# IMAC Sepharose™ High Performance HiTrap™ IMAC HP

Proteins and peptides that have an affinity for metal ions can be purified using immobilized metal ion affinity chromatography (IMAC), a method that has been growing in popularity and effectiveness.

IMAC Sepharose HP is supplied free of metal ions. It is charged by the user with the transition metal ion of choice (e.g. $Cu^{2+}$, $Zn^{2+}$, $Ni^{2+}$, or $Co^{2+}$); these metal ions will bind to the covalently immobilized chelating ligand on the Sepharose. The immobilized metal ions will interact with certain amino acid residues on protein surfaces (mainly histidine, but often also cysteine and tryptophan), if the amino acid side chains are sufficiently exposed. The bound protein can be eluted either with a competitive agent such as imidazole or by lowering the pH.

Numerous characteristics make IMAC Sepharose HP a valuable addition to the GE Healthcare line of affinity chromatography media:

- possible to charge with various metal ions for optimized selectivity
- high protein binding capacity
- compatible with a wide range of additives
- available in the convenient and time-saving prepacked HiTrap format

IMAC Sepharose HP is an excellent choice of medium for the purification of histidine-tagged recombinant proteins. The histidine tag is globally the most used affinity tag, often found as six consecutive histidine residues on recombinant proteins. Histidine and other amino acid residues capable of metal ion interaction are also present on the surface of many non-modified proteins. The strength of binding between a protein and a metal ion is affected by several factors, including the general properties of the target protein, the presence and properties of an affinity tag on the protein, the type of metal ion used, and the pH and ionic strength of buffers. With IMAC Sepharose HP, $Ni^{2+}$ generally provides the strongest binding of histidine-tagged proteins. However, with some histidine-tagged proteins and in many applications with untagged proteins, metal ions other than $Ni^{2+}$ may be more suitable. It could also be worth considering that some metal ions might be problematic for a given target protein due to loss of activity, or due to potential problems with environmental or user exposure.

Since it is not always possible to predict which metal ion will be the most appropriate for purifying a given protein, the availability of uncharged IMAC Sepharose HP provides flexibility and ease in planning, testing, and optimizing a purification scheme.

**Fig 1.** IMAC Sepharose High Performance is an uncharged IMAC medium that, once charged with the metal ion of choice, provides flexible possibilities for optimizing purifications. It is available in 25 mL and 100 mL lab packs as well as prepacked HiTrap IMAC HP 1 mL and 5 mL columns.

# Medium characteristics

IMAC Sepharose HP consists of 34 μm beads of highly cross-linked 6% agarose; its chelating ligand will be charged with metal ions by the user, allowing the medium to selectively retain target proteins.

Sepharose beads display high chemical and physical stability, permitting good flow rates. The small bead size allows high chromatographic resolution with distinctly separated peaks containing concentrated material.

The dynamic binding capacity of IMAC Sepharose HP, evaluated using $Ni^{2+}$-charged medium and two $(histidine)_6$-tagged proteins, was determined to be at least 40 mg of bound protein per mL of medium.

Tables 1 and 2 list the main characteristics of IMAC Sepharose HP and HiTrap IMAC HP columns, respectively.

IMAC Sepharose HP is compatible with all commonly used aqueous buffers, denaturants such as 6 M guanidine hydrochloride and 8 M urea, and a wide range of other additives. It is stable over a broad pH range. See Tables 3 and 4.

**Table 1.** Characteristics of IMAC Sepharose HP

| | |
|---|---|
| Matrix | Highly cross-linked spherical agarose, 6% |
| Mean particle size | 34 μm |
| Metal ion capacity | ~15 μmol $Ni^{2+}$/mL medium |
| Dynamic binding capacity[1] | At least 40 mg $(histidine)_6$-tagged protein/mL medium ($Ni^{2+}$-charged) |
| Recommended flow rate[2] | < 150 cm/h |
| Column hardware pressure limit | 5 bar (0.5 MPa, 70 psi) |
| Compatibility during use | See Tables 3 and 4. |
| Chemical stability (metal ion-stripped medium) | 0.01 M HCl, 0.1 M NaOH. Tested for 1 week at +40°C.<br>1 M NaOH, 70% HAc. Tested for 12 hours.<br>2% SDS. Tested for 1 hour.<br>30% 2-propanol. Tested for 30 min. |
| Avoid in buffers | Chelating agents, e.g. EDTA, EGTA, citrate. |
| pH stability (metal-ion-stripped medium) | |
| Cleaning[3] | 2 to 14 |
| Working[4] | 3 to 12 |
| Storage | 20% ethanol at +4°C to +30°C |

[1] Conditions for determining dynamic binding capacity:

Sample: 1 mg/mL $(histidine)_6$-tagged pure proteins ($M_r$ 28 000 or 43 000) in binding buffer (capacity at 10% breakthrough) and/or $(histidine)_6$-tagged protein bound from *E. coli* extract)

Column volume: 0.25 mL or 1 mL

Flow rate: 0.25 mL/min or 1 mL/min, respectively

Binding buffer: 20 mM sodium phosphate, 0.5 M NaCl, 5 mM imidazole, pH 7.4

Elution buffer: 20 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4

**Note:** Dynamic binding capacity is metal-ion- and protein-dependent.

[2] $H_2O$ at room temperature.

