

# HiTrap–convenient protein purification

Column Guide



## Ion Exchange Chromatography (IEX)

IEX separates proteins with differences in charge. The separation is based on the reversible interaction between a charged protein and an oppositely charged chromatographic medium. Proteins bind as they are loaded onto a column. Conditions are then altered so that bound substances are eluted differentially. This elution is usually performed by increases in salt concentration or changes in pH. Most commonly, samples are eluted with salt (NaCl), using a gradient elution, as shown in Figure 1. Target proteins are concentrated during binding and collected in a purified, concentrated form.

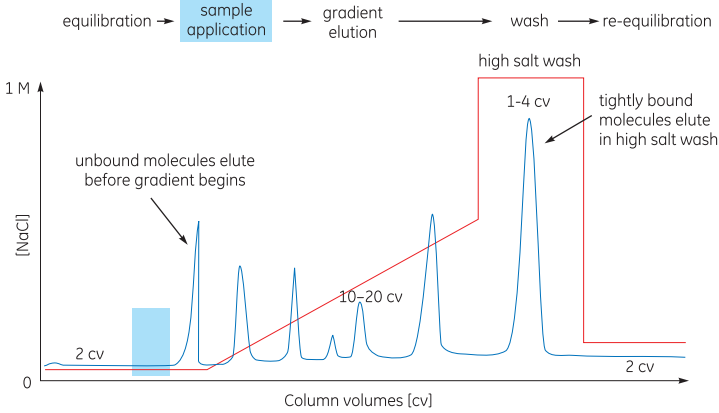


Fig 1. Typical IEX gradient elution.

### Choice of ion exchanger

For most purifications it is recommended to begin with a strong exchanger to allow work over a broad pH range during method development.

Strong ion exchangers

Q (anion exchange), S or SP (cation exchange) are fully charged over a broad pH range (pH 2–12).

Weak ion exchangers

DEAE or ANX (anion exchange) and CM (cation exchange) are fully charged over a narrower pH range (pH 2–9, pH 3–10 and pH 6–10, respectively), but give alternative selectivities.

### Media selection

HiTrap™ IEX Selection Kit, including seven different IEX media, is used for fast screening of IEX ligands and for method optimization.

See also *Ion Exchange Columns and Media Guide*, 18-1127-31.

### Optimization parameters

1. Select ion exchanger.
2. Scout for optimum pH.
3. Select steepest gradient to give acceptable resolution at selected pH.
4. Select highest flow rate that maintains resolution and minimizes separation time.
5. For small scale sample clean up or large scale purifications, transfer to step elution to reduce separation times and buffer consumption, as shown in Figure 2. The different HiTrap IEX columns are ideal for small scale sample clean up.

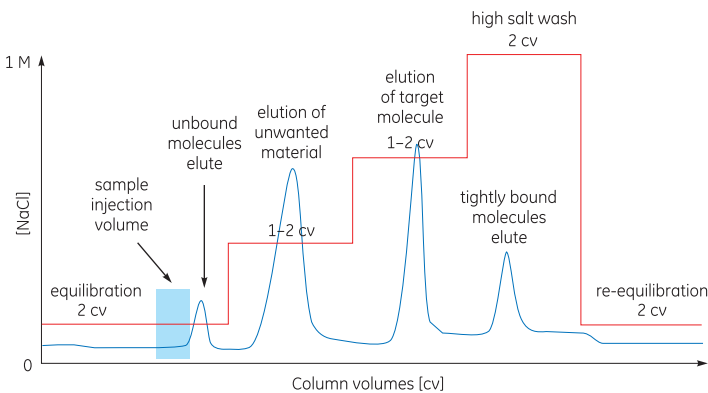


Fig 2. Typical IEX step elution.

## Hydrophobic Interaction Chromatography (HIC)

HIC separates proteins with differences in hydrophobicity. The separation is based on the reversible interaction between a protein and the hydrophobic surface of a chromatographic medium. This interaction is enhanced by high ionic strength buffer, which makes HIC an ideal "next step" for purification of proteins that have been precipitated with ammonium sulphate or eluted in high salt during IEX. Samples in high ionic strength solution (e.g., 1.5 M  $(\text{NH}_4)_2\text{SO}_4$ ) bind as they are loaded onto a column. Conditions are then altered so that the bound substances are eluted differentially. Elution is usually performed by decreases in salt concentration. Changes are made stepwise or with a continuously decreasing salt gradient. Most commonly, samples are eluted with a decreasing gradient of ammonium sulphate concentration. The key stages in a separation are shown in Figure 3. Target proteins are concentrated during binding and collected in a purified, concentrated form.

