Introduction

The Pierce Ion Exchange Spin Columns use membrane-adsorbent technology as a chromatographic matrix to fractionate proteins. Ion exchange chromatography is widely used for pre-fractionation or purification of a target protein from crude biological samples. Such molecules are separated based on differences in their accessible surface charges using relatively mild binding and eluting conditions to produce high protein recovery with intact biological activity.

Membrane-based ion exchange chromatography has advantages over resin-based column chromatography. The membrane adsorbents in the Pierce Ion Exchange Spin Columns are stabilized regenerated cellulose-based with a highly porous structure. The pores are larger than 3,000 nm, which provides proteins easy access to the charged surface. The benefit of adsorptive membranes is the shorter diffusion times than those obtained in resin-based chromatography, as the interactions between molecules and active sites on the membrane occur in convective through-pores, rather than fluid inside the pores of a resin particle. For this reason, adsorptive membranes have the potential to maintain high efficiencies both at high-flow rates and when purifying large biomolecules with low diffusivities.

The Pierce Ion Exchange Spin Columns replace time-consuming, tedious and expensive chromatographic methods for many protein applications. The centrifuge purification format based on membrane adsorbents allows convenient high-yield processing of multiple samples in less than 20 minutes. The purified protein is compatible with downstream applications such as sample fractionation for 1D and 2-D SDS-PAGE, X-ray crystallization and NMR spectroscopy.

Important Product Information

- For ease of use, each Pierce Strong Ion Exchange Spin Column is marked S (strong cation exchanger, sulphonic acid functional groups) or Q (strong anion exchanger, quaternary ammonium functional groups).

- The spin column capacities are 4 mg proteins/peptides for the mini and 60-80 mg protein for the maxi. Actual capacity depends on the specific protein sample, selected pH and salt condition. Capacities were established using 1 mg/ml of BSA in 25 mM Tris•HCl, pH 8.0 for the anion (Q) columns and 1 mg/ml of cytochrome C in 25 mM sodium acetate buffer, pH 5.5 for cation (S) columns.

- Empirically determine the optimal buffer (pH and salt concentration) for purifying and eluting the protein of interest based on the pI of the protein. (See the Additional Information Section for a list of compatible substances.)

- Purification examples: A protein with pI 9 is positively charged at pH 7 and will bind to the cation (S) columns. Increasing the salt concentration or raising the buffer pH above 9 will elute the bound protein. A protein with pI 4 is negatively charged at pH 7 and will bind to the anion (Q) columns. Increasing the salt concentration or lowering the buffer pH below 4 will elute the bound protein. (See Figure 1 for another example.)
Figure 1. Pierce Ion Exchange Spin Column purification example.
Example protein X has a pI of 5.2; therefore, use a purification buffer at pH > 5.2 for the anion (Q) columns or a pH < 5.2 for the cation (S) columns.

- For the anion (Q) columns: Ensure the buffer pH is above the protein’s isoelectric point and the sample does not contain anionic detergents. For purifying the sample, use a buffer with a pH from 5 to 10 and a salt concentration at \( \leq 25 \text{ mM} \). For elution, use step-wise salt gradient to a final salt concentration of 2 M.

- For the cation (S) columns: Ensure the buffer pH is below the protein’s isoelectric point and the sample does not contain cationic detergents. For purifying the sample, use a buffer with a pH from 3 to 8 and a salt concentration at \( \leq 25 \text{ mM} \). For elution, use step-wise salt gradient to a final salt concentration of 2 M.

- Fixed-angle rotors: To achieve even liquid flow through the membrane using a fixed-angle rotor, align the letter printed (e.g., Q, S) on the column toward the rotor center for all chromatography steps (Figure 2).

- To prevent clogging of the spin column, pre-filter samples with a syringe filter (0.45 \( \mu \text{m} \)).

- For effective purification reduce the ionic strength of the protein sample by dilution, dialysis, gel filtration or ultra-filtration before adding to the spin columns. For best results, dilute the sample in loading buffer to \( \leq 25 \text{ mM} \) salt.

Materials Required

- For the Mini Spin Columns use a microcentrifuge that can accommodate 2.0 ml tubes and is capable of 2,000 \( \times \) g

- For the Maxi Spin Columns use a centrifuge that accommodates 50 ml centrifuge tubes and is capable of 500 \( \times \) g

Note: To achieve even liquid flow through the membrane using a fixed-angle rotor, align the printed letter (e.g., Q, S) toward the center of the rotor for all chromatography steps.

