IMAC Sepharose 6 Fast Flow
HiPrep IMAC FF 16/10
HiTrap IMAC FF

Immobilized metal ion affinity chromatography (IMAC) is a widely used separation method for purifying proteins and peptides that have an affinity for metal ions, such as histidine-tagged proteins but also some untagged recombinant or native proteins.

IMAC Sepharose™ 6 Fast Flow is supplied free of metal ions, allowing the user to charge it with the most appropriate metal ion for purification of a target protein (Fig 1). Based on the well-established Sepharose 6 Fast Flow matrix, this medium is a member of the BioProcess™ media family of chromatography products from GE Healthcare.

Key features include:

- Convenient purification of histidine-tagged proteins when Ni^{2+} is not the best choice of metal ion
- Charge with your metal ion of choice to optimize selectivity
- High binding capacity
- BioProcess medium designed to meet manufacturing needs for security-of-supply, robust performance, and regulatory support
- Available in prepacked HiTrap™ columns for convenient purification and prepacked HiPrep™ columns for easy scale-up

Fig 1. IMAC Sepharose 6 Fast Flow is an uncharged IMAC medium for optimizing purifications at both laboratory and process scale.

Immobilized metal ion affinity chromatography

IMAC is based on the specific interaction between transition metal ions (e.g. Cu^{2+}, Zn^{2+}, Ni^{2+} or Co^{2+}) and certain amino acid side chains exposed on the surface of proteins (mainly histidine and to a lesser extent cysteine and tryptophan). Globally, the histidine tag is the most commonly used affinity tag for recombinant proteins. Histidine and other amino acid residues capable of metal ion interaction are also present on the surface of many nonmodified proteins. When the affinity medium is charged with a metal ion, the ions bind to chelating ligands that are covalently linked to an insoluble matrix. Being able to select the metal ion permits control of selectivity, making IMAC a very versatile technique. In addition to purifying histidine-tagged proteins, IMAC can be used to purify a wide range of other biomolecules including interferons, serum and plasma proteins, peptides, peptide hormones, lectins, and nucleic acids.

The strength of the protein or peptide interaction with immobilized metal ions is dependent on the type, number, and spatial distribution of the relevant amino acid side chains, and on the nature of the metal ion. The operating conditions for chromatography (pH, type of salt and concentration, additives, etc.) also contribute to the observed interaction.

The bound protein can be eluted either with a competitive agent such as imidazole or by lowering the pH.

In many cases, it is not possible to predict the most appropriate metal ion for purifying a given protein, and there may be other factors to consider as well in making the choice. Thus, uncharged IMAC Sepharose 6 Fast Flow provides flexibility in planning, testing, and optimizing a purification scheme.

BioProcess chromatography media

BioProcess media are developed and supported for production-scale chromatography. All BioProcess media are produced with validated methods and tested to meet stringent manufacturing requirements. Secure ordering and delivery routines give a reliable supply of media for production scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess media cover all purification steps from capture to polishing.

Chromatography medium characteristics

IMAC Sepharose 6 Fast Flow consists of 90 µm beads of highly cross-linked agarose to which a chelating group has been covalently coupled. This chelating group is charged with suitable metal ions by the user, allowing the medium to selectively retain target proteins.

IMAC Sepharose 6 Fast Flow has a high protein binding capacity. The binding capacity is metal ion- and protein-dependent. The medium is easy to pack and use, and its high flow properties make it excellent for scaling up.

Low metal ion leakage means that the activity of the purified protein will be retained and the risk of precipitation will be reduced, resulting in increased purity, activity, and yield of the target protein. Leakage of metal ions into the eluted target protein pool from IMAC Sepharose 6 Fast Flow is generally low under normal conditions. For critical applications, leakage can be diminished even further by performing a blank run using the selected elution buffer after charging the medium with metal ions. This treatment will remove any weakly bound metal ions that might otherwise be desorbed later during protein elution.

IMAC Sepharose 6 Fast Flow is highly stable and compatible with a wide range of common additives. This gives a broad range of suitable operating conditions, allowing for an increase in product yield while maintaining biological activity.

The key characteristics of the medium are listed in Table 1. A variety of compounds that are compatible with Ni^{2+}-charged IMAC Sepharose 6 Fast Flow are listed in Table 2.

Operation

Recommendations and comprehensive instructions for column packing, operation, optimization, and cleaning are included in the information supplied with IMAC Sepharose 6 Fast Flow. The following details highlight several key aspects.