[3] Refers to the pH interval for regeneration

[4] Refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance

**Table 2.** Characteristics of HiTrap IMAC HP columns

| | |
|---|---|
| Matrix | Highly cross-linked spherical agarose, 6% |
| Mean particle size | 34 μm |
| Metal ion capacity | ~15 μmol $Ni^{2+}$/mL medium |
| Dynamic binding capacity[1] | At least 40 mg $(histidine)_6$-tagged protein/mL medium ($Ni^{2+}$-charged) |
| Column volumes | 1 mL or 5 mL |
| Column dimensions | i.d. × h: 0.7 × 2.5 cm (1 mL)<br>1.6 × 2.5 cm (5 mL) |
| Recommended flow rate[2] | 1 and 5 mL/min for 1 and 5 mL column, respectively |
| Max. flow rates | 4 and 20 mL/min for 1 and 5 mL column, respectively |
| Column hardware pressure limit | 5 bar (0.5 MPa, 70 psi) |
| Compatibility during use | See Tables 3 and 4. |
| Chemical stability (metal-ion-stripped medium) | 0.01 M HCl, 0.1 M NaOH. Tested for 1 week at +40°C.<br>1 M NaOH, 70% HAc. Tested for 12 hours.<br>2% SDS. Tested for 1 hour.<br>30% 2-propanol. Tested for 30 min. |
| Avoid in buffers | Chelating agents, e.g. EDTA, EGTA, citrate |
| pH stability (metal-ion-stripped medium) | |
| Cleaning[3] | 2 to 14 |
| Working[4] | 3 to 12 |
| Storage | 20% ethanol at +4°C to +30°C |

[1] Conditions for determining dynamic binding capacity:

Sample: 1 mg/mL $(histidine)_6$-tagged pure proteins ($M_r$ 28 000 or 43 000) in binding buffer (capacity at 10% breakthrough) and/or $(histidine)_6$-tagged protein bound from *E. coli* extract)

Column volume: 0.25 mL or 1 mL

Flow rate: 0.25 mL/min or 1 mL/min, respectively

Binding buffer: 20 mM sodium phosphate, 0.5 M NaCl, 5 mM imidazole, pH 7.4

Elution buffer: 20 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4

**Note:** Dynamic binding capacity is metal-ion- and protein-dependent.

[2] $H_2O$ at room temperature.

[3] Refers to the pH interval for regeneration

[4] Refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance

**Table 3.** Reducing and denaturing agents compatible with IMAC Sepharose High Performance charged with $Ni^{2+}$ (at the given concentrations)

| |
|---|
| 5 mM DTE[1] |
| 5 mM DTT[1] |
| 20 mM ß-mercaptoethanol[1] |
| 5 mM TCEP[1] (Tris([2-carboxyethyl])phosphine) |
| 10 mM reduced glutathione[1] |
| 8 M urea[2] |
| 6 M guanidine hydrochloride[2] |

[1] For best results, it is recommended to perform a blank run before including reducing agents in the sample/buffer. For details see instructions 28-2046-20 and 28-4046-23.

[2] Tested for 1 week at +40°C.

**Table 4.** Detergents, additives and buffer substances compatible with IMAC Sepharose High Performance charged with $Ni^{2+}$ (at the given concentrations)

| |
|---|
| 2% Triton™ X-100 (nonionic detergent) |
| 2% Tween™ 20 (nonionic detergent) |
| 2% NP-40 (nonionic detergent) |
| 2% cholate (anionic detergent) |
| 1% CHAPS (zwitterionic detergent) |
| 500 mM imidazole |
| 20% ethanol |
| 50% glycerol |
| 100 mM $Na_2SO_4$ |
| 1.5 M NaCl |
| 1 mM EDTA[1] |
| 60 mM citrate[1] |
| 50 mM sodium phosphate, pH 7.4 |
| 100 mM Tris-HCl, pH 7.4 |
| 100 mM Tris-acetate, pH 7.4 |
| 100 mM HEPES, pH 7.4 |
| 100 mM MOPS, pH 7.4 |
| 100 mM sodium acetate, pH 4[2] |

[1] The strong chelator EDTA has been used successfully in some cases, at 1 mM. Generally, chelating agents should be used with caution (and only in the sample, not the buffer). Any metal-ion stripping may be counteracted by addition of a small excess of $MgCl_2$ before centrifugation/filtration of the sample. Note that stripping effects may vary with the applied sample volume.