- Protein sample: Ensure that the pH of the sample is close to the purification buffer’s pH. To reduce the ionic strength of the sample, dilute it in purification buffer. Pre-filter (0.45 \( \mu \text{m} \)) the protein sample before adding to the column to prevent clogging. (See the Additional Information Section for a list of compatible substances.)

- Purification and Elution Buffers: Based on the pI of the target protein, empirically determine the optimal buffers (pH and salt concentration) for purifying and eluting.
General Protocol for using the Mini Spin Columns
1. Add 400 µl of purification buffer to the column and centrifuge at 2,000 × g for 5 minutes. Discard the flow-through.
2. Add ≤ 400 µl of sample and centrifuge at 2,000 × g for 5 minutes. Repeat this step as many times as necessary to process the entire sample.
3. Add 400 µl of purification buffer to the column and centrifuge at 2,000 × g for 5 minutes. Repeat this step once.
4. Add 50-400 µl of elution buffer with increasing salt concentrations (i.e., increase step-wise to a final salt concentration of 2 M) and centrifuge at 2,000 × g for 5 minutes. Elute sample in as many steps as necessary.

General Protocol for using the Maxi Spin Columns
1. Add 5 ml of purification buffer onto the membrane and centrifuge at 500 × g for 5 minutes. Discard the flow-through.
2. Add up to 19 ml of sample for a swinging-bucket or 10.5 ml for a fixed-angle rotor and centrifuge at 500 × g for 5 minutes. Repeat this step as many times as necessary to process the entire sample.
3. Add 10 ml of purification buffer onto the membrane and centrifuge at 500 × g for 5 minutes. Repeat this step once.
4. Add 2-10 ml of elution buffer with increasing salt concentrations (i.e., increase step-wise to a final salt concentration of 2 M) and centrifuge at 500 × g for 5 minutes. Elute sample in as many steps as necessary.

Example Protocol for Purifying Basic Proteins (high pI) with a Mini Cation (S) Column
1. Add 400 µl of 25 mM sodium acetate buffer, pH 5.5 to the column and centrifuge at 2,000 × g for 5 minutes. Discard the flow-through.
2. Add up to 400 µl of sample and centrifuge at 2,000 × g for 5 minutes. Discard the flow-through. Repeat this step as many times as necessary to process the entire sample.
3. Add 400 µl of 25 mM sodium acetate buffer, pH 5.5 onto the membrane and centrifuge at 2,000 × g for 5 minutes. Discard the flow-through. Repeat this step once.
4. To elute the protein, add 50-400 µl of 25 mM sodium acetate buffer, pH 5.5 containing 0.5 or 1.0 M NaCl.

Example Protocol for Purifying Acidic Proteins (low pI) with a Mini Anion (Q) Column
1. Add 400 µl of 25 mM Tris•HCl buffer, pH 8.0 to the column and centrifuge at 2,000 × g for 5 minutes. Discard the flow-through.
2. Add up to 400 µl of sample and centrifuge at 2,000 × g for 5 minutes. Discard the flow-through. Repeat this step as many times as necessary to process the entire sample.
3. Add 400 µl of 25 mM Tris•HCl buffer, pH 8.0 onto the membrane and centrifuge at 2,000 × g for 5 minutes. Discard the flow-through. Repeat this step once.
4. To elute the protein, add 50-400 µl of 25 mM Tris•HCl buffer, pH 8.0 containing 0.5 or 1 M NaCl.
Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>The buffer does not flow through the spin column</td>
<td>The membrane is clogged</td>
<td>Pre-filter the sample</td>
</tr>
<tr>
<td>The binding capacity is low</td>
<td>Ionic strength of the sample is too high Sample contains interfering detergents</td>
<td>Dialyze, desalt or dilute sample in purification buffer with a salt concentration ≤ 25 mM</td>
</tr>
<tr>
<td>The protein did not elute</td>
<td>Eluting buffer ionic strength is too low The pH of the eluting buffer is incorrect A high concentration of salt added to the elution buffer altered its pH</td>
<td>Increase the ionic strength of the elution buffer For the cation (S) columns use a pH above the pI of the protein; for the anion (Q) columns use a pH below the pI of the protein</td>
</tr>
<tr>
<td>Low protein activity</td>
<td>The target protein is not stable in the elution buffer</td>
<td>Use an appropriate buffer (pH) for the specific protein</td>
</tr>
</tbody>
</table>

