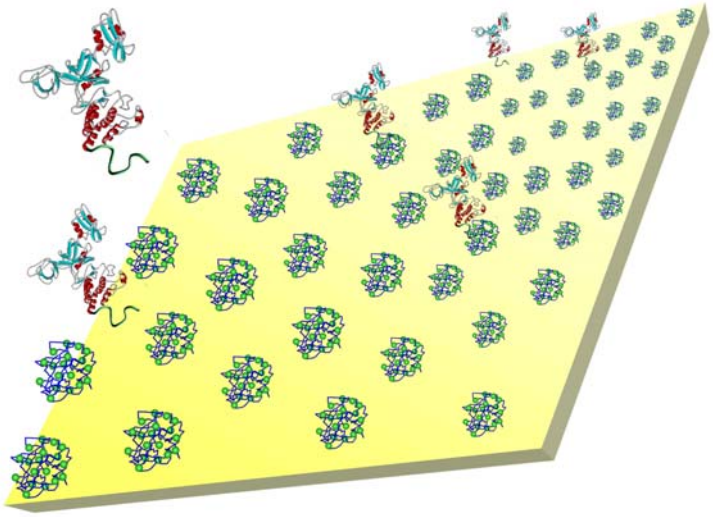
columns

ProPac® IMAC-10 Column
Solutions for Protein and Peptide Analysis

ProPac IMAC-10 Resin Surface Showing Single Nanoparticle-Protein Interactions



HPLC column for high-resolution immobilized metal affinity chromatography (IMAC) separations of metal-binding proteins:

- *State-of-the-art technology for tailored specificity*
- *Protein capture, separation, and elution from one column in a single run*
- *Retention control by imidazole or pH gradient*
- *High purity separations of metal-binding proteins*
- *Wide range of metal-specific applications*

Now sold under the Thermo Scientific brand

Thermo SCIENTIFIC

HPLC Separation of Proteins Using IMAC

Protein structure varies widely, presenting challenges in protein analysis and characterization. As a result of this complexity, a variety of tools have been developed to isolate a single protein, or a small group of proteins from a larger mix, based on a unique characteristic of that single molecule or sub-group. IMAC is one such tool.

IMAC is a powerful purification technique that separates proteins based on their affinity for metals. Proteins with an affinity for the metal immobilized on the resin surface are retained, while other proteins are eluted uninhibited. The Dionex ProPac IMAC-10 column, the latest advancement in IMAC technology, is a true high-pressure, high-resolution column capable of not only separating certain metal-binding proteins from non-metal binding proteins, but also providing resolution of retained proteins from one another, in many cases.

The ProPac IMAC-10 column can be applied to a broad range of applications, including the separation of His-tagged protein aggregation variants, His-tagged protein purification, polishing samples for crystallization experiments, on-column refolding, phosphopeptide analysis, intact protein separations, separation of monoclonal antibodies, and prion peptide analysis.

DIONEX

State-of-the-Art Technology for Tailored Specificity

Typical IMAC stationary phases are based on soft-gel matrices, such as agarose or cross-linked dextran. While commercially available IMAC resins are effective for low-pressure applications, they exhibit low mechanical strength and are not amenable to even HPLC applications. More recently, rigid polymer matrices have been developed that are able to withstand higher backpressures, typical of FPLC. However, the resolution possible with these resins is such that often the captured fractions will require further purification. The Dionex ProPac IMAC-10 column is a true HPLC column capable of performing a large number of injections without losing capacity. When the capacity does start to decrease, it can be recovered simply by recharging the column with metal.

To produce this HPLC IMAC phase Dionex scientists coated 10-µm, nonporous, polymeric beads with a hydrophilic layer, to eliminate secondary hydrophobic interactions with the proteins. Next, using state-of-the-art technology, isolated, poly(IDA) grafts were engineered to the surface of these nonporous, polymeric beads. The poly(IDA) grafts are converted to metal-containing nanoparticles when the column is charged with metal, as illustrated schematically in Figure 1. It is these nanoparticles that act as the IMAC interaction sites for individual proteins.