[2] Tested for 1 week at +40°C.

# Operation

## *Packing in laboratory columns*

IMAC Sepharose HP is supplied preswollen in 25 mL and 100 mL packs. The medium is easy to pack and use in laboratory columns from the Tricorn™ and XK series (see Ordering Information). Full user instructions are supplied with each product package.

## *Availability in HiTrap columns*

The medium is also available in the convenient HiTrap prepacked column format as HiTrap IMAC HP 1 mL and 5 mL columns. These columns bring added time-saving, convenience, and reliability to the purification. The columns are simple to operate with a syringe, a pump, or chromatography systems such as ÄKTA systems. ÄKTA systems include preset method templates for HiTrap columns, which further simplifies operation and provides reproducibility. Figure 2 illustrates purification with a syringe, for which connectors are supplied with each package.

HiTrap columns are made of biocompatible polypropylene. The columns have porous top and bottom frits that allow high flow rates. They are delivered with a stopper on the inlet and a snap-off end on the outlet. Table 2 lists the main characteristics of HiTrap IMAC HP 1 mL and 5 mL. Note that HiTrap IMAC HP columns cannot be opened or repacked.

## *Selecting metal ion*

The following guidelines may be used for preliminary experiments to select the metal ion that is most useful for a given separation:

- $Ni^{2+}$ is commonly used for histidine-tagged recombinant proteins.
- $Co^{2+}$ is also used for purification of histidine-tagged proteins, especially when a somewhat weaker binding of the target proteins is preferred.
- For purification of untagged proteins, $Cu^{2+}$ and $Zn^{2+}$ ions have frequently been used. $Cu^{2+}$ gives strong binding to a range of proteins, and some proteins will only bind to $Cu^{2+}$. Both ions can also be used for histidine-tagged proteins.

In some special applications, $Fe^{3+}$ and $Ca^{2+}$ have been used. When the binding characteristics of a target protein are unknown, it is advisable to test more than one metal ion to establish the most suitable. In some instances, a weak binding to a metal ion can be exploited to achieve selective elution (higher purity) of a target protein.

The medium is charged with metal ions by passing a solution of the appropriate metal salt through the column, e.g. 0.1 M $ZnCl_2$, $NiSO_4$, $CoCl_2$ or $CuSO_4$ in distilled water. The medium should then be washed with water and binding buffer before loading the sample on the column. Detailed information about charging, stripping, cleaning, and operation is included in the instructions accompanying each product.

## *Scaling up*

HiTrap IMAC HP columns can be easily scaled up to increase capacity – just connect two or three 1 mL or 5 mL columns in series; note however, that the backpressure will increase.

If further capacity is needed IMAC Sepharose HP packed in Tricorn or XK columns offers a reliable alternative.

A)

B)

C)

**Fig 2.** Using HiTrap IMAC HP 1 mL with a syringe. (A) Prepare buffers and sample. Remove the column's top cap and snap off the end. Charge with metal ion solution, wash with water and equilibrate. (B) Load the sample and begin collecting fractions. (C) Wash and elute, continue collecting fractions.

| | |
|---|---|
| Columns: | HiTrap IMAC HP, 1 mL, charged with: A) $Cu^{2+}$ B) $Zn^{2+}$ C) $Co^{2+}$ D) $Ni^{2+}$ Conditions were otherwise the same for the four purifications |
| Sample: | *E. coli* extract with APB7, a (histidine)$_6$-tagged protein ($M_r$ ~28 000), including 30 mM imidazole |
| Sample volume: | 10 mL |
| Binding buffer: | 20 mM sodium phosphate, 500 mM NaCl, 30 mM imidazole, pH 7.4 |
| Elution buffer: | 20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4 |
| Flow rate: | 1 mL/min |
| Gradient: | 6% to 60% elution buffer (30–300 mM imidazole) in 25 mL 100% elution buffer (500 mM imidazole) in 5 mL |
| Detection: | Absorbance, 280 nm |
| System: | ÄKTAexplorer 10 |

