ProteoSpin

Application Manual

ProteoSpin™ Abundant Serum Protein Depletion Kit

For Use with P/N 17300

ProteoSpin™ Abundant Serum Protein Depletion Kit

Basic Features	Benefits
Efficient removal of highly abundant proteins	The kit depletes 70% of albumin, 90% of α-antitrypsin, and 50% of transferin and haptoglobin, enabling the visualization of low abundance proteins. Depleted serum profile on 2D is clean and smearless with enrichment of some minor spots.
Fast and easy processing	Efficiently process up to 10 samples in less than 30 minutes using an easy-to-use protocol.
Does not rely on antibodies	Based on an ion exchange mechanism, the kit can be applied to serum and plasma samples from a range of sources, including human and various animals.
Complete kit	All columns and solutions required are provided.
Low price per column	Compared to columns based on antibodies, these ion-exchange columns are much lower in price, yet perform to the same high standards.
Depletion of proteins and salts	Most proteins bind to the column, while salts and many of the major proteins are removed in the flowthrough
Suitable for downstream applications	Final elution suitable for: • SDS-PAGE • 2D gel electrophoresis • LC/MS • Microarrays

General Introduction

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The use of body fluids, including serum and plasma, has become widespread in the area of diagnostics and potential disease marker discovery. The main problem associated with the use of serum and plasma for disease marker identification is the large amount of proteins present, and the enormous range of concentrations of these proteins. Some serum proteins, including albumin and γ-globulins, can constitute up to 70-80% of the total serum proteins present. The high abundance of these serum proteins can result in masking the detection and analysis of less abundant proteins. Thus there is a need to remove as many of these high abundance proteins as possible, in order to be able to analyze the lower abundance proteins of interest.

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 employs an innovative separation material, silicon carbide (SiC). This proprietary processed material acts as an ion exchanger, and is able to remove the high abundance proteins present in serum and plasma based on their pIs. Most of the high abundance proteins do not bind to the column due to their pIs, and thus are eliminated in the flowthrough from the binding step. The majority of lower abundance proteins are able to bind to the column, and therefore remain in the sample. This prepares an enriched serum or plasma sample that is ready for downstream applications.

Ion Exchange Chromatography

In ion exchange chromatography, ions that are electrostatically bound to an insoluble and chemically inert matrix are reversibly replaced by ions in solution. The matrix can be either an anion exchanger that consists of positively charged groups that reversibly bind anions in solution, or it can be a cation exchanger that bears negatively charged groups and binds to mobile cations. Proteins are complex molecules that carry both positive and negative charges, depending on their amino acid composition and the pH of their solutions. In this case, any given protein will bind to both anion and cation exchangers depending on the net charge of the protein.

When purifying a protein, the mobile phase pH, salt concentration and buffer are chosen to bind the desired protein on the selected ion exchanger. The impure protein solution is applied to a column that contains the ion exchanger, and is washed with the same mobile phase solution. Proteins bind to the matrix and are eluted by changing either salt concentration or pH.

Silicon Carbide as an Ion Exchanger

The chromatographic resin used in the ProteoSpin™ Abundant Serum Protein Depletion Kit consists of silicon carbide (SiC), a man-made material noted for its hardness (second only to diamond) and high resistance to chemical change. These properties make SiC highly suitable for spin column chromatography even at extremes of pH.

When processed appropriately, SiC becomes an effective cation exchanger for the purpose of purifying macromolecules. The surface of SiC is negatively charged and can bind positively charged macromolecules. SiC has poor affinity to monovalent and divalent cations, also making it an effective resin for the removal of salts.

Soluble proteins bind to SiC through interaction of the positively charged side-groups on their chain with the negative charges on the SiC surface. As a cation exchanger, SiC has an estimated effective $pK_a \approx 2$ making the resin negatively charged over a wide range of pH. Therefore, with the exception of those peptides that are highly acidic, most soluble polypeptides can be bound to SiC.

ProteoSpin™ Abundant Serum Protein Depletion Kit Components

- Column Activation and Wash Buffer
- Elution Buffer
- Neutralizer
- ProteoSpin™ SiC Spin Columns inserted into 2 mL collection tubes
- Final Collection Tube, 1.7 mL
- ProteoSpin™ Abundant Serum Protein Depletion Kit Application Manual
- ProteoSpin™ Abundant Serum Protein Depletion Kit Protocol Card

Recommended Storage Conditions

For unopened solution containers, the reagents should remain stable for at least one year when stored at room temperature. Once opened, the solution should be stored at 4°C when not in use.

Customer-Supplied Reagents and Equipment

You must have the following in order to use the Proteospin™ Abundant Serum Protein Depletion Kit:

- Benchtop microcentrifuge
- Micropipettors

Procedure

This section describes the procedure associated with the Proteospin™ Abundant Serum Protein Depletion Kit.

Typical Procedure:

The kit utilizes spin columns containing SiC, an ion-exchanger that binds the protein of interest if it retains a net positive charge at the binding pH of 6.5. All the main high abundance proteins will not bind to the column at this pH due to their pIs, and thus will be eliminated in the flowthrough. The majority of low abundance serum proteins will bind to the column at this pH, and will become enriched in the serum sample upon elution. These depleted serum samples are then ready for downstream analysis. Each spin column is able to deplete up to 500 μg of serum proteins.

This kit provides a fast and simple procedure for the effective depletion of major serum proteins including albumin, transferin, haptoglobin and α-antitrypsin, 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.

Protocol

The following procedure describes the depletion of albumin and other high abundance serum proteins from small serum and plasma samples.

Note: All centrifugation steps in these procedures are carried out at 6,700 x g in a microcentrifuge. Performance of the kit is not affected by temperature, and thus the procedure may be performed at room temperature, 4°C, or on ice.

A. Column Activation

1. Open the cap on the pre-assembled spin column with its 2 mL collection tube.
2. Add 500 µL of Column Activation and Wash Buffer to the column and close the cap.
3. Centrifuge for one minute, and discard the flowthrough.
4. Repeat steps 2 and 3 to complete the column activation step.

B. Sample Preparation

It is important that the serum sample be properly prepared before loading it onto the column.

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

C. Protein Binding

During the binding step, the protein solution is passed through the resin bed aided by centrifugation. The high abundance proteins will pass through the column into the flowthrough, while the lower abundance proteins are captured on the resin.

1. Apply the 500 μL of diluted serum sample onto the activated column and centrifuge for one minute.

2. 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.

D. Column Wash

This step removes non-specifically bound debris from the column.

1. Apply 500 μL of Column Activation and Wash Buffer to the column and centrifuge for one minute.

2. Discard the flowthrough and reassemble the spin column with its collection tube.

3. Add another 500 μL of Column Activation and Wash Buffer to the column and centrifuge for one minute.

4. Inspect the column and 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.

E. 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.

1. Add 5 µL of Neutralizer to a fresh 1.7 mL microcentrifuge tube.

2. Transfer the spin column from the Column Wash procedure into the microcentrifuge tube.

3. Apply 100 µL of the Elution Buffer to the column and centrifuge for one minute to elute bound protein.

4. 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.

Frequently Asked Questions and Troubleshooting

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1. What is the black powder that is packed into the spin columns?

The black chromatography material is silicon carbide (SiC). It is processed using proprietary methods to function as an ion exchange chromatography resin.

2. How does the kit deplete albumin and other high abundance proteins from serum and plasma?

The depletion of the major proteins in the serum is based mainly on their pI values. The column binding pH used in this kit depletes most of the major proteins in the flowthrough and retains many of the minor proteins.

3. What high abundance serum proteins can be depleted using this kit?

The Proteospin™ Abundant Serum Protein Depletion Kit allows for the efficient removal of albumin, α-antitrypsin, transferin and haptoglobin.

4. Can the columns be re-used?

The columns are designed for single use only. This minimizes sample carryover.

5. How should I store the Proteospin™ Abundant Serum Protein Depletion Kit solutions?

Once opened, the solution containers should be stored at 4°C when not in use. If unopened, the solution containers can be stored at room temperature.

6. What are the maximum and minimum loads for binding sample volumes that I can load onto the column?

We recommend that no more than 500 μL of sample be loaded onto the column, to ensure that the bottom of the column does not rest in the flowthrough that is caught in the collection tube. The minimum load should be at least 100 μL to completely cover the resin bed.

7. What are the maximum and minimum amounts of protein that I can load onto the column?

We recommend that between 300 and 500 μg of protein be loaded onto the column, or approximately 10 μL of serum, in order to obtain optimal depletion.

8. What are the highest and lowest recommended column microcentrifuge speeds?

We recommend a maximum speed of 6,700 x g be used to spin the columns. Speeds below 6,700 x g may be insufficient to completely move the liquid phase through the resin bed. Additional spinning times may be required to remove this liquid.

9. What is the typical amount of high abundance protein depletion that I can expect with the Proteospin™ Abundant Serum Protein Depletion Kit columns?

Approximately 70% of albumin, 90% of α-antitrypsin, and 50% of transferin and haptoglobin are depleted from the serum sample using the Proteospin™ kit and columns.

10. Can I autoclave the columns?

No. The columns are not designed to be autoclaved as it would cause the plastic in the columns to wrinkle.

11. How can I assess the depletion of albumin and other major serum proteins from my sample?

Save the flowthrough from the binding step and perform a colorimetric protein assay to determine the amount of protein present. This sample can be run on a 1D or 2D gel to determine the specific proteins that have been depleted.

12. I use a colorimetric protein assay to determine concentration of eluted proteins. Is there an accurate way of doing it?

The colorimetric protein assays available in the market are quite accurate, however, there are a number of ways to improve them. First, using a linear regression to fit the points for the standard curve is often less accurate than using a second-degree polynomial. Microsoft Excel™ has full features for curve fitting. The goodness-of-fit for the regression should determine whether first-degree (linear) or second-degree polynomial would be used. Second, the protein used as the standard should closely match the dye-binding characteristics of the protein of interest. For example, using BSA as a standard to determine concentrations of immunoglobulins may provide erroneous results. This is because immunoglobulins do not bind some dyes efficiently. Third, ensure that the protein standard (and standard curve) is in a solution that resembles the solution of the test protein. Sometimes buffer constituents can have drastic effects on dye binding properties.

Troubleshooting Guide

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Each of the following tables discusses a problem which can occur, its possible causes, and presents solutions and explanations.

Problem: Protein Solution Does Not Flow Through the Column

Possible Causes	Solution & Explanation
Centrifugation speed was too low.	Check the centrifuge to ensure that it is capable of generating 6,700 × *g*. Sufficient centrifugal force is required to move the liquid phase through the resin.
Inadequate spin time.	Spin an additional minute to ensure that the liquid is able to flow completely through the column.
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.

Problem: Eluted Protein is Degraded

Possible Causes	Solution & Explanation
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 solution was not neutralized quickly enough.	If eluted protein is 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 protein samples with 0.015 % sodium azide. The Elution Buffer already contains sodium azide.

Problem: Insufficient Depletion of Major Proteins

Possible Causes	Solution & Explanation
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.

Problem: Low Protein Concentration in the Elution

Possible Causes	Solution & Explanation
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.