

Pierce Strong Ion Exchange Spin Columns

90008 90009 90010 90011

2042.0

Number	Description
90008	Pierce Strong Cation Exchange Spin Column, Mini, 24 spin columns and 48 collection tubes
90009	Pierce Strong Cation Exchange Spin Column, Maxi, 8 spin columns and 16 collection tubes
90010	Pierce Strong Anion Exchange Spin Column, Mini, 24 spin columns and 48 collection tubes
90011	Pierce Strong Anion Exchange Spin Column, Maxi, 8 spin columns and 16 collection tubes

Storage: Upon receipt store at room temperature.

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.)

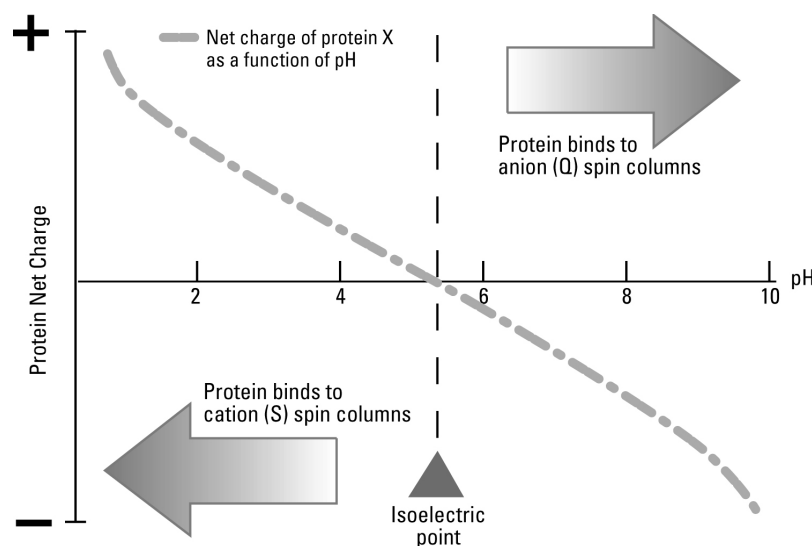


Figure 1. Pierce Ion Exchange Spin Column purification example.
Example protein X has a pI of 5.2; therefore, use a purification buffer at pH > 5.2 for the anion (Q) columns or a pH < 5.2 for the cation (S) columns.

- 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 ≤ 25 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 ≤ 25 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 μ 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 ≤ 25 mM salt.

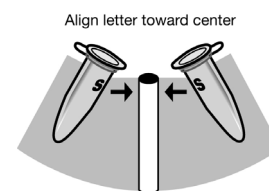


Figure 2. Orientation of columns in a fixed-angle rotor.

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 μ 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 μ l of purification buffer to the column and centrifuge at $2,000 \times g$ for 5 minutes. Discard the flow-through.
2. Add ≤ 400 μ l of sample and centrifuge at $2,000 \times g$ for 5 minutes. Repeat this step as many times as necessary to process the entire sample.
3. Add 400 μ l of purification buffer to the column and centrifuge at $2,000 \times g$ for 5 minutes. Repeat this step once.
4. Add 50-400 μ l of elution buffer with increasing salt concentrations (i.e., increase step-wise to a final salt concentration of 2 M) and centrifuge at $2,000 \times g$ 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 $500 \times g$ 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 $500 \times g$ 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 $500 \times g$ 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 $500 \times g$ 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 μ l of 25 mM sodium acetate buffer, pH 5.5 to the column and centrifuge at $2,000 \times g$ for 5 minutes. Discard the flow-through.
2. Add up to 400 μ l of sample and centrifuge at $2,000 \times g$ for 5 minutes. Discard the flow-through. Repeat this step as many times as necessary to process the entire sample.
3. Add 400 μ l of 25 mM sodium acetate buffer, pH 5.5 onto the membrane and centrifuge at $2,000 \times g$ for 5 minutes. Discard the flow-through. Repeat this step once.
4. To elute the protein, add 50-400 μ l 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 μ l of 25 mM Tris•HCl buffer, pH 8.0 to the column and centrifuge at $2,000 \times g$ for 5 minutes. Discard the flow-through.
2. Add up to 400 μ l of sample and centrifuge at $2,000 \times g$ for 5 minutes. Discard the flow-through. Repeat this step as many times as necessary to process the entire sample.
3. Add 400 μ l of 25 mM Tris•HCl buffer, pH 8.0 onto the membrane and centrifuge at $2,000 \times g$ for 5 minutes. Discard the flow-through. Repeat this step once.
4. To elute the protein, add 50-400 μ l of 25 mM Tris•HCl buffer, pH 8.0 containing 0.5 or 1 M NaCl.

Troubleshooting

Problem	Possible Cause	Solution
The buffer does not flow through the spin column	The membrane is clogged	Pre-filter the sample
The binding capacity is low	Ionic strength of the sample is too high	Dialyze, desalt or dilute sample in purification buffer with a salt concentration ≤ 25 mM
	Sample contains interfering detergents	
The protein did not elute	Eluting buffer ionic strength is too low	Increase the ionic strength of the elution buffer
	The pH of the eluting buffer is incorrect	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
	A high concentration of salt added to the elution buffer altered its pH	
Low protein activity	The target protein is not stable in the elution buffer	Use an appropriate buffer (pH) for the specific protein

Additional Information

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

Solvents	Compatible Concentration	Detergents	Compatible Concentration
Methanol	98%	n-octyl β -D-glucopyranoside SDS Triton [®] X-100 Tween [®] -20	2.0%
Ethanol	99%		2.0%
n-propanol	100%		2.0%
Isopropyl Alcohol	100%		2.0%
Butan-2-ol	99%		2.0%
Glycerol	100%	Reducing Agents	
Ethylene Glycol	20%	Dithiothreitol (DTT)	100 mM
Polyethylene Glycol	20%	β -mercaptoethanol	100 mM
Acetone	100%	Salts & Misc. Reagents	
Methylethyl Ketone	100%	Guanidine•HCl	6 M
Chloroform	100%	Urea	8 M
Dichloromethane	100%	EDTA (sodium salt)	5%
Dimethylformamide	50%	Imidazole	500 mM
Dimethylsulfoxide	100%	Sodium Chloride	5 M
Bases		Oxidizing Agents	
Ammonium Hydroxide	28%	Hydrogen Peroxide	Not Compatible
Sodium Hydroxide	1.0 M	Sodium Hypochlorite	Not Compatible
Acids		Culture Media	
Acetic Acid	1.0 M	DMEM	Norm
Formic Acid	25% (pH 1.0)	RPMI-1640	Norm
Hydrochloric Acid	1.0 M		
Sulphuric Acid	1.0 M		
Trifluoroacetic Acid	2 M		

Related Products

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

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No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current versions of product instructions are available at www.thermo.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

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