



1 mL AcroSep™ IMAC HyperCel™ Columns

- AcroSep IMAC HyperCel resin in pre-packed columns for Immobilized Metal Affinity Chromatography (IMAC) captures protein(s) or other molecules based on their affinity for metal ions. This affinity derives from the formation of coordination bonds between the metal ion and surface exposed molecular moieties on the protein(s) capable of coordination with metal (e.g., histidine).
- Histidine, present in many proteins, forms complexes with transition metal ions such as Cu^{2+} , Zn^{2+} , Ni^{2+} or Fe^{3+} . Typical applications include the purification of his-tagged proteins and metal binding proteins.
- Other amino acids, like cysteine and tryptophan, can interact with metal ions.
- Some glycosylated proteins will also bind IMAC. Additionally, IMAC can be used for fractionation of complex protein mixtures and phosphorylation studies.
- IMAC HyperCel uses tridentate IDA (iminodiacetic acid) as a chelating ligand.
- This resin is uncharged, allowing the user to load the metal ion most appropriate for specific applications.
- This ligand is immobilized on the HyperCel beads, a stable, robust, and well-known resin, used for both research and industrial-scale protein separations.
- IMAC HyperCel is compatible for protein capture under native and denaturing conditions.
- Versatile use:
 - Fully automated in combination with an automated chromatography instrument such as the ÄKTA design* systems.
 - Semi-automated in combination with pumps.
 - Manual use in combination with a syringe.

Ordering Information

<u>Part Number</u>	<u>Description</u>	<u>Color</u>	<u>Column Volume</u>	<u>Pkg</u>
20093-C001	IMAC HyperCel	Cobalt Blue	1 mL	5/pkg

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Note: *The procedures herein are intended only as a guide. Users should always verify product performance with their specific applications under actual use conditions. If you have questions about the information presented in this guide, please contact Pall Life Sciences technical service.*

Specifications

Materials of Construction

Column Housing, Cap, Plug and Adapter: Polypropylene
Column Frit: Polyethylene

IMAC HyperCel Properties

Ligand: Tridentate IDA (iminodiacetic acid)
Particle Size: 80-100 μm
Capacity for metal ions: 40-70 $\mu\text{mol Cu}^{2+}/\text{mL}$ of sorbent
Ionic Capacity: 90-140 $\mu\text{eq/mL}$
Regeneration: 0.5-1.0 M NaOH

Column Geometry

Column Volume: 1.04 mL
Bed Height: 1.48 cm (0.58 in.)
Bed Diameter: 0.94 cm (0.37 in.)

Device Dimensions

Diameter: 1.6 cm (0.6 in.)
Length (Without Plugs): 4.8 cm (1.9 in.)

Connections

Inlet: Threaded female luer
Outlet: Rotating male luer locking hub

Recommended Flow Rates

Metal Loading: 1-6 mL/min; residence time (T_r) = 0.34-0.17 min
Protein Capture: 0.2 to 2.0 mL/min; residence time (T_r) = 2.04-1.02 min

Maximum Column Pressure

Maximum: 3 bar (300 kPa, 43.5 psi)

Storage

2-8 $^{\circ}\text{C}$ (36-46 $^{\circ}\text{F}$); do not freeze

Working Conditions

After preparation, all solutions including sample should be passed through a 0.45 or 0.2 μm filter to remove particulates, which can disrupt flow.

Immobilization of Metal Ions

For protein capture, metal ions should be immobilized on IMAC HyperCel resin prior to use. The choice of the metal ion for immobilization will influence the purification; refer to the literature. The most commonly used metal ions are Cu^{2+} , Ni^{2+} , Zn^{2+} and Co^{2+} (Fe^{+3} and Ga^{+3} for phospho-protein capture).

- Before immobilization of the selected metal ion (Cu^{2+} , Ni^{2+} , Zn^{2+} , Co^{2+} , Fe^{+3} , Ga^{+3} , other...), wash the column extensively with ultrapure water to eliminate ethanol and sodium chloride used as preservatives in the storage solution.
- Load the metal ion solution [$\sim 100 \mu\text{mol/mL}$ (100 mM)] at 1-6 mL/min., until the metal ion solution breaks through (30-150 mM is effective for loading).
- Wash the column extensively with ultrapure water until metal is no longer present in flow through.
- Wash the column with 2-3 column volumes of 1 M NaCl.
- If the resin is not used immediately after loading, equilibrate the column in sample loading buffer.