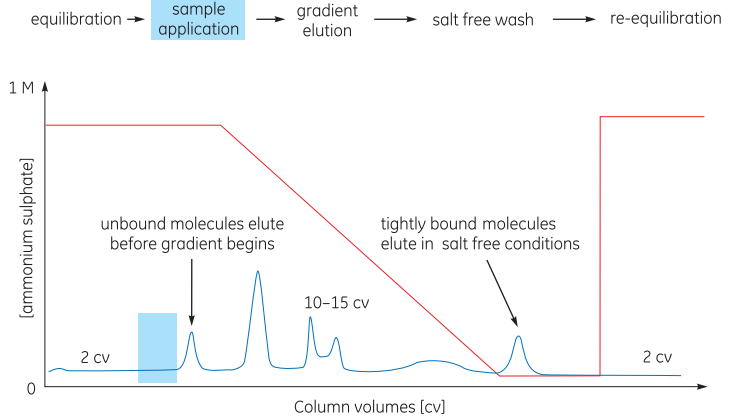


Fig 3. Typical HIC gradient elution.

### Choice of hydrophobic ligand and media selection

The hydrophobicity of a protein is difficult to determine. It is recommended to screen for the most suitable media for each application using HiTrap HIC Selection Kit.

Available hydrophobic ligands vary according to their degree of hydrophobicity:

Increasing hydrophobicity →  
ether → isopropyl → butyl → octyl → phenyl

Highly hydrophobic proteins bind tightly to highly hydrophobic ligands. Note that with HIC the chromatographic matrix as well as the hydrophobic ligand can affect selectivity.

Begin with a medium of low hydrophobicity if the sample is known to have hydrophobic components.

Select the medium that gives the best resolution and loading capacity at a low salt concentration.

See also *RPC & HIC Columns and Media Guide*, 18-1149-96.

### Optimization parameters

1. Select medium.
2. Select optimum gradient to give acceptable resolution. For unknown samples begin with 0%B–100%B (0%B = 1 M ammonium sulphate).
3. Select highest flow rate that maintains resolution and minimizes separation time.
4. For large scale purifications, transfer to step elution to reduce separation times and buffer consumption, as shown in Figure 4.

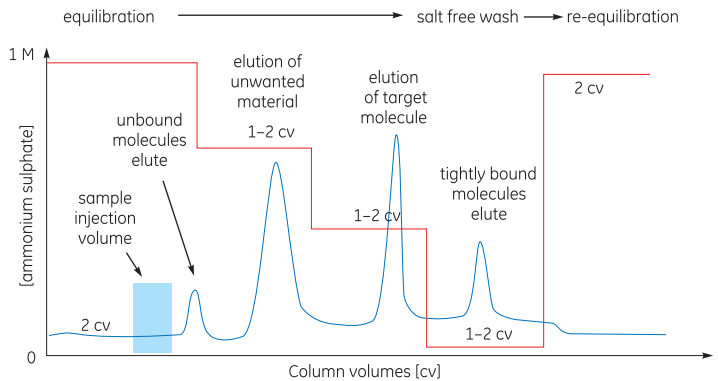


Fig 4. Typical HIC step elution.

## Affinity Chromatography (AC)

AC separates proteins on the basis of a reversible interaction between a protein (or group of proteins) and a specific ligand attached to a chromatographic matrix. AC can be used whenever a suitable ligand is available.

The target protein(s) is specifically and reversibly bound by a complementary binding substance (ligand). The sample is applied under conditions that favor specific binding to the ligand. Unbound material is washed away, and the bound target protein is recovered by changing conditions to those favouring desorption. Elution is performed specifically, using a competitive ligand, or non specifically, by changing the pH, ionic strength or polarity. Proteins are concentrated during binding and collected in a purified, concentrated form. The key stages in a separation are shown in Figure 5.

One important application using AC is purification of tagged recombinant proteins, for example histidine-, GST-, MBP-, and/or Strep(III)-tagged.

AC may also be used to remove specific contaminants. For example, HiTrap Benzamide FF (high sub) removes trypsin-like serine proteases.