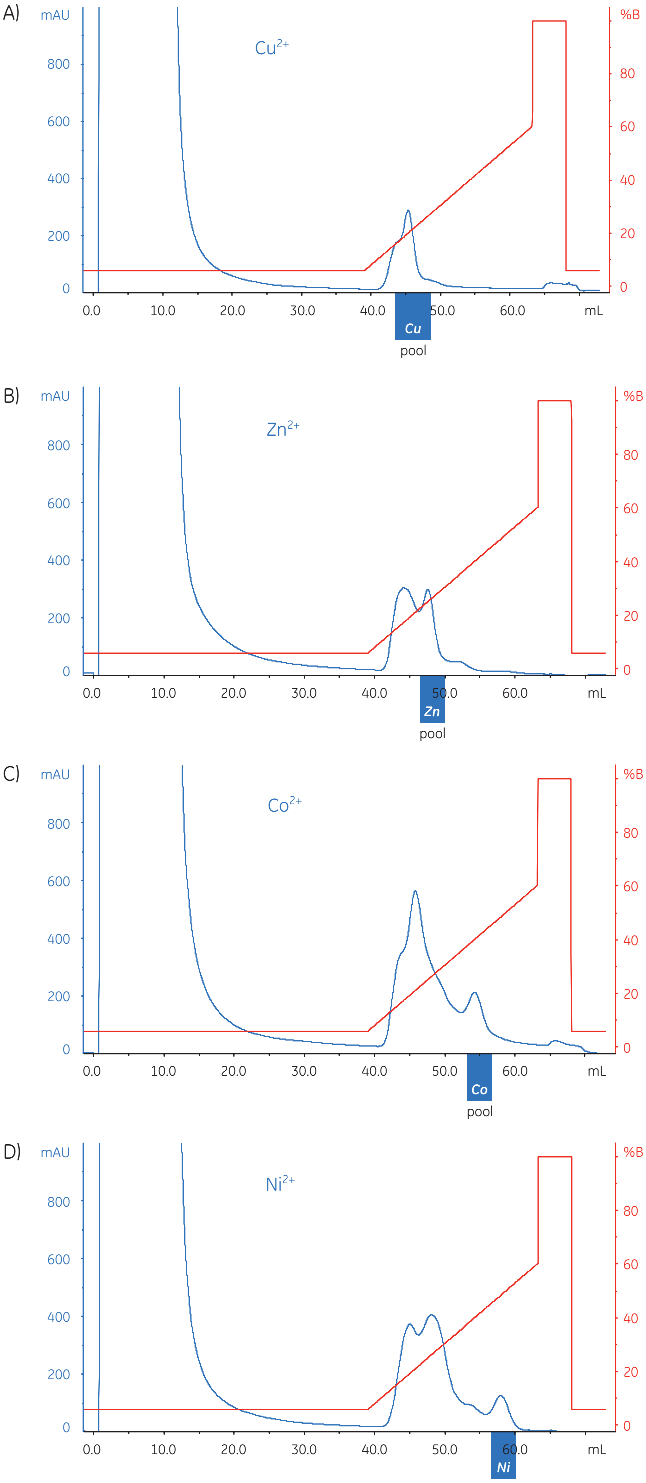



**Fig 3 A-E.** Purification of APB7, a (histidine)$_6$-tagged protein expressed in *E. coli* BL-21, on four different HiTrap IMAC HP 1 mL columns charged separately with metal ions A) $Cu^{2+}$, B) $Zn^{2+}$, C) $Co^{2+}$, or D) $Ni^{2+}$. Pools selected after SDS-PAGE of individual 1 mL fractions (not shown) are indicated.
E) SDS-PAGE analysis: reducing conditions on ExcelGel™ SDS Gradient 8–18; Coomassie™ staining.

# Applications

## *Screening for optimized purity using different metal ions*

A successful IMAC purification depends on several factors, including both the target protein itself and the metal ion used. To achieve the highest purity and yield, screening may have to be performed to select the most suitable metal ion and purification conditions for the specific target protein. Especially for untagged target proteins, choice of metal ion could be crucial.

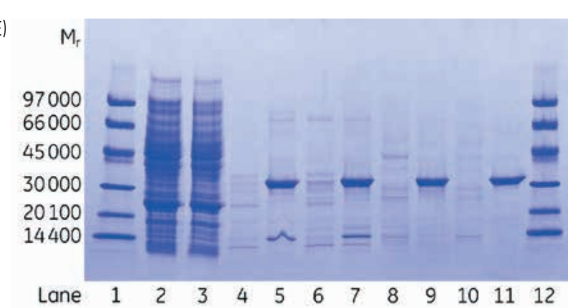
Note that in each of the purification examples below, the collected 1 mL fractions with purified target protein were first analyzed by SDS-PAGE (not shown), to select which fractions to pool for high purity and an acceptable yield.

Also note that, for each series of purifications shown, identical gradient elutions with imidazole were used. Importantly, the results clearly indicate that single-step elution with imidazole would also be possible and would give similar results (purity and yield) as gradient elutions, provided that the imidazole concentration during binding and wash was the appropriate in each case. Should step elution be preferred, exploratory gradient elutions, as the ones shown, can first be used to select that appropriate imidazole concentration.