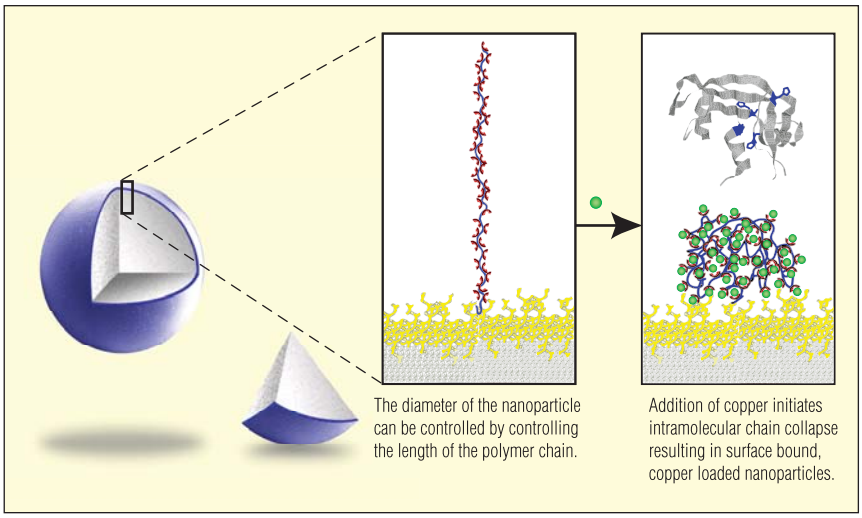


Figure 1. Formation of metal-containing nanoparticles upon charging of the ProPac IMAC-10 column with metal.

The transmission electron microscopy image of the IMAC bead is shown in Figure 2. The image reveals the bead interior, the hydrophilic layer, and the copper nanoparticles. These nanoparticles are covalently bound to the exterior of the hydrophilic layer. Since the average diameter of proteins is similar to the average diameter of the surface bound nano-particles, a single protein interacts with a single nano-particle. This interaction optimizes column performance, on-column refolding applications, and site-specific biotinylation experiments.

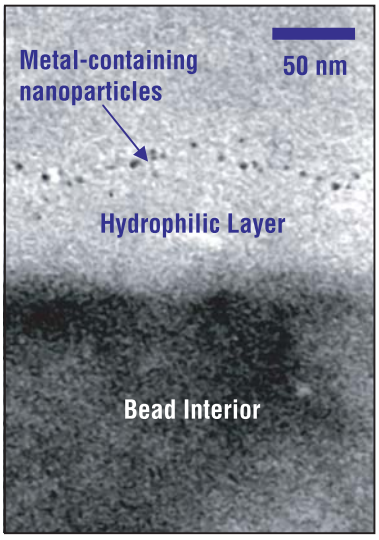


Figure 2. Nano-engineered stationary phase using state-of-the-art technology.

TABLE 1. PROPAC IMAC-10 RESIN CHARACTERISTICS

Characteristic	Value
Substrate	Polystyrene divinylbenzene
Particle size	10 µm
Substrate x-linking	55%
Porosity	Non-porous
Functional Ligand	Iminodiacetate
Chelating metals	Ni, Fe, Cu, others
Metal Ion Capacity	~40 µmol Cu/g resin
Protein Binding Capacity	1–5 mg lysozyme/g resin
pH stability	2–12
Backpressure limit	≤3,000 psi
Temperature limit	≤ 60 °C
Storage solution	20 mM MES, 141.8 mM NaC1, 1 mM EDTA pH = 6.10 + 0.1% NaN_3

The Dionex ProPac IMAC-10 column is stable at pH 2–12 and compatible with most reagents commonly used in protein purification, including denaturants, non-ionic detergents, and reducing agents. It can also be used under native or denaturing conditions using HPLC. The characteristics of the ProPac IMAC-10 resin are listed in Table 1.

Capture, Separate, and Elute in a Single Run

IMAC is typically carried out using a capture/release method for protein and peptide enrichment. Often, the fractions collected contain impurities that require further purification by reversed-phase HPLC, ion-exchange, or size exclusion. Figure 4 shows an example of a His-tagged protein that had previously been purified by this approach. The ProPac IMAC-10 analytical column allows researchers to run gradient separations in the IMAC mode, resulting in highly efficient resolution of proteins and highly pure fractions collected.

