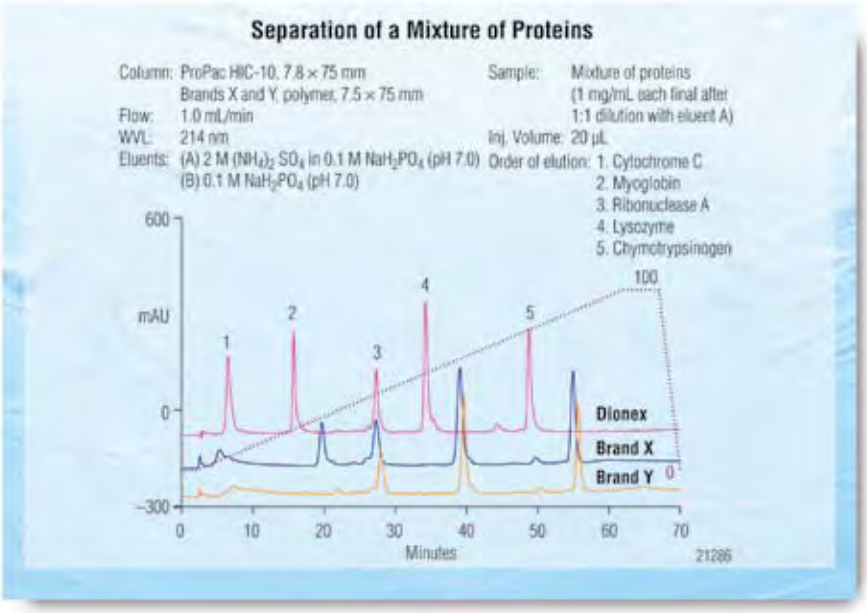
columns

ProPac® HIC-10 Column
Solutions for Protein Analysis



Hydrophobic Interaction Chromatography (HIC) is an important tool for protein chemists separating proteins under gentle conditions that do not cause denaturation. Biomolecules are separated in a decreasing salt gradient, based on differences in their surface hydrophobicity. Since proteins are relatively stable in the salt solutions used as the mobile phase, the biological activity of proteins is usually maintained in HIC, unlike in reversed-phase chromatography where the solvents used for elution often denature the proteins. The separation mechanism of HIC is complementary to those of ion exchange and gel filtration chromatography and thus HIC can be used effectively, in combination with these two other techniques, for high resolution separations of proteins from complex mixtures.

Features

- High-resolution HPLC separations of proteins and peptides using HIC
- Proprietary bonding technology for hydrolytically stable columns
- High-capacity column for purification of proteins
- Separation of proteins under non-denaturing conditions
- Wide range of applications

The ProPac HIC-10 column is a high-resolution, high-capacity, silica-based HIC column that provides greater hydrolytic stability under the highly aqueous conditions used in HIC. The column is suitable for a broad range of protein and peptide purification applications. Examples include the separation of bovine serum proteins, snake venom proteins, human skeletal muscle protein (HSMP), monoclonal antibodies, pancreatin, and thrombin and peptide applications including tryptic digests of cytochrome C and β-casein.

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Hydrolytic stability of the HIC Stationary Phase

The ProPac HIC-10 is a high-efficiency, high-capacity, multi-purpose column, ideal for bioseparations. This novel HIC silica support was developed by applying an innovative bonding technology that improves the hydrolytic stability of bonded silica gel. This superior bonding overcomes the generally low hydrolytic stability of other silica columns functionalized with hydrophilic ligands.

Commercially available HIC columns are often based on either cross-linked polymeric or silica gel particles functionalized with layers of hydrophilic and hydrophobic moieties combined together in certain proportions, and at proper distances. Polymeric HIC phases often provide higher hydrolytic stability and superior longevity as compared to the silica based materials. However, silica columns are well known for their higher efficiencies and better resistance to high pressures, especially, in the presence of organic solvents.

The unique ProPac HIC-10 bonding chemistry results in longer lifetime (tested up to 320 cycles) with less deterioration of its highly hydrophilic surface (Figure 1) while maintaining high efficiency. ProPac HIC-10 provides a solution to a variety of HPLC methods where the longevity of stationary phase is an important feature. The physical and chemical characteristics of the ProPac HIC-10 phase are listed in Table 1.