## *Target protein expressed in* E. coli

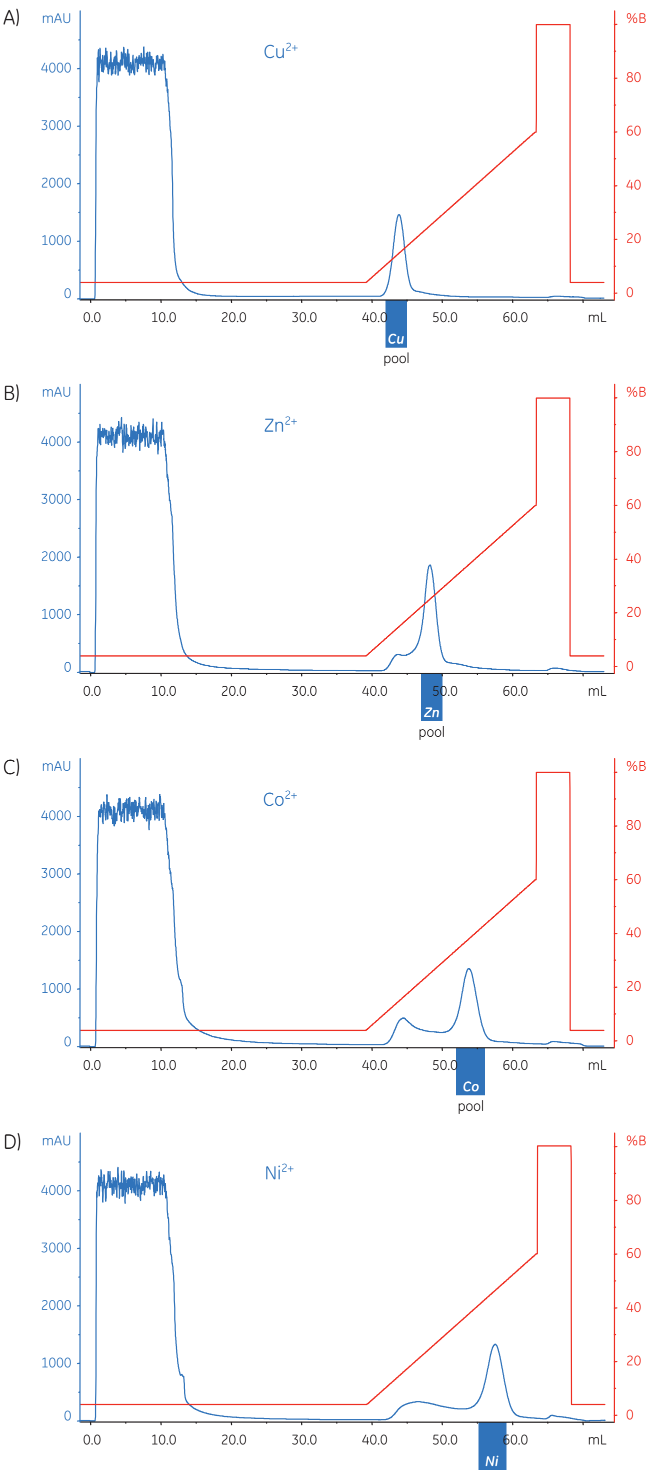
APB7, a (histidine)$_6$-tagged protein ($M_r$ 28 000) expressed in *E. coli*, was purified on four different HiTrap IMAC HP 1 mL columns charged separately with $Cu^{2+}$, $Zn^{2+}$, $Co^{2+}$, or $Ni^{2+}$; conditions were otherwise the same for the four purifications. Chromatograms are shown in Figures 3A–D and the SDS-PAGE analysis of the wash fractions and pooled fractions from each of these four purifications is shown in Figure 3E. Final purity differed sligthly; yields were apparently very

E)




1. LMW markers
2. Start material, diluted 1:10
3. Flowthrough, diluted 1:10, Cu
4. Wash, Cu
5. Eluted pool, Cu
6. Wash, Zn
7. Eluted pool, Zn
8. Wash, Co
9. Eluted pool, Co
10. Wash, Ni
11. Eluted pool, Ni
12. LMW markers

| | |
|---|---|
| Column: | HiTrap IMAC HP 1 mL, charged with: A) $Cu^{2+}$ B) $Zn^{2+}$ C) $Co^{2+}$ D) $Ni^{2+}$ Conditions were otherwise the same for the four purifications |
| Sample: | Histidine-tagged YNR064c, ($M_r$ 33 700) in *Pichia pastoris* extract including 20 mM imidazole |
| Sample volume: | 10 mL |
| Binding buffer: | 20 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.4 |
| Elution buffer: | 20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4 |
| Flow rate: | 1 mL/min |
| Gradient: | 4% to 60% elution buffer (20-300 mM imidazole) in 25 mL 100% elution buffer (500 mM imidazole) in 5 mL |
| Detection: | Absorbance, 280 nm |
| System: | ÄKTAexplorer 10 |