Table 1. Main characteristics of IMAC Sepharose 6 Fast Flow

Matrix	Highly cross-linked 6% spherical agarose
Dynamic binding capacity*	$(Histidine)_6$-tagged: Approx. 40 mg /ml medium (Ni^{2+}-charged)
	Untagged: Approx. 25 mg/ml medium (Cu^{2+}-charged), approx. 15 mg/ml medium (Zn^{2+} or Ni^{2+}-charged)
Metal ion capacity	Approx. 15 µmol Ni^{2+}/ml medium
Average particle size	90 µm
Max. linear flow rate†	600 cm/h (20 ml/min) using XK 16/20 columns with 5 cm bed height
Recommended flow rate†	150 cm/h
Max. operating pressure†	0.1 MPa, (1 bar) (when packed in XK columns; may vary if used in other columns)
Chemical stability‡	0.01 M HCl, 0.1 M NaOH. Tested for 1 week at 40°C. 1 M NaOH, 70% acetic acid. Tested for 12 h. 2% SDS. Tested for 1 h. 30% 2-propanol. Tested for 30 min.
pH stability‡	Short term (at least 2 h): 2–14 Long term (≤ 1 week): 3–12
Storage	4°C to 30°C in 20% ethanol

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

* Conditions for determining dynamic binding capacity.

Samples:	$(Histidine)_6$-tagged proteins: Capacity data were obtained for a protein (M_r 28 000) bound from an *E. coli* extract, and a pure protein (M_r 43 000; applied at 1 mg/ml in binding buffer; capacity at 10% breakthrough).
Untagged protein:	Capacities determined at 10% breakthrough for human apotransferrin applied at 1 mg/ml in binding buffer.
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 (1 mM for untagged protein), pH 7.4
Elution buffer:	20 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole (50 mM for untagged protein), pH 7.4

† H_2O at room temperature.

‡ Metal ion stripped medium.

Column selection

Column packing

IMAC Sepharose 6 Fast Flow is supplied preswollen in 25 ml, 100 ml, 1 l, and 5 l packs. The medium is easy to pack in a wide range of columns (see Table 3). Alternatively, for convenience, IMAC Sepharose 6 Fast Flow is prepacked in HiTrap IMAC FF and HiPrep IMAC FF 16/10 columns.

Prepacked HiTrap columns

IMAC Sepharose 6 Fast Flow is available in the HiTrap prepacked column format as HiTrap IMAC FF 1 ml and 5 ml columns. The columns are simple to operate with a syringe and the supplied Luer connector. Note that HiTrap IMAC FF columns are not designed to be opened or repacked. The columns can be used with a pump or chromatography system such as ÄKTA™ design. ÄKTA design systems include preset method templates for HiTrap columns, which further enhances the simplicity of operation and reproducibility. In addition, multiple HiTrap IMAC FF columns can easily be connected in series for increased purification capacity, although it should be noted that back pressure will increase.

Table 2. IMAC Sepharose 6 Fast Flow charged with Ni^{2+} is compatible with the following compounds (at least at the given concentrations)

Reducing agents	
5 mM DTE	
5 mM DTT	
20 mM β-mercaptoethanol	
5 mM TCEP (Tris [2-carboxyethyl] phosphine)	
10 mM reduced glutathione	
Denaturing agents	
8 M urea*	
6 M Gua-HCl*	
Detergents	
2% Triton™ X-100 (nonionic)	
2% Tween™ 20 (nonionic)	
2% NP-40 (nonionic)	
2% cholate (anionic)	
1% CHAPS (zwitterionic)	
Other additives	
500 mM imidazole	
20% ethanol	
50% glycerol	
100 mM Na_2SO_4	
1.5 M NaCl	
1 mM EDTA†	
60 mM citrate†	
Buffer substances	
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*	

* Tested for 1 week at 40°C.

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

Table 3. Recommended columns for IMAC Sepharose 6 Fast Flow at different scales of operation