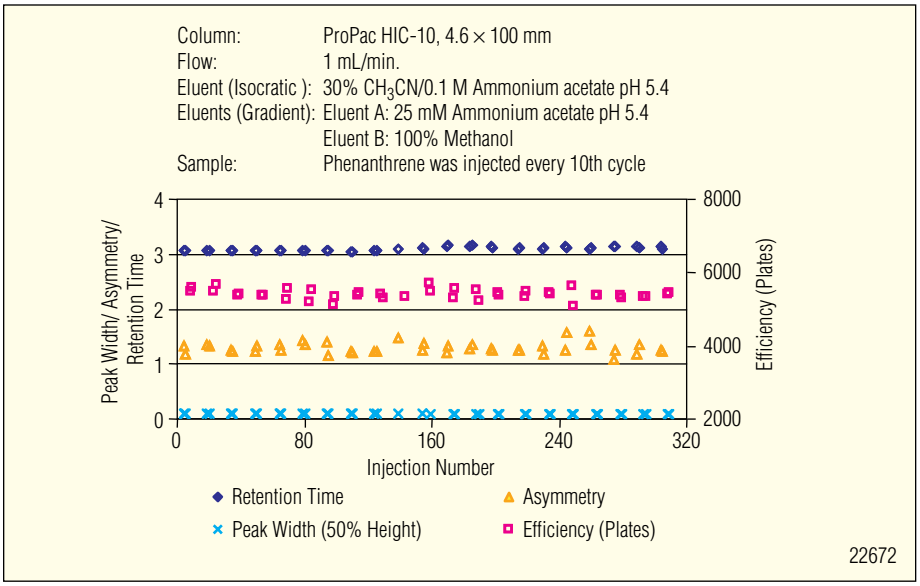

Figure 1. Stability of the bonded phase and mechanical ruggedness.

Table 1. Specifications of ProPac HIC-10 Column

Starting Material	Ultrapure silica
Phase	HIC
Particle Size	5-µm
Particle Shape	spherical
Particle Size Distribution (40/90)	1.2
Metal Impurity (ppm) Na, Fe, Al	<10.0
Pore Volume (mg/L)	0.9
Endcapped	No
Pore Size	300 Å
Surface Area	100 m^2/g
Surface Chemistry	amide/ethyl
Operational Temperature Range	25–40 °C
Protein Loading Capacity for Lysozyme (7.8 x 75 mm column)	340 mg
Solvent Limit	100%
Typical Mobile Phase Components	0.5–2 M ammonium sulfate/ 0.1 M phosphate

High-Capacity Protein Purification

Figure 2 shows a comparison of the protein binding capacity of the ProPac HIC-10 and Brand Y column. As illustrated, under the same elution conditions, the ProPac HIC-10 exhibits almost three times the binding capacity of the other column.

Separation of Proteins Under Non-Denaturing Conditions

Proteins are separated using a variety of chromatographic techniques, including ion-exchange, size exclusion, HIC or reversed-phase. Usually a combination of the above is required. The major advantage of HIC over reversed-phase chromatography is that typical mobile phase conditions in the latter tend to denature protein samples. In HIC, the elution conditions are more gentle, allowing proteins to maintain tertiary structure. Thus, reversed-phase is less suitable for protein purification and HIC is becoming increasingly popular.

Figure 3 shows a 2-D separation of human skeletal muscle protein (HSMP), where HIC is being used in the first dimension. Three-minute fractions were collected, desalted by 10 kDa cut off filter units and individual fractions were injected onto a Dionex ProPac WCX-10 ion-exchange column to separate further in the second dimension. Each fraction from the second dimension was desalted and digested with trypsin. The proteins were identified by fingerprinting using mass spectrometry.