Additional Information

Table 1. Chemical compatibility of the Pierce Strong Ion Exchange Spin Columns.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Compatible Concentration</th>
<th>Detergents</th>
<th>Compatible Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>98%</td>
<td>n-octyl β-D-glucopyranoside</td>
<td>2.0%</td>
</tr>
<tr>
<td>Ethanol</td>
<td>99%</td>
<td>SDS</td>
<td>2.0%</td>
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<tr>
<td>n-propanol</td>
<td>100%</td>
<td>Triton® X-100</td>
<td>2.0%</td>
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<tr>
<td>Isopropyl Alcohol</td>
<td>100%</td>
<td>Tween®-20</td>
<td>2.0%</td>
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<tr>
<td>Butan-2-ol</td>
<td>99%</td>
<td></td>
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<tr>
<td>Glycerol</td>
<td>100%</td>
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</tr>
<tr>
<td>Ethylene Glycol</td>
<td>20%</td>
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</tr>
<tr>
<td>Polyethylene Glycol</td>
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<td></td>
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<tr>
<td>Acetone</td>
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<td></td>
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<tr>
<td>Methylketone</td>
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<td></td>
</tr>
<tr>
<td>Chloroform</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dichloromethane</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Dimethylformamide</td>
<td>50%</td>
<td></td>
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<tr>
<td>Dimethylsulfoxide</td>
<td>100%</td>
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<tr>
<td>Bases</td>
<td>Ammonium Hydroxide</td>
<td>28%</td>
<td>Guanidine•HCl</td>
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<tr>
<td>Sodium Hydroxide</td>
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<td>Urea</td>
<td>8 M</td>
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<tr>
<td>Oxidizing Agents</td>
<td>Hydrogen Peroxide</td>
<td>Not Compatible</td>
<td>Sodium Hypochlorite</td>
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<table>
<thead>
<tr>
<th>Acids</th>
<th>25% (pH 1.0)</th>
<th>DMEM</th>
<th>Norm</th>
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<tr>
<td>Formic Acid</td>
<td>1.0 M</td>
<td>RPMI-1640</td>
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<tr>
<td>Hydrochloric Acid</td>
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<tr>
<td>Sulphuric Acid</td>
<td>1.0 M</td>
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</tr>
<tr>
<td>Trifluoroacetic Acid</td>
<td>2 M</td>
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Related Products

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<tr>
<th>Code</th>
<th>Description</th>
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<tbody>
<tr>
<td>66380</td>
<td>Slide-A-Lyzer® Dialysis Cassette, 10K MWCO, 0.5-3 ml capacity, 10 pack</td>
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<tr>
<td>66810</td>
<td>Slide-A-Lyzer Dialysis Cassette, 10K MWCO, 3-12 ml capacity, 8 pack</td>
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<tr>
<td>66830</td>
<td>Slide-A-Lyzer Dialysis Cassette, 10K MWCO, 12-30 ml capacity, 6 pack</td>
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<tr>
<td>66003</td>
<td>Slide-A-Lyzer Dialysis Cassette, 20K MWCO, 0.5-3 ml capacity, 10 pack</td>
</tr>
<tr>
<td>66012</td>
<td>Slide-A-Lyzer Dialysis Cassette, 20K MWCO, 3-12 ml capacity, 8 pack</td>
</tr>
<tr>
<td>66030</td>
<td>Slide-A-Lyzer Dialysis Cassette, 20K MWCO, 12-30 ml capacity, 6 pack</td>
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<tr>
<td>89891</td>
<td>Zeba™ Desalt Spin Columns, 5 ml, 0.5-2 ml samples, 5 columns</td>
</tr>
<tr>
<td>89893</td>
<td>Zeba Desalt Spin Columns, 10 ml, 1.5-4 ml samples, 5 columns</td>
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<tr>
<td>23225</td>
<td>Pierce BCA Protein Assay Kit, working range of 20-2,000 µg/ml</td>
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<tr>
<td>23250</td>
<td>Pierce BCA Protein Assay Kit-Reducing Agent Compatible, working range of 125-2,000 µg/ml</td>
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<tr>
<td>23226</td>
<td>Pierce Coomassie Plus (Bradford) Protein Assay Kit, working range of 1-1,500 µg/ml</td>
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