INSTRUCTIONS

Compat-Able™ Protein Assay Preparation Reagent Set

23215

1308.3

Number	Description
23215	**Compat-Able Protein Assay Preparation Reagent Set**, sufficient reagents to quickly and easily pre-treat up to 500 samples to remove interfering substances prior to total protein quantitation.
	Kit Contents:
	Compat-Able Protein Assay Preparation Reagent 1, 250mL
	Compat-Able Protein Assay Preparation Reagent 2, 250mL
	Storage: Upon receipt store kit reagents at room temperature. Product shipped at ambient temperature.

Table of Contents

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 Thermo Scientific BCA Protein Assay Kit and the Coomassie Plus 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 Thermo Scientific 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, total protein concentration can be determined using either the BCA Protein Assay Kit (Product No. 23227) or the Coomassie Plus™ Protein Assay Kit (Product No. 23236).

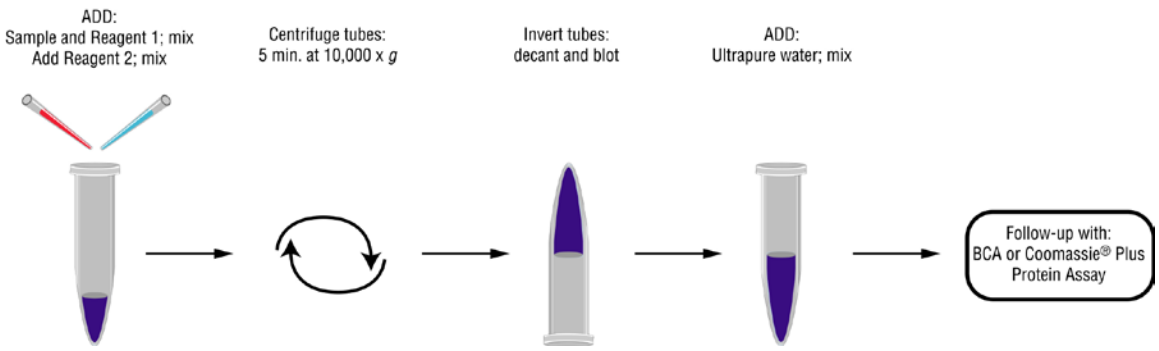

Figure 1. Procedure Summary for the Thermo Scientific Compat-Able Protein Assay Preparation Reagent Set.

Important Note: Pre-treat each of dilution of the 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

- Disposable glass culture tubes or disposable plastic microcentrifuge tubes
- Centrifuge or microcentrifuge capable of generating 10,000 × *g*
- Pipettors and disposable pipette tips
- Clean paper towels

Test Tube Procedure for Sample Pre-treatment

Note: For optimal results, follow these instructions. Be sure to pre-treat the protein standards to be used later in the protein assay exactly the same as the samples to be analyzed.

1. In duplicate, dispense 100µL (0.100mL) of each sample or diluted protein standard to be treated into a test tube.
2. Add 500µL (0.5mL) 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 500µL (0.5mL) 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. If needed, a pipette can be used to carefully remove excess liquid.

   Note: The protein pellet may be difficult to see as it may form a thin layer on the walls of the tube. If the protein assay will not be performed immediately, the tubes may be covered and stored refrigerated for up to 1 week. Before using the stored pellet, warm it to room temperature and carefully examine the pellet for microbial growth. If necessary, repeat the pre-treatment procedure on a fresh sample.
5. When ready to perform the protein assay, dissolve the protein pellet in the original sample volume (100µL) of ultrapure water. To avoid sample transfer errors, perform the protein assay in the tube containing the dissolved protein pellet. Vortex vigorously to solubilize the pellet.

   Note: To aid complete solubilization, the protein pellet may be dissolved in the original sample volume (100µL) of either the BCA Working Reagent or the Coomassie Plus™ Protein Assay Reagent.
6. Perform the total protein assay per the selected protein assay kit instructions. Use 100µL of ultrapure water for each protein assay blank tube. Follow the standard test tube assay protocol as given in the instruction booklet for the appropriate protein assay reagent.

Note: 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 and 3 using 500µL of Reagent 1 and 160µL of Reagent 2. Then, continue with steps 4 and 5 and repeat the protein assay.

Microcentrifuge Tube (2.0mL) Procedure for Sample Pre-treatment

Note: This protocol uses less sample volume, which reduces the volume of protein assay reagent needed allowing the protein assay to be done in the same microcentrifuge tube. For optimal results, follow these instructions. Be sure to pre-treat the protein standards to be used later in the protein assay exactly the same as the samples to be analyzed.

1. In duplicate, dispense 50µL of each sample or diluted protein standard to be treated into a 2.0mL microcentrifuge tube.
2. Add 500µL of Compat-Able Protein Assay Preparation Reagent 1 to each microcentrifuge tube. Mix each tube and allow the tubes to stand at room temperature for at least five minutes.
3. Add 500µL of Compat-Able Protein Assay Preparation Reagent 2 to each microcentrifuge tube. Mix each tube and centrifuge at a minimum of 10,000 × *g* for at least 5 minutes.
4. Invert each microcentrifuge tube and discard the supernatant. Blot the open end of the inverted tube on clean paper toweling to completely remove the supernatant. If needed, a pipette can be used to carefully remove excess liquid.

   Note: The protein pellet may be difficult to see as it may form a thin layer on the walls of the tube. If the protein assay will not be performed immediately, the tubes may be covered and stored refrigerated for up to 1 week. Before using the stored pellet, warm it to room temperature and carefully examine the pellet for microbial growth. If necessary, repeat the pre-treatment procedure on a fresh sample.
5. When ready to perform the protein assay, dissolve the protein pellet in the original sample volume (50µL) of ultrapure water. To avoid sample transfer errors, perform the protein assay in the tube containing the dissolved protein pellet. Vortex vigorously to solubilize the pellet.