Column	Inner diam (mm)	Bed volume (ml or l)	Bed height max (cm)
Lab scale:			
Tricorn™ 5/20	5	up to 0.55 ml	2.8
Tricorn 5/50	5	up to 1.1 ml	5.8
Tricorn 10/20	10	up to 2.2 ml	2.8
Tricorn 10/50	10	up to 4.5 ml	5.8
Tricorn 10/100	10	up to 8.5 ml	10.8
XK 16/20	16	up to 30 ml	15
XK 16/40	16	up to 70 ml	35
XK 26/20	26	up to 80 ml	15
XK 26/40	26	up to 190 ml	35
XK 50/20	50	up to 275 ml	15
XK 50/30	50	up to 510 ml	25
Production scale:			
BPG™ 100/500	100	up to 2.0 l	26
BPG 140/500	140	up to 4.0 l	26
BPG 200/500	200	up to 8.2 l	26
BPG 300/500	300	up to 18.0 l	26
BPG 450/500	450	17.2 to 34.4 l	23
Chromaflow™ 400/100–300	400	13–38 l	30
Chromaflow 600/100–300	600	28–85 l	30
AxiChrom™ 50/300	50	0.20-0.59 l	30
AxiChrom 70/300	70	0.38-1.15 l	30
AxiChrom 100/300	100	0.79-2.36 l	30
AxiChrom 140/300	140	1.54-4.62 l	30
AxiChrom 200/300	200	3.14-9.4 l	30
AxiChrom 400/300	400	13-38 l	30
AxiChrom 600/300	600	28-85 l	30
AxiChrom 800/300	800	50-151 l	30
AxiChrom 1000/300	1000	79-236 l	30

HiTrap columns are made of biocompatible polypropylene. The porous top and bottom frits are fully compatible with the high flow rate capabilities of IMAC Sepharose 6 Fast Flow. Columns are delivered with a stopper on the inlet and a snap-off end on the outlet. Table 4 lists the main characteristics of HiTrap IMAC FF columns.

Prepacked HiPrep columns

A bed volume of 20 ml combined with the built-in convenience of the HiPrep prepacked column format make HiPrep IMAC FF 16/10 a sound choice for scaling up to levels beyond those attainable with the smaller HiTrap columns. HiPrep IMAC FF 16/10 is simple to operate and compatible with single-pump chromatography configurations as well as ÄKTA design systems (which include preset method templates).

HiPrep 16/10 columns are made of transparent, biocompatible polypropylene. Table 5 lists the main characteristics of HiPrep IMAC FF 16/10 columns. Note that the columns cannot be opened or repacked.

Table 4. Main characteristics of HiTrap IMAC FF columns

Column data	
Medium	IMAC Sepharose 6 Fast Flow (Table 1)
Column volumes	1 ml or 5 ml
Column dimensions (i.d. × H)	0.7 × 2.5 cm (1 ml column) 1.6 × 2.5 cm (5 ml column)
Recommended flow rate*	1 ml/min (1 ml column) 5 ml/min (5 ml column)
Maximum flow rates*	4 ml/min (1 ml) 20 ml/min (5 ml)
Maximum pressure over the packed bed during operation	1.5 bar (0.15 MPa, 22 psi)
Column hardware pressure limit	3 bar (0.3 MPa, 45 psi)
Storage	4 to 30°C in 20% ethanol

* H_2O at room temperature.

Table 5. Main characteristics of HiPrep IMAC FF 16/10 columns

Column data	
Medium	IMAC Sepharose 6 Fast Flow (Table 1)
Bed volume	20 ml
Bed height	100 mm
Inner diameter	16 mm
Column hardware	Polypropylene
Recommended flow rate*	1 to 10 ml/min (30 to 300 cm/h)
Maximum flow rate*	10 ml/min (300 cm/h)
Maximum pressure over the packed bed during operation	1.5 bar (0.15 MPa, 22 psi)
Column hardware pressure limit	5 bar (0.5 MPa, 73 psi)
Storage	4°C to 30°C in 20% ethanol

* H_2O at room temperature.

Selecting the metal ion and charging the column

Since IMAC Sepharose 6 Fast Flow is supplied free of metal ions, it needs to be charged by the user with a suitable ion before use. The choice of metal ion is dependent on the type of application and the specific protein to be purified. In addition, other considerations might also be important such as environmental or user exposure. The following guidelines may be used for preliminary screening experiments to select the most appropriate metal ion for a given separation:

- Ni^{2+} is commonly used for histidine-tagged recombinant proteins. It generally provides the strongest binding of such proteins. However, with some histidine-tagged proteins, metal ions other than Ni^{2+} may be more suitable – due to selectivity or for other reasons (e.g. environmental and health concerns).
- For purification of untagged proteins and especially in process scale, Cu^{2+} and Zn^{2+} ions are frequently used. Cu^{2+} binds relatively strongly to a range of proteins, and some proteins will only bind to Cu^{2+}. Zn^{2+} ions often give a weaker binding, and in some cases this can be exploited to achieve selective elution of the target protein. Both of these metal ions can also be used for histidine-tagged proteins.