Retention Control by Imidazole or pH Gradient

The protein adsorption step in IMAC is performed between pH 6 and pH 9; nonspecific adsorption is reduced by adding high concentrations of salt to the binding buffer. The elution step can be performed in one of three ways: using a pH gradient; introduction of a competitor ligand; or by stripping the metal from the column. The Dionex ProPac IMAC-10 column is stable at pH 2–12 and thus compatible with a pH gradient. However, if a decreasing pH gradient is chosen, protein sensitivity to low pH must be considered.

The recommended method for eluting bound protein from a Dionex ProPac IMAC-10 column is a linear imidazole gradient, as illustrated in Figure 3. Using a linear gradient rather than a step gradient allows for not only the elution of bound proteins, but also the separation of different bound fractions at different imidazole concentrations.

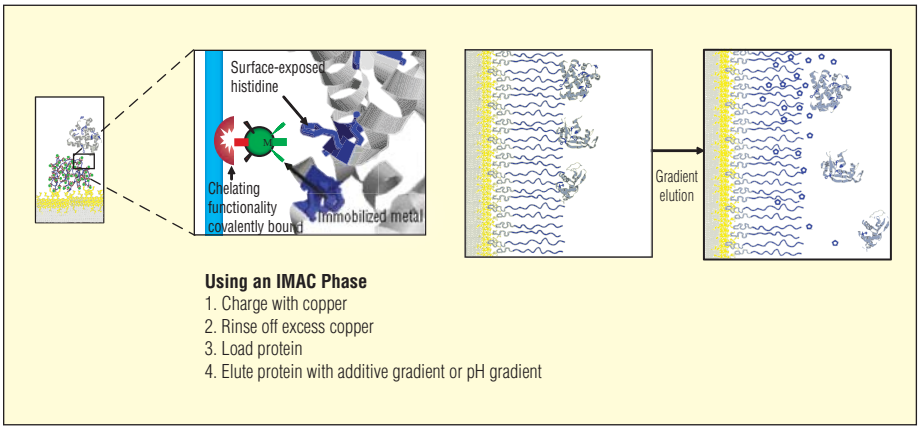


Figure 3. Retention control by imidazole or pH gradient.

High-Resolution Separation of Proteins

Dionex columns are all characterized by their ability to deliver high resolution separations, and the ProPac IMAC-10 is no exception. The use of small, non-porous resin particles results in narrow, high efficiency peaks, with minimal band broadening due to dispersion. The hydrophilic layer surrounding the resin particle eliminates peak broadening due to non-specific hydrophobic interactions.

The capacity of the column is maintained by grafting the polymer chains that carry the functional ligand onto the resin. This design maximizes the number of sterically accessible ligands for binding to the proteins, thereby ensuring that the proteins remain tightly bound during the separation step. Together, these design features result in high-efficiency peaks, enabling the ProPac IMAC-10 to resolve proteins that cannot be resolved on other IMAC resins.

Wide Range of Metal-Specific Applications

His-tagged Protein Aggregation Variants

His-tagged proteins are usually purified with an IMAC cartridge by applying a capture/release protocol. The purity of the released fraction is typically assessed by SDS-Page. SDS-Page often reveals the presence of protein impurities from the host system (i.e., *Escherichia coli*) after IMAC cartridge purification (Figure 4). The amount of impurity can be reduced by optimization of rinse conditions; however, impurities with affinity similar to, or greater than, His-tagged protein can not be excluded by this approach. Using the Dionex ProPac IMAC-10 column and a linear imidazole gradient, resolution between different prion peptides can be achieved as shown in Figure 4. Monitoring the separation with a UV detector allows collection of the fraction containing His-tagged protein, while the impurities are passed on to waste.

Aggregation is a problem for some His-tagged proteins. The chromatogram in Figure 4 shows an imidazole gradient separation of a 55-kD His-tagged protein that is known to polymerize, and was previously purified by IMAC cartridge capture/release. The separation was carried out using non-denaturing conditions that mimic protein polymerization conditions. The multiple peaks shown in the trace were verified as aggregation variant peaks by on-line light-scattering detection.