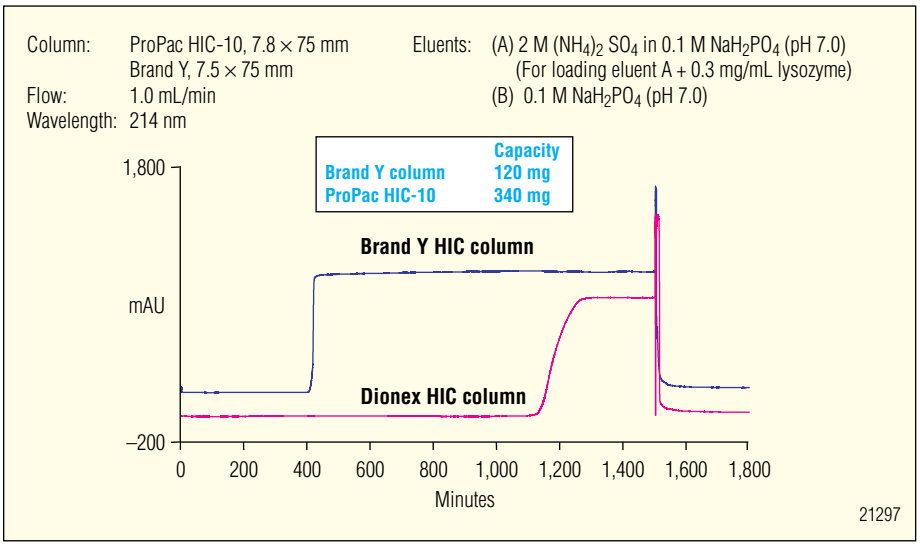

Figure 2. Capacity of Dionex ProPac HIC-10 columns.

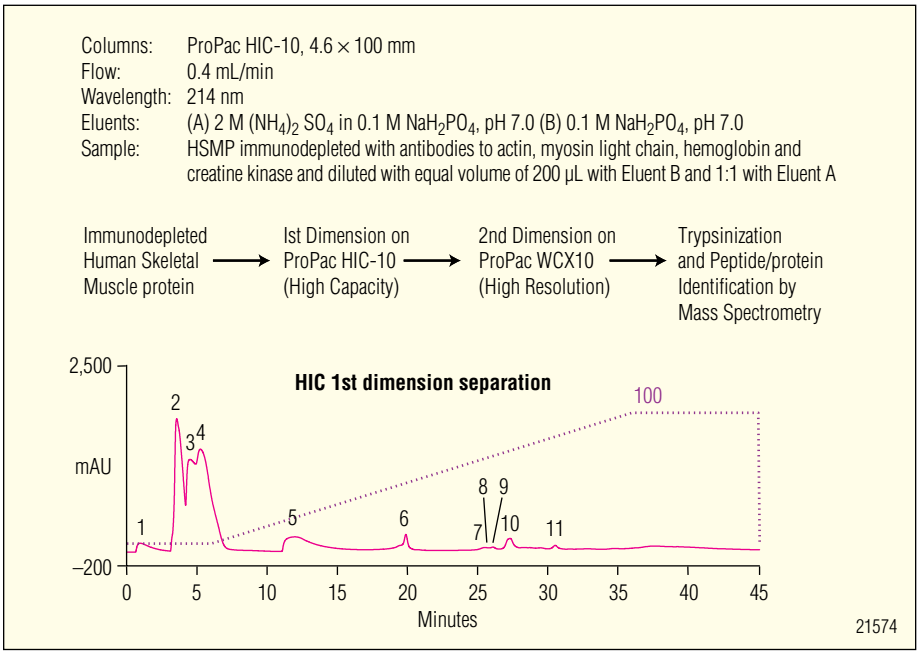

Figure 3. Separation of human skeletal muscle protein using HIC in the first dimension.

Wide Range of Applications

Albumin and IgG are the major protein constituents of serum, comprising about 75% of the total protein content. As a result of their high abundance, these proteins interfere with the isolation and purification of the minor, low abundance proteins. If serum albumin and IgG could be removed from the sample, separation and isolation of the low abundance proteins would become much easier. Figure 4 shows the separation of bovine serum proteins using the ProPac HIC-10 column and compares the separation to those obtained using two other commercially available HIC columns. Using the ProPac HIC-10 column, BSA and IgG are separated from each other and from a series of low abundance components more readily than on other columns under the same elution conditions.

Figure 5 shows another comparison of the separation of a complex protein mix, snake venom proteins, using the ProPac HIC-10 column and two other HIC columns. Snake venoms are rich in hydrolytic enzymes, which are a complex mixture of polypeptides, peptidases, and nucleases. Since some of these components contribute to the toxicity of the venom, their isolation, identification and characterization is important for the development of anti-venom therapies. Figure 5 shows good separation of several components. Compared with the other two columns, the peaks eluting from the ProPac HIC-10 are relatively narrow and symmetrical facilitating collection of the eluting fractions.

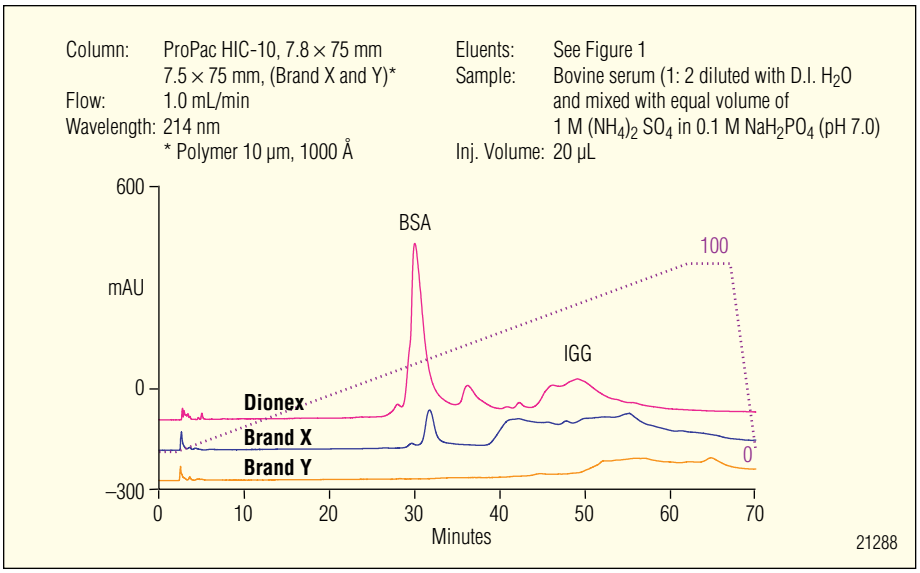

Figure 4. Separation of bovine serum proteins.

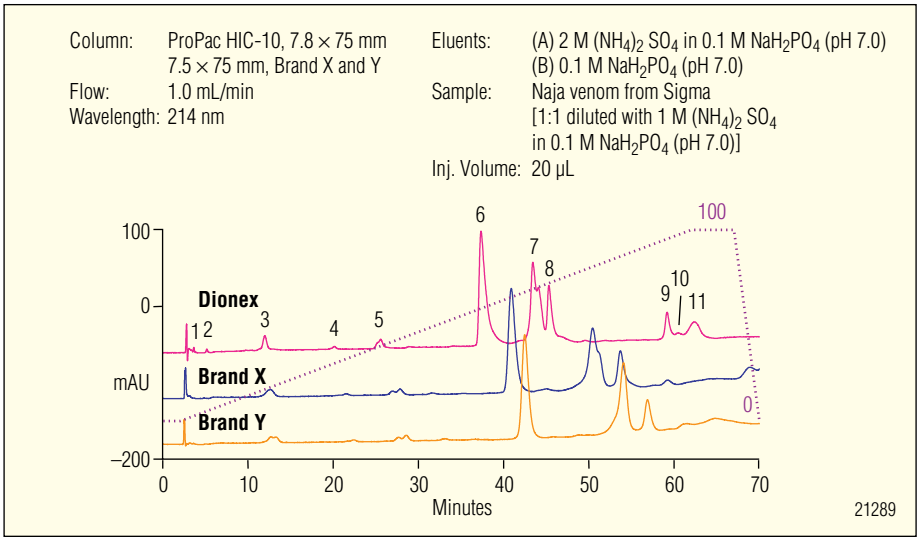

Figure 5. Separation of snake venom proteins/peptides.

The ProPac HIC-10 provides narrow, high-efficiency peaks, as shown in Figure 6. The advantage of narrow peaks is that better resolution is possible between closely eluting peaks, which facilitates integration and fraction collection. In this experiment, a protein standard mixture was separated on the ProPac HIC-10 and the separation compared to that using a commercially available porous (300 Å) silica column of similar dimension. The protein mixture contained cytochrome C, myoglobin, ribonuclease A, lysozyme, and chymotrypsinogen. All five proteins are baseline resolved on the ProPac HIC-10 column, with sharp, symmetrical peaks. In contrast, the separation on the Brand Z porous silica column shows broader, less efficient peaks, with poor resolution between some peaks and poor peak shape on others. In addition, 214-nm absorbing material leaches from the Brand Z column as shown with an arrow.

The separation of pancreatin on the ProPac HIC-10 column (7.8 x 75 mm) is shown in Figure 7. The chromatography is compared with columns from other vendors. Pancreatin contains a variety of enzymes including proteases like trypsin, pepsin, amylases, and lipases. When pancreatin is separated on the HIC column, the chromatography shows an unbound and bound fraction. Each fraction consists of several resolved peaks. An enlarged version of the bound fraction is shown as an inset to illustrate the resolving ability of ProPac HIC column. The identities of individual peaks are not assigned.

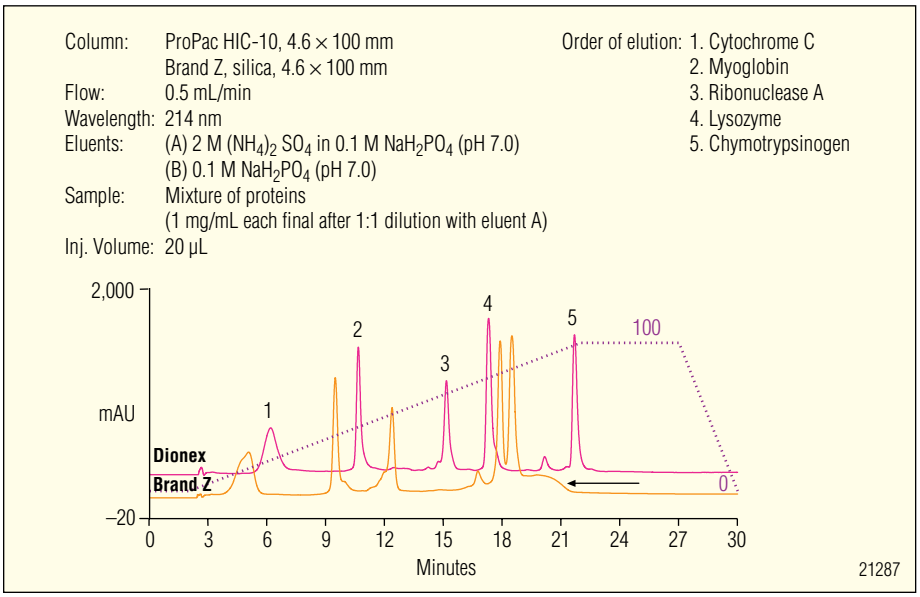

Figure 6. Separation of a mixture of proteins.

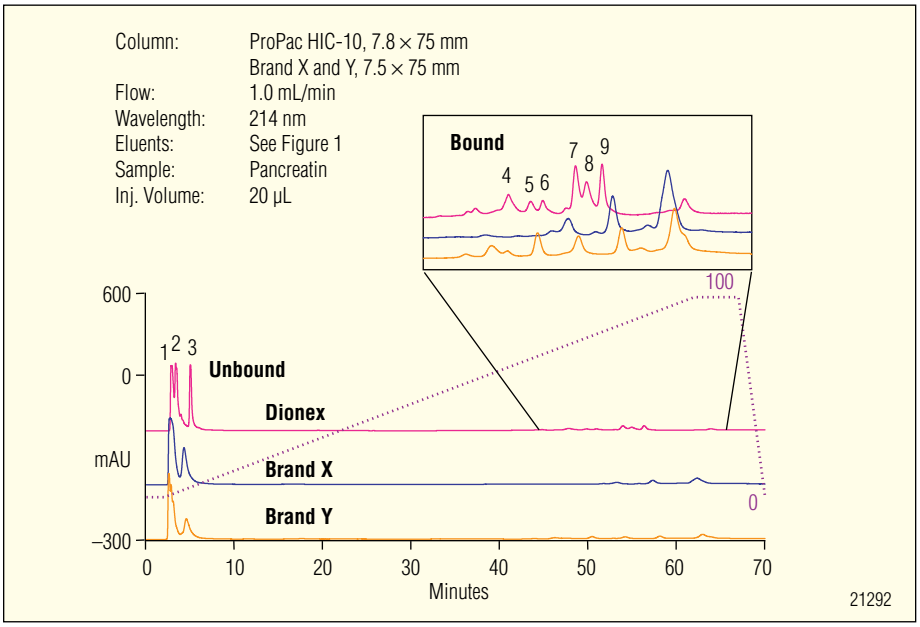

Figure 7. Separation of pancreatin.

Monoclonal antibody (MAb) microheterogeneity (e.g., glycosylation, oxidation, mutation, phosphorylation, amino terminal modifications, incomplete processing of the C-terminus, and asparagine deamidation) plays a major role in influencing the potency and immunogenicity of the specific antibody. Thus, the ability to isolate various isoforms is essential for the development of active therapies. In Figure 8A, the ProPac HIC-10 was used to purify a MAb; separation of the acidic variants was subsequently improved upon by changing the gradient conditions (Figure 8B). Note that the ammonium sulfate concentration (0.5 M) required for this application was much less than the ammonium sulfate concentration (2 M) routinely used for other HIC applications. This helps ease the desalting efforts for further downstream purification schemes. The ProPac HIC-10 can also be used to separate the accelerated forced methionine oxidation variants to assess the stability of antibodies (see the ProPac HIC-10 manual for application details).

Guaranteed performance

The unique chemistry of the ProPac HIC-10 offers exceptional selectivity and hydrolytic stability over the range of pH 2.5–7.5. Its design ensures long column life and easy cleanup. The column manufacturing process is carefully controlled to ensure that every Dionex ProPac HIC-10 column delivers reproducible performance. ProPac HIC-10 columns are batch tested to ensure that the stationary phase delivers the same separation, lot after lot, and individually tested to ensure that each column provides exceptional peak shape and resolution.

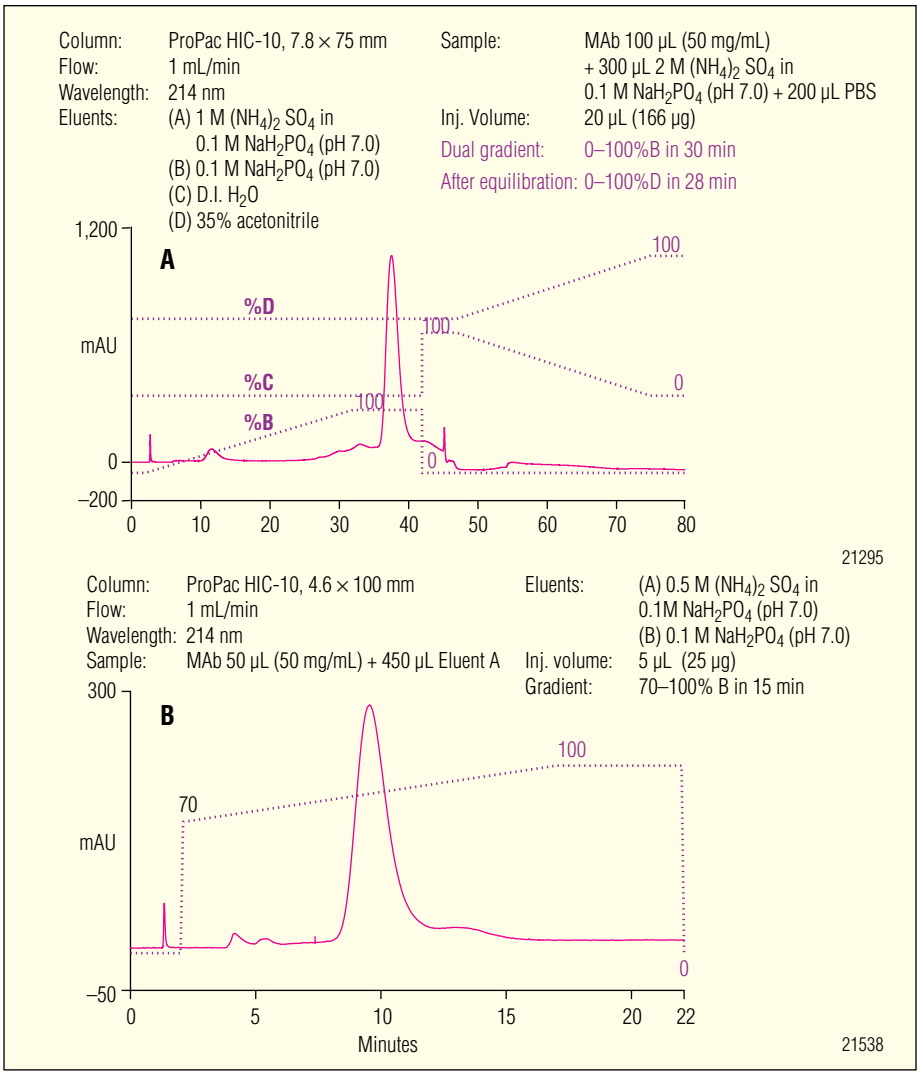

Figure 8. Separation of MAb variants.

ORDERING INFORMATION

In the U.S. call 1-800-346-6390, or contact the Dionex Regional Office nearest you. Outside the U.S., order through your local Dionex office or distributor. Refer to the following part numbers.

Product Description	Part Number
ProPac HIC-10 Column (2.1 × 100 mm)	063653
ProPac HIC-10 Column (4.6 × 100 mm)	063655
ProPac HIC-10 Column (7.8 × 75 mm)	063665

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