When the binding characteristics of a target protein are unknown, it is advisable to test more than one metal ion in order to determine the most suitable one. The optimal binding and elution conditions could be dependent on the metal ion. In addition to the metal ions mentioned above, Co^{2+} is used for purification of histidine-tagged proteins, especially when a somewhat weaker binding than with Ni^{2+} is preferred to achieve selective elution (high purity) of the target protein. In some special applications, Fe^{3+} and Ca^{2+} have also been used.

Prepacked HiTrap IMAC FF columns are suitable for screening metal ions for a specific application.

The medium is charged with metal ions by passing a solution of the appropriate metal salt through the column (e.g., 0.1 M $ZnCl_2$, $NiSO_4$, or $CuSO_4$ in distilled water). The medium should then be washed with water and binding buffer before loading the sample on the column.

Sample binding

The choice of binding buffer depends on the metal ion and on the binding properties of the sample molecules.

Protein binding to an immobilized metal ion usually occurs in the pH range of 5.5 to 8.5. Binding is often strongest at the upper end of this range.

For histidine-tagged protein applications, imidazole at low concentrations is commonly used in the samples as well as in the binding/wash buffer to minimize binding of unwanted host cell proteins. The concentration of imidazole that will give optimal purification results is protein-dependent. For IMAC Sepharose 6 Fast Flow charged with Ni^{2+} or Co^{2+}, 20 to 40 mM in the sample as well as in the binding and wash buffer is a good starting point for optimization.

Elution

Elution is performed by reducing the pH or by competitive displacement, using for example imidazole. The most frequently used elution procedure for histidine-tagged proteins is based on a linear or stepwise increase of the imidazole concentration.

Elution by reducing pH can be performed using a linear or stepwise gradient. Most untagged proteins can be eluted between pH 6 and 4. Prepacked HiTrap IMAC FF columns are an excellent choice for screening to establish the optimal chromatographic conditions.

Regeneration and cleaning

When performing repeated purification cycles, the need for stripping and re-charging is dependent on the sample properties, sample volumes, metal ion, etc.

In some applications, substances such as denatured proteins or lipids are not removed during the regeneration procedures. These substances can be removed by Cleaning-In-Place (CIP). The column should be cleaned when the back pressure increases, or to avoid cross-contamination between samples/target proteins. Recommendations for CIP are included in the instructions enclosed with each pack of medium.

Applications

A successful IMAC purification depends on several factors, including the nature of the target protein, the metal ion used, and the binding and elution conditions. 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.

Screening for optimal conditions using different metal ions in the purification of a histidine-tagged protein

The protein used in these experiments was APB7, a $(histidine)_6$-tagged protein (M_r 28 000) expressed in *E. coli* BL-21. The protein was purified on HiTrap IMAC FF 1 ml columns charged separately with Cu^{2+}, Zn^{2+}, or Ni^{2+}. Screening experiments were performed to determine the optimal conditions using different concentrations of imidazole. At concentrations that are too high, imidazole can decrease the binding of histidine-tagged proteins. The imidazole concentration used in the sample and during the wash before elution must therefore be optimized to ensure the best balance of high purity (low binding of unwanted host cell proteins) and high yield (binding of all the histidine-tagged protein). The optimal concentration is protein-dependent and also metal ion-dependent. Figures 2 and 3 show the chromatographic and SDS-PAGE results, respectively, from these screening experiments. Chromatograms from the experiment using 10 mM imidazole are shown in Figure 2 (chromatograms from 20 mM imidazole and 5 mM imidazole experiments are not shown). Wash fractions and eluted protein fractions were analyzed, the former to monitor loss of target protein. The results from each of these three purifications indicated:

- At 20 mM imidazole, there was significant leakage of target protein in the wash with Cu^{2+} and Zn^{2+}, showing that the imidazole concentration was too high to allow a maximal yield. When Ni^{2+} was used, very little leakage of target protein could be seen (Fig 3A). The purity was excellent in all three cases.
- At 10 mM imidazole, the leakage of target protein in the wash with Cu^{2+} and Zn^{2+} was significantly reduced. The purity of the target protein in the eluted pool was similar with all three metal ions (Fig 3B), but not as good as with 20 mM imidazole.
- At 5 mM imidazole, practically no leakage of target protein in the wash could be seen from any of the metal ions. Here, the Ni^{2+}-charged column provided the purest protein (Fig 3C) although not as pure as with 20 mM imidazole.

