Introduction

The Thermo Scientific Pierce Top 12 Abundant Protein Depletion Spin Columns are used for single-step removal of twelve high-abundance proteins from 10µL of human serum or plasma. The resin uses highly specific immobilized antibodies for protein removal, providing minimal nonspecific interactions with other proteins.

Plasma proteome studies are limited by the vast dynamic range (10 orders of magnitude) of protein abundance. Many proteins of pharmaceutical interest are present at low concentrations in the plasma and are difficult to detect in the presence of high-abundant proteins. The proteins listed in the table below constitute ~95% of all plasma proteins. The Pierce Top 12 Abundant Protein Depletion Spin Columns remove > 95% of these 12 high-abundant proteins from 10µL of serum or plasma samples and enable the identification and quantitation of low-abundant proteins in samples by mass spectrometry (MS).

Table 1. List of the top 12 proteins removed by the Thermo Scientific Pierce Top 12 Abundant Protein Depletion Spin Columns.

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>85164</td>
<td>Pierce Top 12 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</td>
</tr>
<tr>
<td>85165</td>
<td>Pierce Top 12 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</td>
</tr>
</tbody>
</table>

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

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 Top 12 Protein Removal

**Note:** The concentration of serum/plasma proteins can vary widely depending on the origin of the sample. Each 810µL of antibody resin slurry is optimized to process 10µL (600µg) of serum or plasma. 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 60 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 the top 12 proteins 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

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incomplete removal of albumin or IgG</td>
<td>Sample exceeds binding capacity</td>
<td>Reduce amount of sample processed</td>
</tr>
<tr>
<td>Incomplete binding</td>
<td></td>
<td>Increase incubation time</td>
</tr>
<tr>
<td>Sample is not mixed during incubation</td>
<td></td>
<td>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.</td>
</tr>
</tbody>
</table>

Related Thermo Scientific Products

- Pierce Albumin/IgG Removal Kit (89875)
- Pierce Top 2 Abundant Protein Depletion Spin Columns, 6 columns (85161)
- Pierce Top 2 Abundant Protein Depletion Spin Columns, 24 columns (85162)
- Pierce Normal Human Serum (31876)
- Pierce Protein Concentrators, 9K MWCO, 7mL (87748)
- BCA Protein Assay Kit (23225)
- GelCode™ Blue Stain Reagent, 500 mL (24590)
- Pierce MS Sample Prep Kit (84840)

General Reference

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