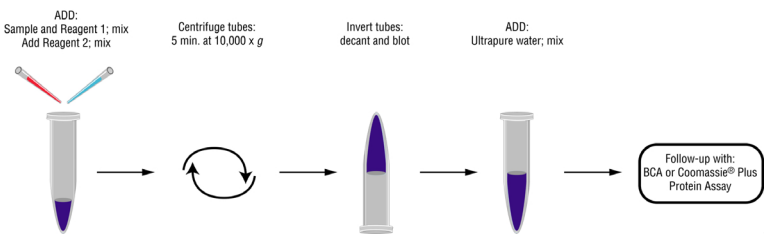
Introduction

Samples for total protein quantitation may contain substances that interfere. Since every protein assay method differs with respect to which substances interfere, it may be possible to use an alternative protein assay method. For this reason, most researchers have more than one total protein assay kit available in the lab for routine use. The BCA Total Protein Assay Kit and the Coomassie® Plus Total Protein Assay Kit are excellent choices to meet the need for protein assay methods that are compatible with most reagents and buffers used in protein samples. However, there are times when the presence of one or more substances makes the sample incompatible with either protein assay. In those cases, some sample pre-treatment to remove the interfering substances is required.

If the total protein concentration is high, simple dilution may decrease the concentration of the substance so that it no longer interferes. When sample dilution alone will not suffice or if sample dilution is not practical because total protein concentration is low, dialysis may be used to remove the interfering substance. Alternatively, a desalting media or gel filtration media packed into an appropriate column may be used to exchange the sample into another buffer or reagent that does not interfere in the protein assay method. Other than simple dilution, the methods previously available to remove interfering substances are considered to be tedious and time-consuming.

The Compat-Able™ Protein Assay Preparation Reagent Set allows the pre-treatment of up to 500 samples to remove interfering substances prior to total protein quantitation. After sample pre-treatment, it is recommended that the total protein concentration be determined using either the Pierce BCA Total Protein Assay Kit (Product No. 23227) or the Pierce Coomassie® Plus Total Protein Assay Kit (Product No. 23236).

Figure 1: Pictorial summary of the pre-treatment protocol



Procedure for Sample Pre-treatment

Pre-treat each of the diluted protein standards (BSA or BGG) to be used in the subsequent protein assay along with the samples to ensure that the standards and samples are treated exactly alike.

Additional materials required

a. Disposable glass culture tubes or disposable plastic microfuge tubes
b. Centrifuge or microfuge capable of generating 10,000 x *g*
c. Pipettors and disposable pipette tips
d. Clean paper toweling

Note: For optimal results, follow these instructions

1. *In duplicate*, dispense 100 µl (0.100 ml) of each sample or diluted protein standard to be treated into a test tube.
2. Add 500 µl (0.500 ml) of Compat-Able™ Protein Assay Preparation Reagent 1 to each tube. Mix each tube and allow the tubes to stand at room temperature for at least five minutes.
3. Add 500 µl (0.500 ml) of Compat-Able™ Protein Assay Preparation Reagent 2 to each tube. Mix each tube and centrifuge at 10,000 *g* for at least 5 minutes.
4. Invert each tube and discard the supernatant. Blot the open end of the inverted tube on clean paper toweling to completely remove the supernatant.
5. Dissolve the protein pellet in 100 µl (0.100 ml) of ultrapure water. *Vortex mixing in short bursts may help to solubilize the protein pellet. If the protein assay will not be performed immediately, the tubes may be covered and stored refrigerated for up to 1 week. Just prior to running the protein assay, carefully examine such stored samples for microbial growth. If a stored sample is suspected of being contaminated with microbial growth, repeat the sample pre-treatment procedure on a fresh sample.*
6. Perform the total protein assay per the kit instructions. *The Coomassie® Plus Protein Assay Reagent or the BCA Working Reagent may be added directly to each tube containing a well mixed, solubilized protein pellet.*

Note 1: For convenience, the sample pre-treatment may be performed in 1.5 ml microfuge tubes. Use ½ of each volume used in the tube protocol (50 µl of sample and 250 µl each of Reagents 1 and 2). After dissolving the protein pellet in ultrapure water, the protein assay may be done in the microfuge tubes by adding 1.0 ml of the BCA Working Reagent or 1.5 ml of the Coomassie® Plus Protein Assay Reagent to each tube. Proceed with the protein assay per the standard tube assay described in the appropriate kit's instruction booklet.