This series of experiments illustrates that an increase in target protein purity obtained by increasing the imidazole concentration might be accompanied by a lower yield. For any given metal ion, the user can adjust these variables, to either achieve a higher yield or a higher purity, or choose a successful compromise. For the APB7 protein, Ni^{2+} and 20 mM imidazole gave practically no loss in recovery and high purity. With the other two metal ions, no compromise as good as that found with Ni^{2+} could be reached. An imidazole concentration of 20 mM led to high purity with Cu^{2+} and Zn^{2+}, whereas 5 mM gave a very high yield.

10 mM imidazole

Column:	HiTrap IMAC FF, 1 ml, charged with Cu^{2+}, Zn^{2+} or Ni^{2+}
Sample:	*E. coli* extract with APB7, a $(histidine)_6$-tagged protein (M_r ~28 000), including 10 mM imidazole
Sample volume:	5 ml (25 mg protein APB7)
Binding buffer:	20 mM sodium phosphate, 0.5 M NaCl, 10 mM imidazole pH 7.4
Elution buffer:	20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole pH 7.4
Flow rate:	1 ml/min (150 cm/h)
Gradient:	2 to 60% elution buffer (10 to 300 mM imidazole) in 25 ml 100% elution buffer (500 mM imidazole) in 5 ml
System:	ÄKTAexplorer™ 10
Detection:	Absorbance, 280 nm

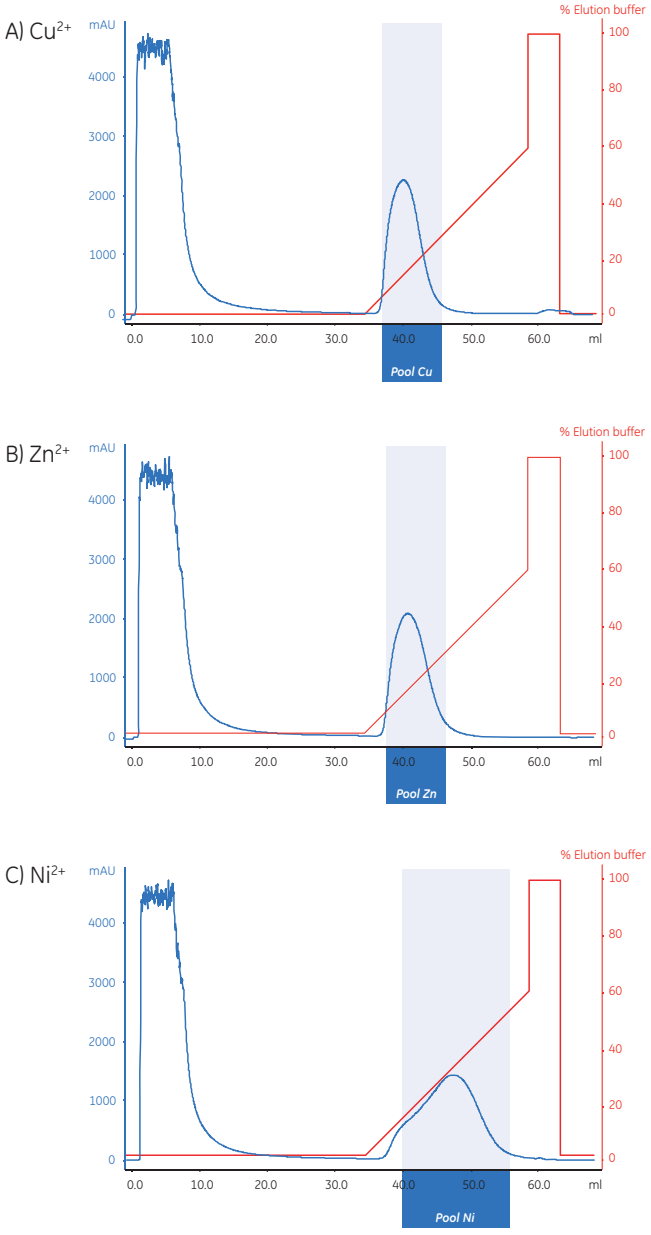
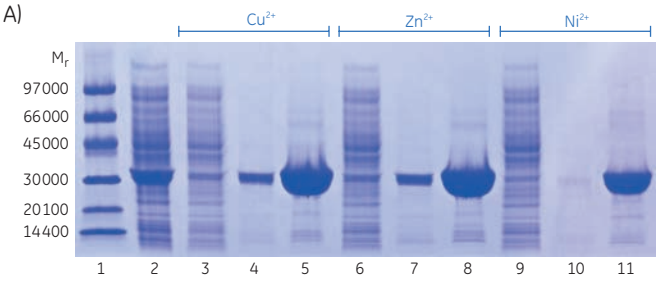