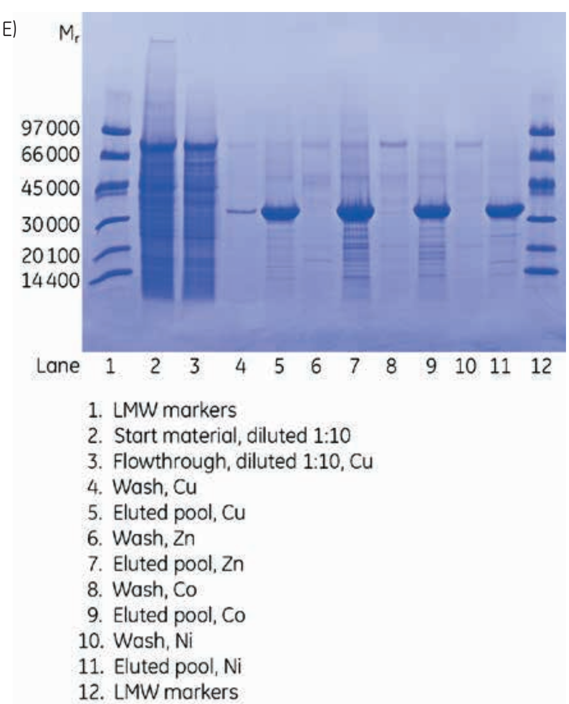



similar. For this specific target protein, the highest purity was achieved when the HiTrap IMAC HP column was charged with $Ni^{2+}$ or $Co^{2+}$, but the differences compared to the results with $Zn^{2+}$ or $Cu^{2+}$ were small.

## *Target protein expressed in Pichia pastoris*

Another example is presented for the target protein YNR064c ($M_r$ 33 700), expressed in *Pichia pastoris*. This (histidine)$_6$-tagged protein was purified in the same way as the protein above, i.e. using HiTrap IMAC HP 1 mL columns charged separately with $Cu^{2+}$, $Zn^{2+}$, $Co^{2+}$, or $Ni^{2+}$; conditions were otherwise the same for the four purifications. See Figures 4A–F for the resulting chromatograms, SDS-PAGE analysis of pooled fractions and an image of the metal ion charged HiTrap IMAC HP columns.

The results show that for this (histidine)$_6$-tagged target protein, the highest purity was achieved with $Ni^{2+}$ or $Cu^{2+}$, although $Cu^{2+}$, at the conditions used, apparently gave a small loss of target protein (see Figure 4E, lane 4).




F)

**Fig 4 A-E.** Purification of (histidine)$_6$-tagged YNR064c expressed in *Pichia pastoris* on four different HiTrap IMAC HP 1 mL columns charged separately with metal ions A) $Cu^{2+}$, B) $Zn^{2+}$, C) $Co^{2+}$, or D) $Ni^{2+}$. Pools selected after SDS-PAGE of individual 1 mL fractions (not shown) are indicated. E) SDS-PAGE analysis: reducing conditions on ExcelGel SDS Gradient 8–18; Coomassie staining. F) HiTrap IMAC HP 1 mL columns charged with $Cu^{2+}$, $Zn^{2+}$, $Co^{2+}$ and $Ni^{2+}$, respectively.

## Comparison of different IMAC media

In another study, the performance of a HiTrap IMAC HP 1 mL column charged with $Co^{2+}$ was compared with media precharged with $Co^{2+}$, His-Select™ Cobalt from Sigma-Aldrich Co., and TALON® Superflow from Clonetech Laboratories Inc.

An *E. coli* extract containing (histidine)$_6$-tagged APB7 protein was used in all three purifications to compare final purity and yield. In addition, all media were packed in HiTrap 1 mL columns for a reliable comparison. Also other conditions were the same for the three purifications. The superimposed chromatograms are shown in Figure 5A, and Figure 5B shows SDS-PAGE analysis of the wash fractions and pooled fractions from each of these three purifications.

For this specific (histidine)$_6$-tagged target protein, the results show that the yields were very similar and that the eluted pool from the $Co^{2+}$-charged HiTrap IMAC 1 mL column contained the purest target protein.

## Performance benefits

IMAC Sepharose High Performance and the prepacked HiTrap IMAC HP 1 mL and 5 mL columns provide numerous performance benefits. Whether the goal is to purify histidine-tagged recombinant proteins, untagged recombinant or native proteins, the benefits of low concentration of metal ions in purified pools, high stability and protein binding capacity will translate into higher protein purity, yield, and activity, plus greater operational flexibility.

The wide interest in purifying histidine-tagged recombinant proteins makes medium stability and compatibility a key issue. Table 3 summarizes the stability of $Ni^{2+}$-charged IMAC Sepharose HP in common reducing and denaturing agents. For best results, we recommend performing a blank run without reducing agents before applying samples and buffers containing reducing agents (see product instructions).

Table 4 summarizes the compatibility of $Ni^{2+}$-charged IMAC Sepharose HP with a range of detergents, additives, and buffers. Little, if any change is seen in protein purity or recovery when such additives or buffer substances are used.