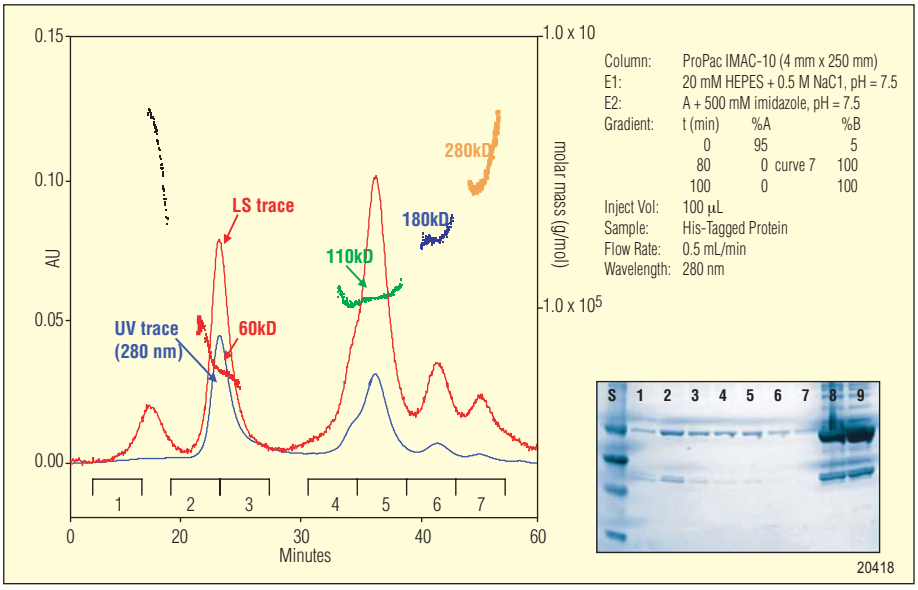


Figure 4. Separation of His-tagged protein aggregation variants.

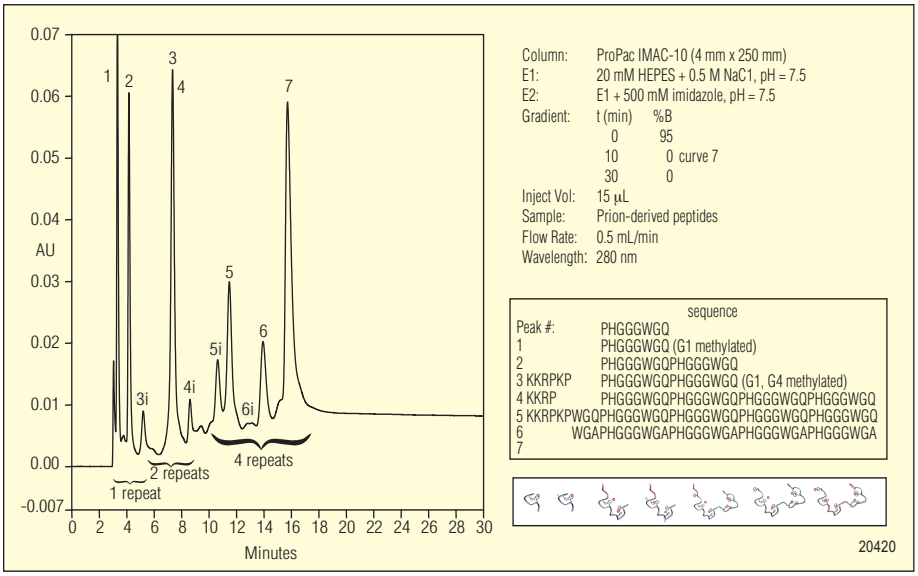


Figure 5. Separation of prion related peptides.

Prion Peptides

The ability to capture and separate peptides with affinity for immobilized copper in one run and on one column is advantageous for proteomics applications. The proteomics approach involves identification of large numbers of proteins in very complex mixtures. Typically, the mixtures are simplified by class separation. IMAC can be used to capture the copper binding peptide class.