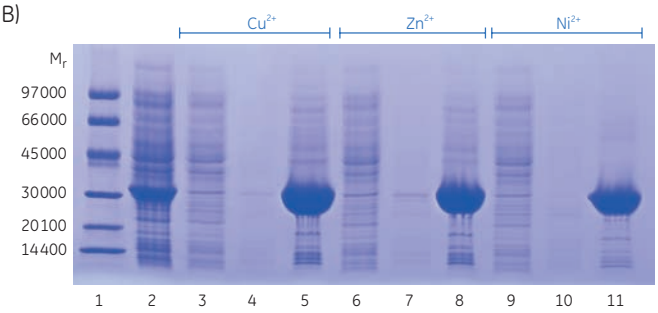
Fig 2. Purification of APB7 with IMAC Sepharose 6 Fast Flow charged with either Cu^{2+}, Zn^{2+}, or Ni^{2+} and with 10 mM imidazole in the sample. A–C: chromatograms using Cu^{2+}, Zn^{2+}, and Ni^{2+}, respectively.

A)



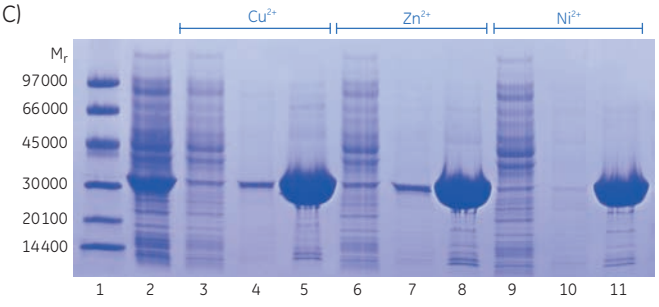
Lanes
1. LMW markers (LMW-SDS Marker Kit)
2. Start material APB7, diluted 1:10
3. Flowthrough, diluted 1:10, Cu^{2+}
4. Wash, Cu^{2+}
5. Eluted pool, Cu^{2+}
6. Flowthrough, diluted 1:10, Zn^{2+}
7. Wash, Zn^{2+}
8. Eluted pool, Zn^{2+}
9. Flowthrough, diluted 1:10, Ni^{2+}
10. Wash, Ni^{2+}
11. Eluted pool, Ni^{2+}

B)



Lanes
1. LMW markers
2. Start material APB7, diluted 1:10
3. Flowthrough, diluted 1:10, Cu^{2+}
4. Wash, Cu^{2+}
5. Eluted pool, Cu^{2+}
6. Flowthrough, diluted 1:10, Zn^{2+}
7. Wash, Zn^{2+}
8. Eluted pool, Zn^{2+}
9. Flowthrough, diluted 1:10, Ni^{2+}
10. Wash, Ni^{2+}
11. Eluted pool, Ni^{2+}

C)



Lanes
1. LMW markers
2. Start material APB7, diluted 1:10
3. Flowthrough, diluted 1:10, Cu^{2+}
4. Wash, Cu^{2+}
5. Eluted pool, Cu^{2+}
6. Flowthrough, diluted 1:10, Zn^{2+}
7. Wash, Zn^{2+}
8. Eluted pool, Zn^{2+}
9. Flowthrough, diluted 1:10, Ni^{2+}
10. Wash, Ni^{2+}
11. Eluted pool, Ni^{2+}

Fig 3. SDS-PAGE analyses (reducing conditions) of fractions from the APB7 purification using IMAC Sepharose 6 Fast Flow, charged with either Cu^{2+}, Zn^{2+} or Ni^{2+}, and with (A) 20 mM imidazole in the sample, (B) 10 mM imidazole in the sample, or (C) 5 mM imidazole in the sample. The gels were Coomassie™ stained.

Note that, for each purification series 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) to those obtained with gradient elution, provided that the imidazole concentration during the binding and wash steps was appropriate in each case.

In the purification examples above, individual 1 ml fractions were first analyzed by SDS-PAGE (not shown), to discard very impure fractions before pooling.

Scale-up purification of an untagged protein using IMAC Sepharose 6 Fast Flow

Experiments were performed to assess the efficiency of scaling up the capture step in the purification of recombinant bovine carbonic anhydrase II (r-BCA, M_r 30 000), a protein that naturally contains exposed histidine residues. Initial experiments were designed to determine the optimal metal ion and elution method for r-BCA purification.

