Ion exchange chromatography

TR0062.0

Ion exchange chromatography is a process for separating proteins and other molecules in a solution based on differences in net charge. Negatively charged molecules bind to positively charged solid supports and positively charged molecules bind to negatively charged supports. To ensure that a protein has a particular net charge, dissolve it in a buffer that is either above or below its isoelectric point (pI). For example, a protein with a pI of 5 will have a net negative charge if it is in a buffer at pH 7. In this case, the protein could bind to a positively charged solid support like diethylaminoethanol (DEAE) or Pierce® Strong Anion Exchange Columns (see the Related Products Section). Conversely, a protein with a pI of 7 will have a positive charge in a buffer at pH 5 and can bind to a negatively charged solid support such as the Pierce Strong Cation Exchange Columns.

Whether using a cation or anion column, sodium chloride can elute the bound protein. In an anion application, the counter ion is chloride because the chloride anion is exchanged for the target, which is then released. In a cation application, the counter ion is sodium because the sodium cation is exchanged for the target. The strength of the electrostatic interaction between a target and a solid support is a function of the difference in the target pI and the buffer that contains the target. Therefore, a protein with a pI of 5 will bind more tightly to the anion exchange column if the protein is in a buffer at pH 8 rather than at pH 7. A protein with a pI of 7 will bind more tightly to the cation column if its buffer is pH 3 rather than pH 4. The greater the electrostatic charge is, the more concentrated the sodium chloride must be to elute the target.

An alternative to eluting by increasing the salt concentration is to appropriately alter the pH of the buffer. For instance, a protein with a pI of 5 bound to an anion column at pH 7 will elute by decreasing the pH to below 5. Likewise, a protein with a pI of 7 bound to a cation column at pH 5 will elute by increasing the pH above 7. Ion exchangers, whether they be salts or buffering agents, differ in their effectiveness for particular applications. An salt is considered a strong ion exchanger if it remains almost fully ionized over a wide pH range, and it is considered a weak ion exchanger if it is ionized over a narrow pH range.

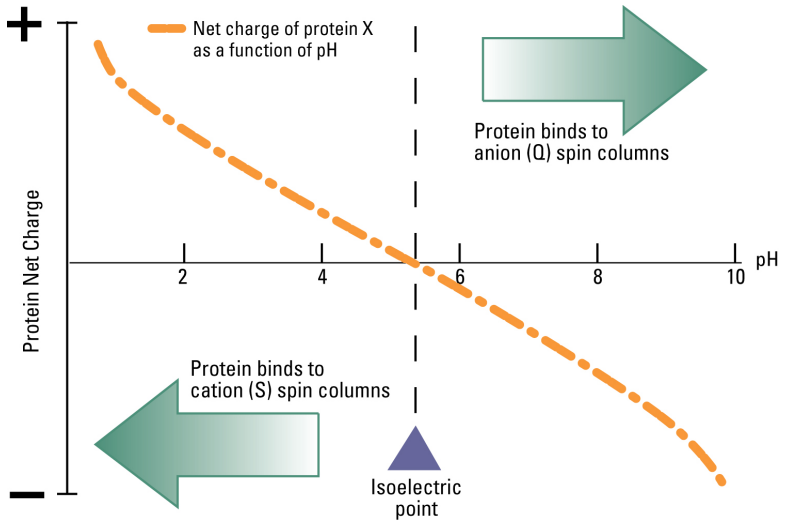

Figure 1. Pierce Ion Exchange Spin Column purification example. Example protein X has a pI of 5.2; therefore, use a purification buffer at pH > 5.2 for the anion (Q) columns or a pH < 5.2 for the cation (S) columns.

Pierce Biotechnology
3747 N. Meridian Road
PO Box 117
Rockford, IL 61105 USA
(815) 968-0747
(815) 968-7316 fax
www.thermo.com/pierce

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