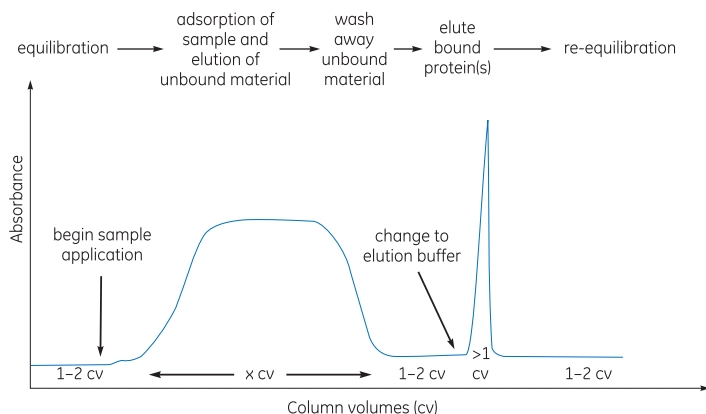


Fig 5. Typical affinity separation.

### Media selection

Parameters such as scale of purification and commercial availability of affinity matrices should be considered when selecting affinity media.

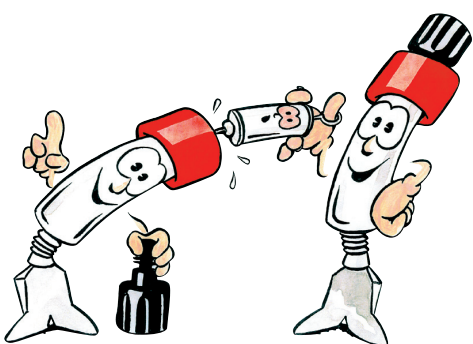
HiTrap affinity columns are ideal for method optimization or small scale purification of target proteins using well-established protocols.

Affinity media can be prepared by coupling a ligand to a selected gel matrix. HiTrap NHS-activated HP is designed specifically to facilitate this process and is supplied with a recommended coupling procedure for coupling primary amines.

See also *Affinity Chromatography Columns and Media Guide*, 18-1121-86.

### Optimization parameters

1. Select correct specificity for target protein.
2. Follow manufacturer's recommendations for binding or elution conditions.
3. Select optimum flow rate for sample application to achieve efficient binding.
4. Select optimum flow rate for elution to maximize recovery.
5. Select maximum flow rate for column re-equilibration to minimize run times.



## Gel Filtration Chromatography (GF)

Gel filtration (size exclusion) chromatography separates proteins with differences in molecular size. Samples are eluted isocratically (single buffer, no gradient). Since buffer composition does not directly affect resolution, the buffer conditions can be varied to suit the sample type or the requirements for the next purification, analysis or storage step. Proteins are collected in purified form in the chosen buffer.

### Sample clean up

Sephadex™ G-25, is ideal for rapid clean up of protein samples.

HiTrap Desalting columns (prepacked with Sephadex G-25) enable fast sample clean up in less than 5 minutes for sample volumes from 0.25 to 1.5 ml, as shown in Figure 6. To increase the maximum sample volume capacity to 3 ml simply connect two columns in series.

HiTrap Desalting columns are ideal for desalting, buffer exchange, and removal of salts, co-factors, labels or other small molecules.

Sample volumes up to 30% of total column volume are loaded when using gel filtration for desalting. The high sample volume gives a separation with minimal sample dilution. Larger sample volumes can be applied but resolution will be reduced.

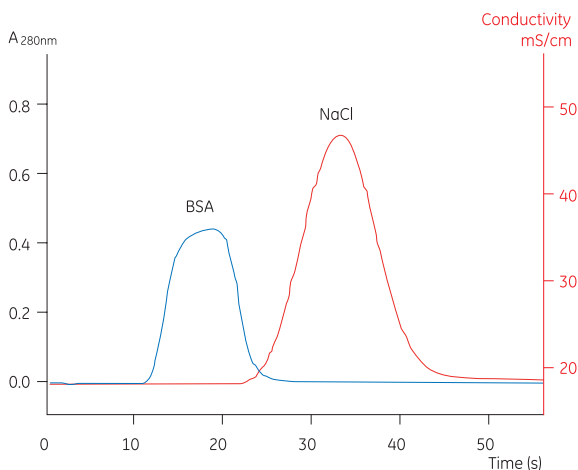


Fig 6. Typical desalting separation.

### High resolution separations

For high resolution separations the technique should be used when sample volumes have been minimized. Figure 7 shows a typical high resolution gel filtration separation.