Figure 5 shows a separation of six synthetically prepared copper binding peptides related to prion protein. The peptides contain octapeptide repeats where each repeat has been found to bind one copper atom. Using the ProPac IMAC-10 column, prion-related peptides differing in the number of octapeptide repeat units (PHGGGWGQ) (peak 1), (PHGGGWGQ)2 (peak 3), and (PHGGGWGQ)4 (peak 5) were separated. The column is also able to separate several prion-related peptide mixtures where the peptides contained the same number of copper binding sites but differed in the presence of a hydrophilic tail (peak 5 and peak 6) or Q_A mutation (peak 6 and peak 7).

Monoclonal Antibodies

Monoclonal antibodies (MAbs) are used in a wide variety of applications, each of which has its own set of purity requirements. Figure 6 shows the chromatographic trace of an very pure MAb sample. The ability to isolate individual proteins on individual nanoparticles is expected to result in higher purity fractions from site-directed biotinylation experiments

Intact Protein Separations

Analytical IMAC is capable of separating proteins based on the extent of surface exposed histidine residues. The chromatogram in Figure 7 shows resolution of three standard proteins separated on the ProPac IMAC-10 column using an imidazole gradient.

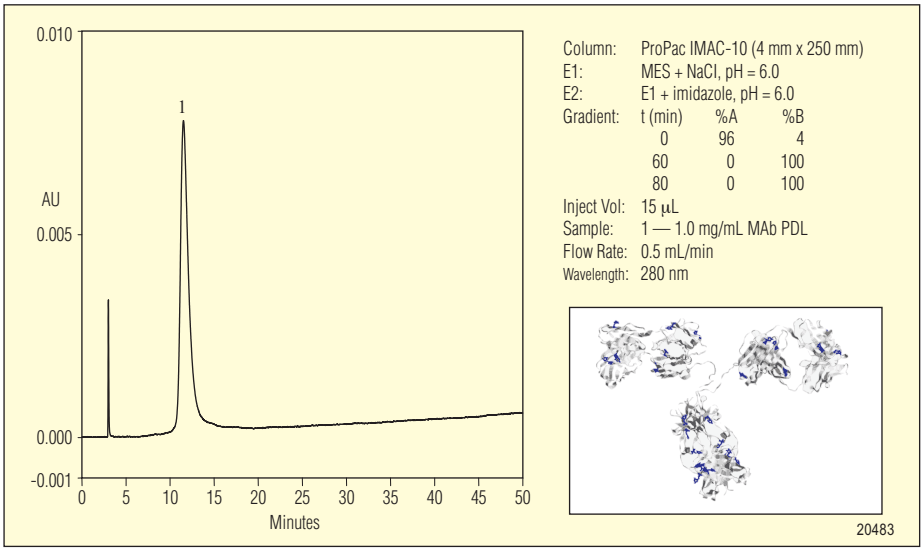


Figure 6. Monoclonal antibody.

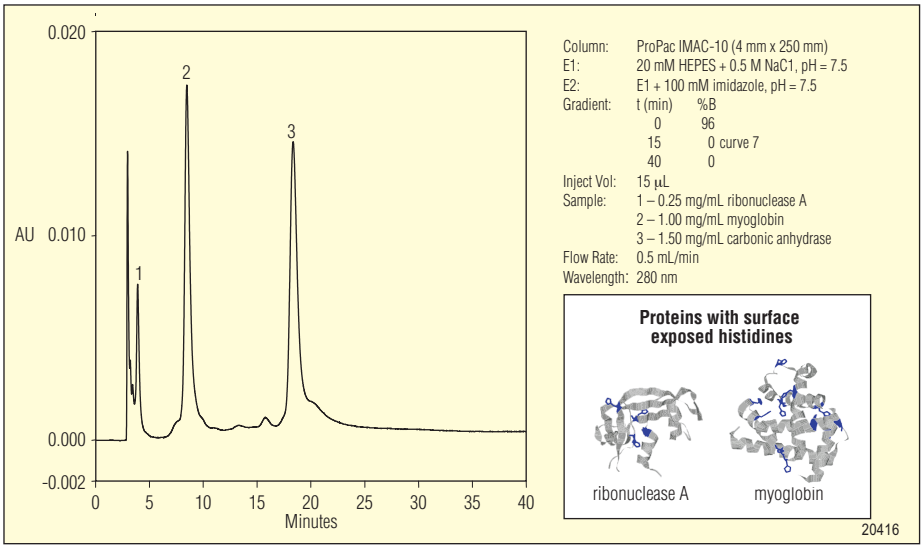


Figure 7. Separation of standard proteins within the same class.

