Ion Exchange Chromatography Selection Guide

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Introduction and Product Selection Table

Ion exchange chromatographyseparates compounds based on net surface charge. Molecules are classified as either anions (having a negative charge) or cations (having a positive charge). Some molecules (e.g., proteins) may have both an anionic and cationic group. A positively-charged support (anion exchanger) will bind a compound with an overall negative charge. Conversely, a negatively-charged support (cation exchanger) will bind a compound with an overall positive charge.

Ion exchange matrices can be further categorized as either strong or weak. Strong ion exchange matrices are charged (ionized) across a wide range of pH levels. Weak ion exchange matrices are ionized within a narrower pH range. The four most common ion exchange chemistries are shown here:

Type of Ion Exchanger	Common Abbreviation	Functional Group	Pall Product
Strong Anion	Q	Quarternary Ammonium	Mustang® Q Q Ceramic HyperD® F Acrodisc® with Mustang Q AcroPrep™ with Mustang Q AcroSep™ with Q Ceramic HyperD® F HyperCel STAR AX
Weak Anion	DEAE	Diethylaminoethyl	DEAE Ceramic HyperD® F AcroSep with DEAE Ceramic HyperD® F
Strong Cation	S	Sulfonic Acid	Mustang S Acrodisc with Mustang S AcroPrep with Mustang S
Weak Cation	CM	Carboxymethyl	CM Ceramic HyperD® F AcroSep with CM Ceramic HyperD® F

Pall offers ion exchange resins, pre-packed columns, and membranes. In many areas, chromatography resins are the media of choice for chromatography applications, but in some cases where resin-based methods have limitations (e.g., purification of viruses or large molecules) membranes have proven to be a robust, scalable, and economic alternative. Membranes perform well in such applications because of their faster flow rates compared to resins.

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What Flow Rate to Use?

Mustang membrane chromatography devices are designed to run at flow rates of at least 10 column volumes per minute. Initial optimization of buffer selection, pH, capacity, and elution conditions can all be performed at this flow rate. Faster flow rates for equilibration, loading, and washing will give better throughput; a slower flow rate during binding and elution may give better resolution for some processes.

The open structure of Mustang membranes does not require diffusion into pores, and therefore normally permits high flow rates.

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What Buffer to Use?

Typically, ion exchange chromatography matrices are loaded in low ionic strength buffers. Under these conditions, charged macromolecules will be retained by the stationary phase bearing the opposite charge. Macromolecules bearing the same charge as the stationary phase will simply flow through without binding. The ion exchange matrix is washed with additional low ionic strength buffer to completely wash out any remaining unbound species, and the bound species are differentially eluted by buffers containing increasing amounts of salt. As the ionic strength of the mobile phase increases, salt ions compete for binding to the charges on the ion exchange matrix, displacing the bound macromolecule, and allowing them to elute from the matrix. To avoid difficulty, use anionic (negatively-charged) buffers for cation exchange (Mustang S membrane), and cationic (positively-charged) buffers for anion exchange (Mustang Q membrane).

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What pH to Use?

Although pH does not influence the charge of a strong ion exchange matrix, it will influence the charge on the macromolecules in solution. The operating pH in ion exchange chromatography is selected to maximize the resolution of the target molecule from the contaminant background. In some cases, a pH is selected to provide maximum binding of the target molecule and minimum binding of the contaminants (positive mode). Elution of the target molecule is accomplished by increasing the salt concentration. In other cases, a pH is selected to provide maximum binding of the contaminants and minimal or no binding of the target molecule (negative mode). The target molecule ends up in the flow through, and the contaminants are separated away by binding to the matrix. Through careful selection of both the ion exchange matrix and the operating pH, both yield and purity can be maximized for a single step. However, it is never possible to achieve 100% purity in a single step, which is why multiple steps must be sequenced together to take advantage of the variety of chemical differences between the target molecule and background contaminants.

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What Salt to Use for Elution?

After binding, salt concentrations for elution are chosen so the target molecule does not co-elute with contaminants that have also bound to the ion exchange matrix. Ions of the eluting salt must displace other molecules from the charged groups on the stationary phase with either a gradient or step in the 0 to 1.0 M range.

The effectiveness of displacement for commonly used cations is: $Ca^{2+} > Mg^{2+} > Na^{+} > K^{+} > NH_4^{+}$

The order of displacement effectiveness for commonly used anions: $PO_4^{3-} > SO_4^{2-} > COO^{-} > Cl^{-}$

These rankings correlate with the Hofmiester series, and the strongest eluting salt is not always best. Ideally, several salts should be tested, and finding optimum elution conditions often involves trial and error. Most users will start with either NaCl or KCl simply because they are readily available in the lab. However, $CaCl_2$ or $MgCl_2$ may be used. For some proteins, those salts may actually end up being a better choice. Regardless of eluting salt selection, the effect on the purity, stability, and activity of the target molecule will have to be assessed.

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