### Media selection

Refer to *Gel Filtration Columns and Media Guide*, 18-1124-19.

### Optimization parameters for high resolution separations

1. Select medium that gives the best separation of target proteins from contaminants.
2. Select the highest flow rate that maintains resolution and minimizes separation time. Lower flow rates improve resolution of high molecular weight components, whereas faster flow rates may improve resolution of low molecular weight components.
3. Determine the maximum sample volume that can be loaded without significant reduction in resolution (sample volume should be 0.5 to 5% of total column volume).
4. To improve resolution further, increase column length by connecting two columns in series.

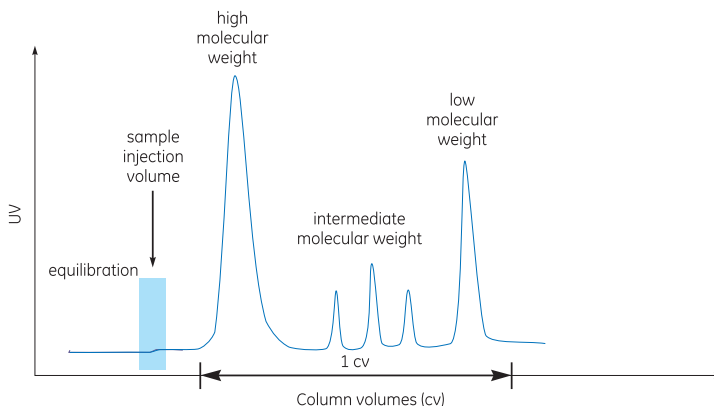


Fig 7. Typical high resolution GF separation.



BioProcess, HiTrap, ÄKTAFPLC, ÄKTApurifier, ÄKTAprime, ÄKTAexplorer, ÄKTAexpress, Sepharose, Sephadex, MAbTrap, MabSelect, MabSelect Xtra, MabSelect SuRe, GSTrap, HisTrap, Capto, MBPTrap, StrepTrap and Drop Design are trademarks of GE Healthcare companies. GE, imagination at work and GE monogram are trademarks of General Electric Company.

All third party trademarks are the property of their respective owners.

All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare which supplies them. GE Healthcare reserves the right, subject to any regulatory and contractual approval, if required, to make changes in specifications and features shown herein, or discontinue the product described at any time without notice or obligation. Contact your local GE Healthcare representative for the most recent information.

A license for the commercial use of GST gene fusion vectors must be obtained from Chemicon International Incorporated, 28820 Single Oak Drive, Temecula, CA 92590, USA.

Separating viral particles with Capto Q products may require a license under United States patent 6,537,793 B2 and foreign equivalents owned by Centelion SAS. Such a license is not included with the purchase of Capto Q but is included with the purchase of Capto ViralQ products.

With the purchase of Capto ViralQ the customer is granted a free limited license under US patent 6,537,793 B2 and foreign equivalents owned by Centelion SAS to separate viral particles solely through use of the product purchased.

Purification and preparation of fusion proteins and affinity peptides comprising at least two adjacent histidine residues may require a license under US pat 5,284,933 and US pat 5,310,663, including corresponding foreign patents (assigne: Hoffman La Roche, Inc).

StrepTrap HP has been manufactured by GE Healthcare and contains Strep-Tactin, manufactured by IBA GmbH, which has been immobilized to GE Healthcare's chromatography media. Strep-Tactin is covered by US patent number 6,103,493 and equivalent patents and patent applications in other countries. The purchase of StrepTrap HP includes a license under such patents limited to internal use, but not re-sale. Please contact IBA for further information on licenses for commercial use of Strep-Tactin.

© 2011 General Electric Company - All rights reserved.  
First published Nov. 1996.

GE Healthcare Bio-Sciences AB, a General Electric Company.

GE Healthcare Europe GmbH  
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare UK Ltd  
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp  
800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA

GE Healthcare Bio-Sciences KK  
Sanken Bldg. 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan



**Distributor**  
GE Healthcare



**In the United States:**  
For customer service, call 1-800-766-7000.  
To fax an order, use 1-800-926-1166.  
To order online: [www.fishersci.com](http://www.fishersci.com)

**In Canada:**  
For customer service, call 1-800-234-7437.  
To fax an order, use 1-800-463-2996.  
To order online: [www.fishersci.ca](http://www.fishersci.ca)