Phosphopeptide Capture and Release

Detection of phosphorylation in proteins is necessary to the understanding of their biological functions, but the abundances are usually low, making this a challenging analytical problem. IMAC in the ferric form has been used on enzymatic digests of phosphorproteins to selectively fractionate phosphorylated from nonphosphorylated peptides. Tryptic digests of beta-casein were evaluated to determine nonspecific binding of nonphosphorylated peptides and recovery of phosphopeptides, as shown in Figure 8. Fractions were analyzed by reversed-phase HPLC. The chromatograms in Figure 8 show the un-fractionated digest, released fraction (phosphopeptide enriched), and flow-through fraction (nonphosphorylated peptides). The captured peptides were verified as phosphopeptides by dephosphorylating and monitoring run time shifts.

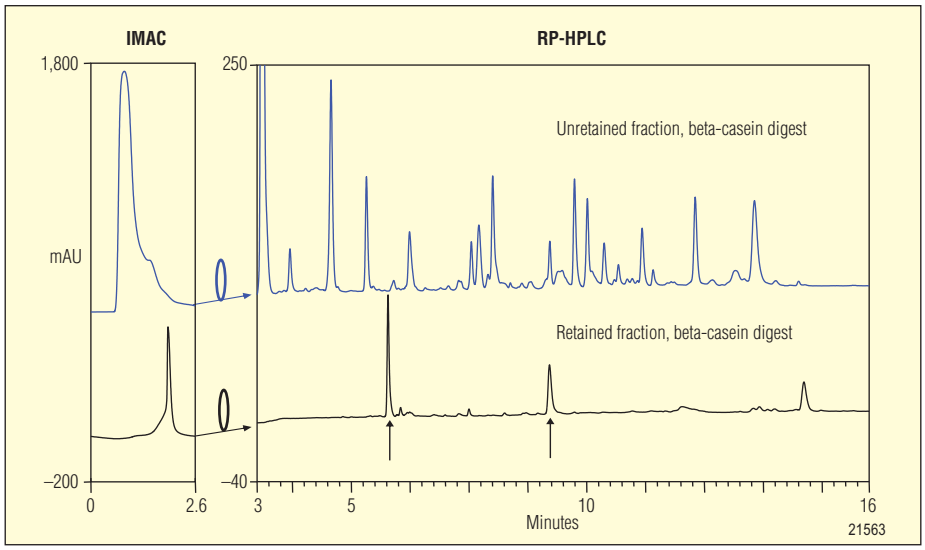


Figure 8. Phosphopeptide analysis.

Guaranteed performance

The unique pellicular resin of the ProPac IMAC-10 columns offers exceptional selectivity and stability over the entire pH range. Its highly crosslinked structure ensures long column life and easy cleanup. The entire manufacturing process (resin synthesis, synthesis of the polymer chains, and packing and testing of the chromatographic columns) is carefully controlled to ensure that every Dionex ProPac IMAC-10 column delivers reproducible performance. ProPac IMAC-10 columns are tested in the copper-loaded mode with a standard protein mix to ensure lot-to-lot reproducibility.

PROPAC IMAC-10 COLUMNS ORDERING INFORMATION

In the U.S., call 1-800-346-6390, order on-line at http://dstore.dionex.com, 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 IMAC-10 Column (1 × 50 mm)	063617
ProPac IMAC-10 Column (2 × 50 mm)	063272
ProPac IMAC-10 Column (4 × 50 mm)	063276
ProPac IMAC-10 Column (9 × 50 mm)	063615
ProPac IMAC-10 Column (4 × 250 mm)	063278
ProPac IMAC-10 Column (9 × 250 mm)	063280
ProPac IMAC-10 Column (22 × 250 mm)	063282

DIONEX

www.dionex.com ON THE WEB

Dionex Corporation
1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

Dionex Corporation
Salt Lake City Technical Center
1515 West 2200 South, Suite A
Salt Lake City, UT
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(801) 972-9292

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* Designed, developed, and manufactured under an NSAI registered ISO 9001 Quality System.

LPN 1731 10M 08/05