   Note: To aid complete solubilization, the protein pellet may be dissolved in the original sample volume (50µL) of either the BCA Working Reagent or the Coomassie Plus Protein Assay Reagent.
6. Perform the total protein assay per the selected protein assay kit instructions. Use 50µL of ultrapure water for each protein assay blank tube. Use half the volume of BCA Working Reagent (i.e., 1.0mL/microcentrifuge tube) or half the volume of the Coomassie Plus Protein Assay Reagent (i.e., 1.5mL/microcentrifuge tube) as described in standard test tube assay protocol as given in the instruction booklet for the appropriate protein assay reagent.

Note: 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 and 3 using 500µL of Reagent 1 and 160µL of Reagent 2. Then, continue with steps 4 and 5 and repeat the protein assay.

Troubleshooting

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

Additional Information

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

Typical BSA response curves (Figure 2) before and after pre-treatment with either the BCA or the Coomassie Plus Protein Assay demonstrate the quality of the pre-treatment. The difference observed between the before and after response curves illustrate the need to also pre-treat the protein standards. Pre-treatment of the protein standards will improve the accuracy of the protein quantification.

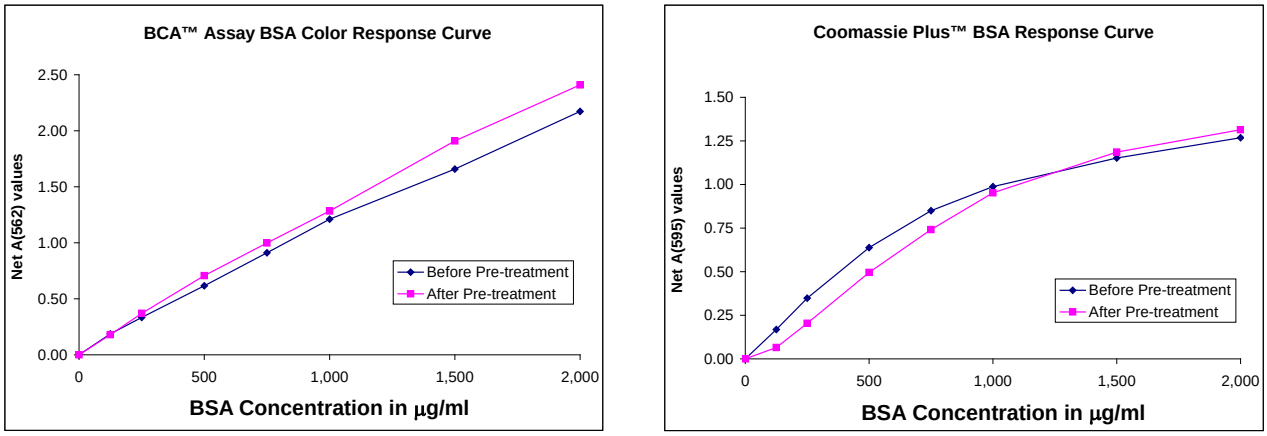

Figure 2. Color response curves for BSA Standard in BSA and Thermo Scientific Coomassie Plus Protein Assays before and after pre-treatment with the Thermo Scientific Compat-Able Protein Assay Preparation Reagent Set.

After pre-treatment with the Compat-Able Protein Assay Preparation Reagent Set, human serum samples containing one or more of the several substances were assayed at two concentrations using both the BCA Protein Assay Kit and the Coomassie Plus Protein Assay Kit (Table 1). 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.)

Table 1. Maximum amounts of protein assay-interfering substances sufficiently removed in one round of pre-treatment using the Thermo Scientific Compat-Able Protein Assay Preparation Reagent Set.

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

Related Thermo Scientific Products

23227	**BCA Protein Assay Kit**
23236	**Coomassie Plus Protein Assay Kit**
23208	**Pre-Diluted Protein Standards Set (BSA),** 7 × 3.5mL
23213	**Pre-Diluted Protein Standards Set (BGG),** 7 × 3.5mL

Tween is a trademark of Croda International PLC.

Triton is a trademark of The Dow Chemical Company.

Products are warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation"). No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than Buyer. Any model or sample furnished to Buyer is merely illustrative of the general type and quality of goods and does not represent that any Product will conform to such model or sample.

NO OTHER WARRANTIES, EXPRESS OR IMPLIED, ARE GRANTED, INCLUDING WITHOUT LIMITATION, IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR ANY PARTICULAR PURPOSE, OR NON INFRINGEMENT. BUYER'S EXCLUSIVE REMEDY FOR NON-CONFORMING PRODUCTS DURING THE WARRANTY PERIOD IS LIMITED TO REPAIR, REPLACEMENT OF OR REFUND FOR THE NON-CONFORMING PRODUCT(S) AT SELLER'S SOLE OPTION. THERE IS NO OBLIGATION TO REPAIR, REPLACE OR REFUND FOR PRODUCTS AS THE RESULT OF (I) ACCIDENT, DISASTER OR EVENT OF FORCE MAJEURE, (II) MISUSE, FAULT OR NEGLIGENCE OF OR BY BUYER, (III) USE OF THE PRODUCTS IN A MANNER FOR WHICH THEY WERE NOT DESIGNED, OR (IV) IMPROPER STORAGE AND HANDLING OF THE PRODUCTS.

Unless otherwise expressly stated on the Product or in the documentation accompanying the Product, the Product is intended for research only and is not to be used for any other purpose, including without limitation, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses, or any type of consumption by or application to humans or animals.

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

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce