INSTRUCTIONS

Pierce™ Top 2 Abundant Protein Depletion Spin Columns

85161 85162 2605.0

Number	Description
85161	**Pierce Top 2 Abundant Protein Depletion Spin Columns,** 6 columns, each column contains 62% slurry in 10mM PBS, 0.15M NaCl, 0.02% sodium azide, pH 7.4
85162	**Pierce Top 2 Abundant Protein Depletion Spin Columns,** 24 columns, each column contains 62% slurry in 10mM PBS, 0.15M NaCl, 0.02% sodium azide, pH 7.4

Storage: Upon receipt store at 4°C. Product shipped with an ice pack. Do not freeze product.

Introduction

The Thermo Scientific Pierce Top 2 Abundant Protein Depletion Spin Columns are used for the removal of human serum albumin (HSA) and immunoglobulins (IgG) from human serum, plasma or spinal fluids. These depletion spin columns use highly specific immobilized anti-HSA and anti-IgG antibodies for protein removal, providing minimal nonspecific interactions with other proteins.

Analysis of human fluids is often complicated by the presence of high concentrations of albumin and IgG that represent more than 70% of the total serum protein. Removal of these proteins is often essential for the study of low-abundant proteins. The Pierce Top 2 Abundant Protein Depletion Spin Columns can deplete > 95% of HSA and > 90% of IgG. Samples are processed in a convenient disposable spin format and can be completed in ~40 minutes. The depletion of high-abundant proteins enables the identification and quantitation of low-abundant proteins in samples by mass spectrometry (MS).

Important Product Information

- The depletion spin columns contain a storage solution used as a dilution and binding buffer for direct processing of 10µL of serum or plasma.

  Note: No other additions or solvent exchanges are required before protein depletion.

- Each depletion spin column processes a maximum of 10µL of human serum or plasma. To process larger serum sample volumes, use multiple depletion spin columns. For serum samples containing abnormally high amounts of albumin or IgG, the sample load may need to be reduced.
- The depletion spin columns are designed for single use. Do not reuse the resin.
- The depletion spin columns are designed for use with human serum or plasma samples and have not been tested on other species. Alternative human biological fluids (e.g., cerebrospinal fluid or amniotic fluid) may be used with this product, but may require optimization.

Additional Materials Required

- Microcentrifuge capable of operating at 1000 × *g*
- 2mL collection tubes
- End-over-end mixer

Spin Column Procedure for HSA and IgG Removal

Note: Because of the high concentration of albumin present in serum, each 600µL of antibody resin slurry binds sufficient albumin and IgG to process 10µL (600µg) of serum or plasma. However, the amount of HSA and IgG in serum or other fluid samples will vary considerably. For best results, optimize the ratio of sample to slurry volume for each specific application.

1. Equilibrate the depletion spin column to room temperature.
2. Remove the column screw cap and add 10µL of sample directly to the resin slurry in the column.
3. Cap the column and invert the column several times until the resin is completely suspended in the solution.
4. Incubate the mixture in the column with gentle end-over-end mixing for 30 minutes at room temperature. Make sure the sample mixes with the resin during the incubation period. Alternatively, vortex every 5 minutes.
5. Twist off the bottom closure and loosen the cap. Place column into a 2mL collection tube and centrifuge at 1000 × *g* for 2 minutes.
6. Discard the column containing the resin.
7. Filtrate contains sample with albumin and IgG removed. Use for further processing or store for later use. The depleted sample will be in 10mM PBS, 0.15M NaCl, 0.02% azide, pH 7.4.
8. Sample processing will depend on the type of downstream analysis and may require buffer exchange and/or concentration for 2D gel electrophoresis and MS analysis. Use Thermo Scientific Pierce Protein Concentrators for buffer exchanging and/or concentration (see Related Thermo Scientific Products Section).

Troubleshooting

Problem	Possible Cause	Solution
Incomplete removal of albumin or IgG	Sample exceeds binding capacity	Reduce amount of sample processed
	Incomplete binding	Increase incubation time
	Sample is not mixed during incubation	Mix the sample with resin by gentle end-over-end mixing and make sure that the sample is mixing with the resin during the incubation period.

Related Thermo Scientific Products

89875 **Pierce Albumin/IgG Removal Kit**

85164 **Pierce Top 12 Abundant Protein Depletion Spin Columns,** 6 columns

85165 **Pierce Top 12 Abundant Protein Depletion Spin Columns,** 24 columns

31876 **Pierce Normal Human Serum**

87748 **Pierce Protein Concentrators, 9K MWCO, 7mL**

23225 **BCA Protein Assay Kit**

24590 **GelCode™ Blue Stain Reagent, 500 mL**

84840 **Pierce MS Sample Prep Kit**

General Reference

Anderson, N.L. and Anderson, N.G. (2002). The human plasma proteome: History, character, and diagnostic prospects. *Mol Cell Proteomics* **1:**845-67.

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