Low metal ion leakage means that the activity of the purified protein will be retained and the risk of precipitation will be reduced, which results in increased purity, activity, and yield of the target protein. Leakage of metal ions in the eluted target protein pool from IMAC Sepharose High Performance is generally low under normal conditions. For applications where very low leakage during purification is critical, it can be diminished even further by performing a blank run using the selected elution buffer after charging the medium with metal ions. Such treatment will remove any weakly bound metal ions that might otherwise be desorbed later during protein elution.

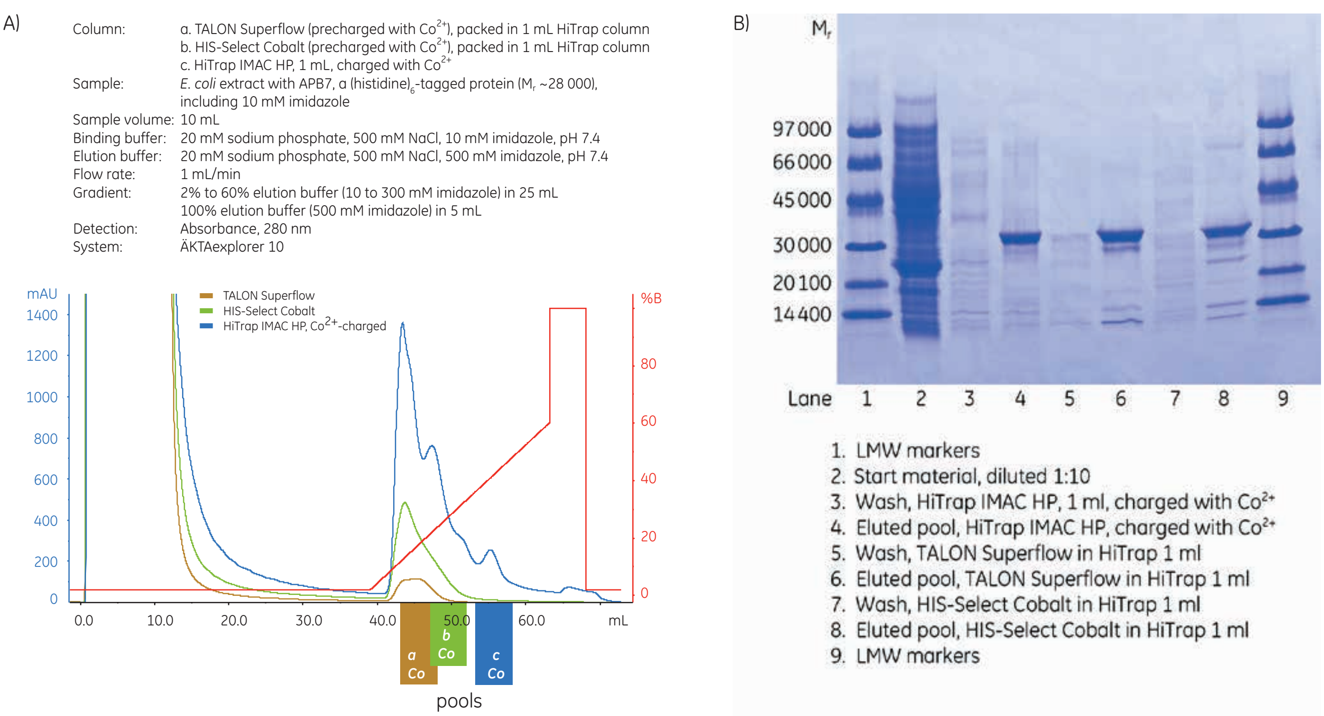



**Fig 5 A-B.** Comparison of different IMAC media charged with $Co^{2+}$. HiTrap IMAC HP 1 mL charged with $Co^{2+}$, TALON Superflow, and HIS-Select Cobalt (both precharged). Pools selected after SDS-PAGE of individual 1 mL fractions (not shown) are indicated in the superimposed chromatograms. SDS-PAGE analysis: reducing conditions on ExcelGel SDS Gradient 8–18; Coomassie staining.

## Summary

Whether the target protein is a histidine-tagged recombinant or an untagged protein with an affinity for metal ions, the ability to charge IMAC Sepharose HP with the metal ion of choice provides flexibility in planning, testing, and optimizing a purification scheme.

The high binding capacity of IMAC Sepharose HP means greater efficiency. More sample can be applied, and the target protein is obtained more concentrated at a lower cost/mg and in less time.

The medium's high chemical stability extends its use to other challenging purification environments. The overall high chemical stability of IMAC Sepharose HP also applies to the medium in its HiTrap column format. This prepacked column format expands the range of conditions in which the medium can be used, as well as helping maintain biological activity and increasing product yield.

