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ProteoSpin™ Total Protein Detergent Clean-Up Micro Kit Product Insert

Product # 23300

The ProteoSpin™ Total Protein Detergent Clean-Up Micro Kit provides a fast and simple procedure for the removal of SDS, Triton® X-100 and other detergents from total protein samples, including lysates. Detergents are extensively used to prepare protein samples; however, these detergents must often be removed prior to downstream analysis because of their undesirable effects. These include extraneous peaks in mass spectrometry, artifacts with chromatography and electrophoresis, interference with microinjection into cells and interference with protein immunization.

The ProteoSpin™ Total Protein Detergent Clean-Up Micro Kit can remove greater than 95% of detergents from total protein samples while maintaining high protein recovery. The kit is able to remove all types of detergents including ionic, non-ionic and zwitterionic detergents. It is designed to remove detergents from protein solutions either in their free form or bound form, as when complexed with the protein. Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The purified proteins can be used in a number of downstream applications including mass spectrometry, SDS-PAGE, isoelectric focusing, and NMR spectroscopy.

The ProteoSpin™ Total Protein Detergent Clean-Up Micro Kit contains all the solutions and columns for the processing of 20 total protein samples. Each spin column is able to process small detergent-containing samples containing up to 200 µg of protein. Detergents including SDS, Triton® X-100, CHAPS, NP-40 and Tween 20 can be removed using the kit, with protein recoveries of 80 – 95% for most proteins. Preparation time for 12 samples is only 20 minutes. The kit has a shelf life of at least 1 year when stored as suggested.

Kit Components

Component	Product # 23300 (20 samples)
Binding Buffer	5 mL
Wash Buffer I	20 mL
Wash Buffer II	12 mL
Elution Buffer	6 mL
Neutralizer	1 mL
Micro Spin Columns	20
Collection Tubes	20
Elution tubes (1.7 mL)	20
Product Insert	1

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. Once opened, the solutions should be stored at 4°C. All the reagents should remain stable for at least 1 year in their unopened containers.

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use.

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- Micropipettors
- 1.5 mL microcentrifuge tubes
- Isopropanol
- Milli-Q® water

Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge at 6,700 x g (~10,000 RPM) except where noted. Please check your microcentrifuge specifications to ensure proper speed. Performance of the kit is not affected by temperature, and thus the procedure may be performed at room temperature, 4°C, or on ice.

Notes prior to use:

- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Ensure that no more than 500 µL or 200 µg of protein sample is used per column.
- Prepare a working concentration of **Wash Buffer I** by adding 20 mL of isopropanol (to be provided by the user) to the supplied bottle containing **Wash Buffer I**. This will give a final volume of 40 mL. The label on the bottle has a box that can be checked to indicate that isopropanol has been added.

1. Sample Preparation

This step ensures that the protein solution is at the proper pH for column binding.

a. Obtain protein sample and determine the volume.
b. Adjust the volume to 500 µL using Milli-Q® water.
c. Add 40 µL of **Binding Buffer** to the 500 µL protein sample prepared above.
d. Add 540 µL of isopropanol (provided by the user) and mix contents well.
e. Verify that the pH is between 3.5 – 4, and adjust with more **Binding Buffer** if needed.

Note: In some concentrated protein samples, precipitation may occur with the addition of the Binding Buffer. This precipitate includes proteins, and thus should not be discarded. The precipitate should be resuspended as much as possible, and loaded onto the column with the rest of the sample.

2. Column Activation

a. Assemble a spin column with a provided collection tube. Open the cap on the column.
b. Apply 500 µL of **Wash Buffer I** to the column and close the cap.
c. Centrifuge for one minute at 6,700 x g, and discard the flowthrough.
d. Repeat steps **2b** and **2c** to complete the column activation step.

3. Protein Binding

a. Apply a maximum of 650 µL of protein solution (from the Sample Preparation step) onto the column and centrifuge for two minutes at 6,700 x g. Inspect the column to ensure that the entire sample has passed through into the collection tube. If necessary, spin for an additional three minutes.

Note: If the sample still has not passed into the collection tube after five minutes, the speed may be increased to 14,000 x g and the column spun for another two minutes.

b. Discard the flowthrough. Reassemble the spin column with its collection tube.
c. Repeat steps **3a** and **3b** once more until the entire protein sample has been applied to the column.
d. Discard any remaining flowthrough and reassemble the spin column with its collection tube.

Note: You can save the flowthrough in a fresh tube for assessing your binding efficiency.

4. Column Wash

a. Apply 500 µL of **Wash Buffer I** to the column and centrifuge for one minute.
b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Apply 500 µL of **Wash Buffer II** to the column and centrifuge for two minutes.
d. Inspect the column to ensure that the liquid has passed through into the collection tube. There should be no liquid in the column. If necessary, spin for an additional minute to dry.

5. Protein Elution and pH Adjustment

The supplied Elution Buffer consists of 10 mM sodium phosphate pH 12.5.

a. Add 9.3 µL of Neutralizer to a fresh 1.7 mL Elution Tube.
b. Transfer the spin column from the Column Wash procedure into the Elution Tube.
c. Apply 100 µL of **Elution Buffer** to the column and centrifuge for two minutes to elute bound proteins.

Note: Approximately 90% of bound total proteins are recovered in the first elution. If desired, a second elution using 100 µL of Elution Buffer may be carried out. This should be collected into a different tube (to which 9.3 µL of Neutralizer is pre-added) to prevent dilution of the first elution.

Total proteins are now ready for downstream applications.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Protein solution does not flow through the column during the binding step	Centrifugation speed was too low	Check the centrifuge to ensure that it is capable of generating 6,700 x g. Sufficient centrifugal force is required to move the liquid phase through the resin. Centrifugation speeds may be increased to 14,000 x g, but this speed should not be exceeded.
	Inadequate spin time	Spin an additional two minutes to ensure that the liquid is able to flow completely through the column.
	Cellular debris is present in the protein solution.	Prior to the sample preparation step, filter the sample with a 0.45 µM filter or spin down insoluble materials. Solid, insoluble materials can cause severe clogging problems.
Poor protein recovery	Incorrect pH adjustment of protein sample.	It is important that the proper amount of Binding Buffer be added to the total protein sample in order to adjust the pH prior to loading onto the column.
	Initial volume of sample applied to column was too low.	Ensure that 400-500 µL of the pH-adjusted protein sample is loaded onto the column at one time in order to capture a large portion of the proteins present in the sample.
Eluted protein is degraded	Eluted protein solution was not neutralized.	Add 9.3 µL of Neutralizer to each 100 µL of eluted total proteins in order to adjust the pH to neutral. Some proteins are sensitive to high pH, such as the elution buffer at pH 12.5.
	Eluted protein was not neutralized quickly enough.	If eluted proteins are not used immediately, degradation will occur. We strongly suggest adding Neutralizer in order to lower the pH.

Related Products	Product #
ProteoSpin™ Detergent Clean Up Micro Kit	10200, 10500
ProteoSpin™ Detergent Clean Up Maxi Kit	17100

Technical Support
Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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