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ProteoSpin™ Abundant Serum Protein Depletion Kit Product Insert

Product # 17300

The ProteoSpin™ Abundant Serum Protein Depletion Kit provides a fast and simple procedure for the effective depletion of major serum proteins including albumin, α-antitrypsin, transferin and haptoglobin from serum and plasma samples. The kit is unique in that it is based on an ion-exchange mechanism and not the use of specific antibodies. As a result, the kit can be used to deplete serum proteins from a wide variety of samples, including human and various animals. Albumin has been found to be depleted by 70%, transferin and haptoglobin by 50% and α-antitrypsin by 90%. The complexity of the sample is thus greatly reduced, allowing for the detection of less abundant proteins present in the sample. Eluted samples are ready for use in various downstream applications including 2D gel electrophoresis, LC/MS and microarrays.

The ProteoSpin™ Abundant Serum Protein Depletion Kit contains sufficient materials for 25 preparations. Each spin column can deplete up to 500 µg of abundant serum proteins. Preparation time for 10 samples is less than 30 minutes. The kit has a shelf life of at least 2 years when stored as suggested.

Kit Components

Component	Product # 17300 (25 samples)
Column Activation and Wash Buffer	90 mL
Elution Buffer	12 mL
Neutralizer	0.5 mL
Mini Spin Columns	25
Collection Tubes	25
Elution tubes (1.7 mL)	25
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 2 years 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

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:

- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- 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.
- Each column is able to deplete up to 500 μg of serum protein. We recommend that between 200 μg and 500 μg of protein be applied to each column. Thus it is necessary to determine the amount of protein present in your sample prior to starting the procedure.

1. Column Activation

a. Assemble a spin column with a provided collection tube, and open the cap on the column.
b. Add 500 μL of Column Activation and Wash Buffer to the column and close the cap.
c. Centrifuge for one minute and discard the flowthrough.
d. Repeat steps **1b** and **1c** to complete the column activation step.

2. Sample Preparation

a. Dilute 10 μL of the serum sample in 490 μL of Column Activation and Wash Buffer.
b. Mix well.

3. Protein Binding

a. Apply the 500 μL of diluted serum sample onto the activated column and centrifuge for one minute.
b. Discard the flowthrough. Reassemble the spin column with its collection tube.

Note: You can save the flowthrough in a fresh tube for assessing albumin and high abundance protein depletion.

4. Column Wash

- **a.** Apply 500 μL of Column Activation and Wash Buffer to the column and centrifuge for one minute.
- **b.** Discard the flowthrough and reassemble the spin column with its collection tube.
- **c.** Add another 500 μL of Column Activation and Wash Buffer to the column and centrifuge for one minute.
- **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. It is necessary to adjust the pH of the eluted serum sample to neutral by pre-adding Neutralizer to the elution tube. This step is necessary before running downstream applications including 2D gel electrophoresis.

- **a.** Add 5 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 the Elution Buffer to the column and centrifuge for one minute to elute bound proteins.
- **d.** Add another 100 μL of Elution Buffer and centrifuge for one minute into the same microcentrifuge tube.

Note: Approximately 70% of albumin, 90% of α-antitrypsin, and 50% of transferin and haptoglobin are depleted from the serum sample at this point.

Serum samples are now ready for downstream applications.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Protein solution does not flow through the column	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.
	Protein solution is too viscous.	Dilute protein solution as described in protocol.
	Cellular debris is present in protein solution.	Filter the sample in a 0.45 μM filter or spin down insoluble materials and transfer liquid portion to the column. Solid, insoluble materials can cause severe clogging problems.
	Inadequate spin time.	Spin an additional minute to ensure that the liquid is able to flow completely through the column.
Insufficient depletion of major proteins	Column was overloaded with proteins.	Decrease the amount of serum that is loaded onto the column.
	Improper sample preparation.	Ensure that the serum sample is properly prepared by diluting it in the provided Column Activation and Wash Buffer.
Eluted protein is degraded	Eluted protein solution was not neutralized.	Add 5 μL of Neutralizer to each 200 μL of eluted protein in order to adjust the pH to neutral. Some proteins are sensitive to high pH, such as the elution buffer at pH 12.
	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.
	Proteases may be present.	Use protease inhibitors during all steps of sample preparation, and during storage of the serum, if desired.
	Bacterial contamination of the protein solution.	Prepare the serum samples with 0.015% sodium azide. The Elution Buffer already contains sodium azide.
Low protein concentration in the elution	Low levels of serum proteins present in the initial sample.	Increase the amount of serum that is loaded onto the column. The input amount can be verified using a reliable colorimetric assay.

Related Products	Product #
ProteoSpin™ CBED Micro Kit	10100
ProteoSpin™ Detergent Clean-Up Micro Kit	10200

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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