IMAC Sepharose HP is easy to pack and use in laboratory columns such as Tricorn and XK columns. However, the greatest savings in time and ease-of-use come when the medium is prepacked as HiTrap IMAC HP columns. Prepacked columns also mean consistently high quality and, above all, greatly increased reproducibility.

### *Acknowledgement*

The recombinant clones used here were obtained through cooperation with SGX Pharmaceuticals, Inc., San Diego, CA, 92121 USA (protein APB7) and Dr. Mikael Widersten, Protein Engineering & Redesign, Dept. of Biochemistry, Uppsala University, Sweden (protein YNR064c).

## Ordering information

| Products | Quantity | Code number |
|---|---|---|
| IMAC Sepharose High Performance | 25 mL* | 17-0920-06 |
| IMAC Sepharose High Performance | 100 mL* | 17-0920-07 |
| HiTrap IMAC HP | 5 × 1 mL | 17-0920-03 |
| HiTrap IMAC HP | 5 × 5 mL | 17-0920-05 |

* Larger quantities are available. Please contact your local representative for more information.

| Related products | Quantity | Code number |
|---|---|---|
| IMAC Sepharose 6 Fast Flow | 25 mL* | 17-0921-07 |
| IMAC Sepharose 6 Fast Flow | 100 mL* | 17-0921-08 |
| HiTrap IMAC FF | 5 × 1 mL | 17-0921-02 |
| HiTrap IMAC FF | 5 × 5 mL | 17-0921-04 |
| HiPrep™ IMAC FF 16/10 | 1 × 20 mL | 17-0921-06 |
| Ni Sepharose High Performance | 25 mL* | 17-5268-01 |
| Ni Sepharose High Performance | 100 mL* | 17-5268-02 |
| HisTrap™ HP | 1 × 1 mL | 29-0510-21 |
| HisTrap HP | 5 × 1 mL | 17-5247-01 |
| HisTrap HP | 100 × 1 mL† | 17-5247-05 |
| HisTrap HP | 1 × 5 mL | 17-5248-01 |
| HisTrap HP | 5 × 5 mL | 17-5248-02 |
| HisTrap HP | 100 × 1 mL† | 17-5248-05 |
| HiTrap Desalting | 1 × 5 mL | 29-0486-84 |
| HiTrap Desalting | 5 × 5 mL | 17-1408-01 |
| HiTrap Desalting | 100 × 5 mL† | 11-0003-29 |
| PD-10 Desalting Column | 30 | 17-0851-01 |
| HiPrep 26/10 Desalting | 1 × 53 mL | 17-5087-01 |
| HiPrep 26/10 Desalting | 4 × 53 mL | 17-5087-02 |

* Larger quantities are available. Please contact your local representative for more information.

† Pack size available by special order. Please contact your local representative.

| Empty lab-scale columns | Quantity | Code number |
|---|---|---|
| Tricorn 5/20 column | 1 | 18-1163-08 |
| Tricorn 5/50 column | 1 | 18-1163-09 |
| Tricorn 10/20 column | 1 | 18-1163-13 |
| Tricorn 10/50 column | 1 | 18-1163-14 |
| Tricorn 10/100 column | 1 | 18-1163-15 |
| XK 16/20 column | 1 | 18-8773-01 |
| XK 16/40 column | 1 | 18-8774-01 |
| XK 26/20 column | 1 | 18-1000-72 |
| XK 26/40 column | 1 | 18-8768-01 |

| Accessories | Quantity | Code number |
|---|---|---|
| 1/16" male/luer female* | 2 | 18-1112-51 |
| Tubing connector flangeless/M6 female | 2 | 18-1003-68 |
| Tubing connector flangeless/M6 male | 2 | 18-1017-98 |
| Union 1/16" female/M6 male | 6 | 18-1112-57 |
| Union M6 female /1/16" male | 5 | 18-3858-01 |
| Union luerlock female/M6 female | 2 | 18-1027-12 |
| HiTrap/HiPrep, 1/16" male connector for ÄKTA | 8 | 28-4010-81 |
| Stop plug female, 1/16"† | 5 | 11-0004-64 |
| Fingertight stop plug, 1/16"‡ | 5 | 11-0003-55 |

* One connector included in each HiTrap package.
† Two, five, or seven stop plugs female included in HiTrap packages depending on products.
‡ One fingertight stop plug is connected to the top of each HiTrap column at delivery.

| Related literature | Quantity | Code number |
|---|---|---|
| The Recombinant Protein Handbook | 1 | 18-1142-75 |
| Affinity Chromatography Handbook | 1 | 18-1022-29 |
| Affinity Chromatography Columns and Media Product Profile | 1 | 18-1121-86 |
| HiTrap Column Guide | 1 | 18-1129-81 |

For local office contact information, visit
**www.gelifesciences.com/contact**

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