Note 2: Some interfering substances may require a second washing of the protein pellet for complete removal. Repeat Steps 1-4 of the pre-treatment protocol on a fresh sample. Immediately following Step 4, repeat Steps 2-3 using 500 µl of Reagent 1 and 160 µl of Reagent 2. Then, do Steps 4 and 5 and repeat the protein assay.

Troubleshooting

Problem:	Possible Cause:	Solution:
1. Less color than expected from the protein assay (one or more tubes - except the blank)	1A. Incomplete precipitation	1A. After adding Reagent 1, mix and wait at least 5 min.
	1B. Loss of protein pellet during decantation	1B. Centrifuge at 10,000 x *g* for at least 5 min.
	1C. Protein pellet not dissolved	1C. Vortex mix and/or heat slightly to dissolve the pellet.
	1D. Sample(s) contain chelator at high concentration	1D. Repeat the pre-treatment using a second wash.
2. More color than expected from the protein assay (one or more sample tubes)	2. Sample(s) contain substance(s) that interfere in the protein assay at high concentration	2. Repeat the pre-treatment using a second wash.

Related Products

23227, BCA Protein Assay Reagent Kit

23236, Coomassie® Plus Protein Assay Reagent Kit

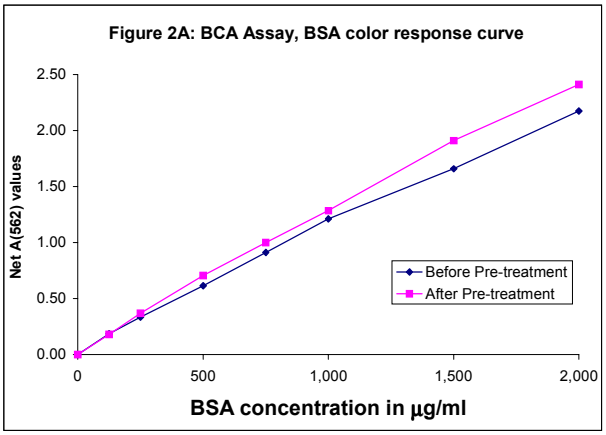
23208, Pre-Diluted Protein Standards Set (BSA), 7 x 3.5 ml

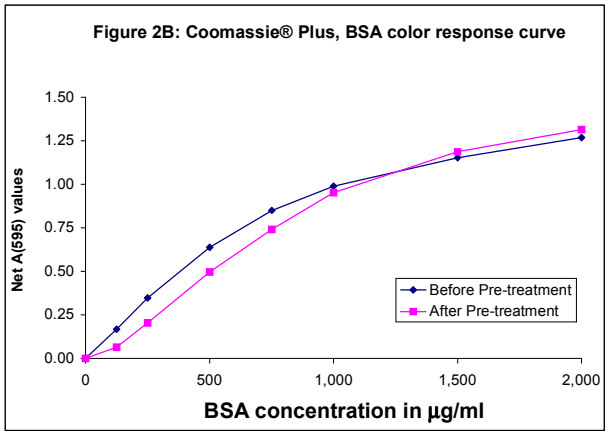
23213, Pre-Diluted Protein Standards Set (BGG), 7 x 3.5 ml

Additional information

Please visit the Pierce web site for additional information on protein assays and protein standards including instruction booklets.

Figure 2 shows typical BSA response curves before and after pre-treatment with either the BCA (Figure 2A) or the Coomassie® Plus Protein Assay (Figure 2B). The difference observed between the before and after response curves illustrates the need to also pre-treat the protein standards. Pre-treatment of the protein standards will improve the accuracy of the protein quantitation.





After pre-treatment with the Compat-Able™ Protein Assay Preparation Reagent Set, human serum samples containing one or more of the following substances were assayed at two concentrations using both the BCA Protein Assay Reagent Kit and the Coomassie® Plus Protein Assay Kit. Two controls consisting of the human serum diluted 1:100 and 1:50 in 0.9% saline were included with each run. Successful removal of the interfering substance was accorded if the color produced by both dilutions of the pre-treated samples was within 10% of the color produced by the controls. (The color was measured at the appropriate wavelength.)

Up to 3.0 M tris	Up to 125 mM sodium citrate
Up to 20% glycerol	Up to 200 mM glucose
Up to 4% SDS	Up to 200 mM sodium acetate
Up to 3.6 M magnesium chloride	Up to 20 mM arginine, pH 10
Up to 1.25 M sodium chloride	Up to 20 mM lysine, pH 10
Up to 350 mM dithiothreitol (DTT)	Up to 5% β-mercaptoethanol
Up to 5% Triton® X-100	Up to 200 mM EDTA
Up to 5% Tween® 20	Up to 1.0 M imidazole, pH 10.4