Three metal ions (Cu^{2+}, Ni^{2+}, and Zn^{2+}) were assessed using HiTrap IMAC FF 1 ml columns. Scale-up studies were then performed using HiTrap IMAC FF 5 ml column and HiPrep IMAC FF 16/10 (20 ml) column.

The results showed that high purity was obtained with all three metal ions tested and binding strength decreased in the order $Zn^{2+} = Ni^{2+} > Cu^{2+}$ (data not shown). Due to its low toxicity, zinc is often the preferred metal ion to use in process scale, and thus it was chosen for the additional experiments. Results also demonstrated excellent recovery and purity in both elution methods tested – imidazole and pH (data not shown). However, because pH elution is the least expensive, it is the preferred elution method for process scale, and for this reason, it was chosen for the scale-up studies.

The data from the scale-up studies, shown in Figures 4 and 5, show high yields (> 90%) with both HiTrap IMAC FF 5 ml and HiPrep IMAC FF 16/10 (20 ml) columns. The loading was approximately 74% of maximum binding capacity. Table 6 shows that no significant change in recovery and purity was seen between the different scales. The recovery of the enzymatic activity was determined using an esterase activity assay, and was found to be approximately 90% in all cases (Table 6).

Metal ion leakage from the medium was investigated as this is an important issue when applying IMAC in an industrial scale. Total leakage of zinc was found to be very low, less than 3% in the HiPrep IMAC FF 16/10 scale. It should be noted that r-BCA needs one zinc ion in the active site for its enzymatic activity. By using a simple desalting step (HiPrep 26/10 Desalting), all metal ions were removed except for the zinc anchored in the active site of the protein. For further details about this scale-up study, refer to application note 28-4044-80.

The results—together with the regulatory and process-related support that is available for the medium—demonstrate that IMAC Sepharose 6 Fast Flow is well-adapted for industrial applications.

Acknowledgement

The recombinant clone with APB7 was obtained through cooperation with SGX Pharmaceuticals, Inc., San Diego, CA, USA.

Columns: HiTrap IMAC FF (1 ml and 5 ml) and HiPrep IMAC FF 16/10 (20 ml) charged with Zn^{2+}
Sample: 2.4, 12 and 49 ml of clarified *E. coli* extract containing 12.5, 62.4 and 255 mg r-BCA, respectively
Binding buffer: 20 mM sodium phosphate, 0.5 M NaCl, pH 7.4
Elution buffer: 20 mM sodium acetate, 0.5 M NaCl, pH 4.0
Flow rate: 150 cm/h in all cases
Experimental: After sample application, each column was washed with 20 column volumes (CV) Binding buffer followed by stepwiseelution with 15 CV 100% Elution buffer.
Detection: Absorbance, 280 nm

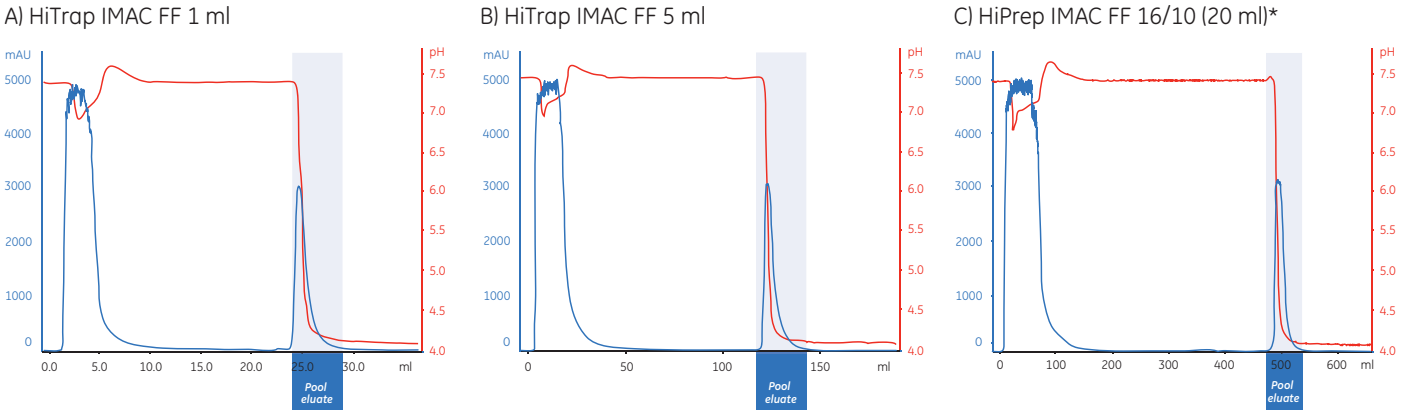
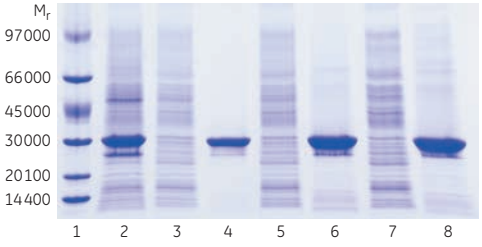


Fig 4. Chromatograms showing scale-up of purification from HiTrap IMAC FF 1 ml to HiTrap IMAC FF 5 ml and HiPrep IMAC FF 16/10 (20 ml) columns. Sample was 2.4, 12, and 49 ml clarified extract of *E. coli* containing 12.5, 62.4, and 255 mg of r-BCA, respectively. The load was approximately 74% of maximum binding capacity.

* Note: Data was obtained using first-generation HiPrep 16/10 columns.



Lanes
1. LMW markers (LMW-SDS Marker Kit)
2. Start material, clarified *E. coli* extract, diluted 1:33
3. Flowthrough HiTrap IMAC FF 1 ml, diluted 1:4
4. Eluted pool HiTrap IMAC FF 1 ml, diluted 1:4
5. Flowthrough HiTrap IMAC FF 5 ml, diluted 1:4
6. Eluted pool HiTrap IMAC FF 5 ml, diluted 1:5
7. Flowthrough HiPrep IMAC FF 16/10, 20 ml, diluted 1:4
8. Eluted pool HiPrep IMAC FF 16/10, 20 ml, diluted 1:4

Fig 5. Non-reduced SDS-PAGE analysis on ExcelGel™ Gradient 8–18 of the main fractions from the scale-up experiments. The gel was stained with a 1% solution of PhastGel™ Blue R (Coomassie).

Table 6. Data and results from the scale-up purification of r-BCA on IMAC Sepharose 6 Fast Flow. Comparisons of r-BCA yields and recoveries for the different runs show scalability of the application

Column	Fraction	Amount applied (mg)	Amount eluted (mg)	Recovery of protein	Recovery of r-BCA activity
HiTrap IMAC FF 1 ml	Clarified *E. coli* extract	12.5	—	—	—
	Eluted pool	—	11.7	94%	93%
HiTrap IMAC FF 5 ml	Clarified *E. coli* extract	62.4	—	—	—
	Eluted pool	—	56.1	90%	84%
HiPrep IMAC FF 16/10 (20 ml)	Clarified *E. coli* extract	255	—	—	—
	Eluted pool	—	235	92%	90%

Ordering Information

Product	Quantity	Code No.
IMAC Sepharose 6 Fast Flow	25 ml	17-0921-07
	100 ml	17-0921-08
	1 l	17-0921-09
	5 l*	17-0921-10
HiPrep IMAC FF 16/10	1 × 20 ml	28-9365-52
HiTrap IMAC FF	5 × 1 ml	17-0921-02
	5 × 5 ml	17-0921-04

* Larger quantities are available. Please contact GE Healthcare for more information.

Related products	Quantity	Code No.
Ni Sepharose 6 Fast Flow	5 ml	17-5318-06
	25 ml	17-5318-01
	100 ml	17-5318-02
	500 ml	17-5318-03
	1 l	17-5318-04
	5 l*	17-5318-05
HisTrap™ FF	5 × 1 ml	17-5319-01
	5 × 5 ml	17-5255-01
HisTrap FF crude	5 × 1 ml	11-0004-58
	5 × 5 ml	17-5286-01
HisPrep™ FF 16/10	1 × 20 ml	28-9365-51

* Larger quantities are available. Please contact GE Healthcare for more information.

Empty lab-scale columns	Quantity	Code No.
Tricorn 10/20 column	1	28-4064-13
Tricorn 10/50 column	1	28-4064-14
Tricorn 10/100 column	1	28-4064-15
XK 16/20 column	1	18-8773-01
XK 26/20 column	1	18-1000-72
XK 50/20 column	1	18-1000-71
XK 50/30 column	1	18-8751-01

Related literature	Code No.
Recombinant Protein Purification Handbook, Principles and Methods	18-1142-75
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Ni Sepharose and IMAC Sepharose, Selection Guide	28-4070-92
Affinity Chromatography Columns and Media, Selection Guide	18-1121-86
Prepacked chromatography columns for ÄKTA design systems, Selection Guide	28-9317-78

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

www.gelifesciences.com/protein-purification
www.gelifesciences.com/bioprocess
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