Examples of Reagents to be Used for Metal Ion Immobilization (other salts are also effective)

Metal Ion	Reagent Example	MW (g/mol)
Cu^{2+}	$\text{CuSO}_4, 5 \text{ H}_2\text{O}$	250
Ni^{2+}	$\text{Ni}(\text{CH}_3\text{CO}_2)_2, 4 \text{ H}_2\text{O}$	249
Zn^{2+}	$\text{Zn}(\text{CH}_3\text{CO}_2)_2, 4 \text{ H}_2\text{O}$	219
Co^{2+}	$\text{Co}(\text{CH}_3\text{CO}_2)_2, 4 \text{ H}_2\text{O}$	249
Ag^+	AgNO_3	170
Ga^{3+}	$\text{Ga}_2(\text{SO}_4)_3$	428
Fe^{3+}	$\text{FeCl}_3, 6 \text{ H}_2\text{O}$	270

Caution: Metal ions used in IMAC may be toxic and/or cause allergic reactions. Additionally there may be specific metal ion disposal requirements.

Working Conditions *(continued)*

Sample Loading

The choice of loading buffer depends on the selected metal and on the sample binding properties. Sodium phosphate and Tris buffers are most commonly used, although others will also work. For most applications, binding at neutral or slightly basic pH is optimal (up to pH 9.0). NaCl is required to minimize any potential ionic interaction which occurs as a secondary mode of capture with IMAC chemistry. Minimally 150 mM, up to 1000 mM can be used (300-500 mM is most common). The optional inclusion of low amounts of imidazole (5-25 mM) in the binding buffer will reduce the capture of lower affinity IMAC binding proteins. This can be especially useful when purifying his-tagged proteins, which have high IMAC affinity, from crude samples which are likely to contain multiple proteins with low to moderate IMAC affinity. The amount of imidazole most effective for selective target capture is highly dependent on the target and other proteins present in the sample, and thus must be tested on a case-by-case basis.

In general, for soluble proteins, load up to 10 mg of total cell lysate; whereas, for insoluble proteins, load up to 6 mg of total cell lysate. Be aware that the sample loading amount will vary on a case-by-case basis since the optimum loading is dependent on the expression level of protein and its affinity for a particular metal ion in addition to other factors. Protein binding efficiency is often improved with lower flow rates.

Note: *EDTA or EGTA and other chelating agents should be avoided in sample loading buffers.*

Working Conditions *(continued)*

Sample Elution Buffers

Elution should be optimized on a case-by-case basis. Typically, elution can be achieved through competitive binding of imidazole (gradient or stepwise), pH reduction, or a combination of both.

Imidazole-based Elution

The concentration of imidazole needed for protein elution depends on the target protein and the choice of metal ion. Typically, 200 mM of imidazole is used for elution, but this can be optimized for specific samples using 50-250 mM imidazole in the presence of 0-1.0 M NaCl. In general, his-tagged proteins have a higher affinity for IMAC than endogenously expressed proteins and often require relatively higher concentrations of imidazole for elution. Although NaCl is not required during elution, it will prevent ionic interactions, thus limiting the possible re-binding of eluted proteins. To fully elute or strip any remaining protein from the column after target protein elution, even higher concentrations of imidazole can be used.

Note: *Many commercially available imidazole preparations contain a contaminant that will absorb at OD₂₈₀. If using OD₂₈₀ to monitor imidazole-based protein elution, we recommend testing your elution buffer first.*

Working Conditions *(continued)****pH-dependent Elution***

Sodium acetate or phosphate, or other appropriate buffers, can be used for pH elution (dependent on desired elution pH). Citrate buffers are not recommended as they are mildly chelating. The inclusion of salt during pH elution may be beneficial. Depending on the type of immobilized ion, acidic elution is not always complete and can be exploited for further fractionation: for example, with Cu^{+} , pH 6 elution may allow for the separation of IgG from other serum proteins (e.g., transferrin).

Note: *For maximum binding with repeated use, the IMAC HyperCel may require reloading of metal ion after stripping/cleaning procedures.*

If imidazole needs to be removed from the eluted target protein, use either Pall Trisacryl® GF 05 (MWCO = ~3 kDa) or Ultrogel® AcA 202 (MWCO = ~20kDa) resins with the desalting protocol. These resins will also remove salt and low MW contaminating proteins with no sample dilution. Choice of resin will depend on the size of the target protein.

Instructions for Use for Protein Purification – Automated or Pumped Chromatography Systems

Materials Required

- System (e.g., ÄKTA design, pump, or equivalent)
- Filtered, degassed buffers

Automated System Protocol

1. Attach column to pre-primed system. To prevent air from getting into the column, fill the neck of the column dropwise while system is running very slowly. Allow the buffer to flow through the column until all bubbles at the bottom of the column have been evacuated.
2. Wash with 5-10 column volumes (CV) of ultrapure water.
3. Load 4-6 CV of 100 mM chosen metal solution.
4. Wash with 2-3 CV of 1M NaCl.
5. Wash column with 20 CV of ultrapure water.
6. Equilibrate with 5-10 CV of running buffer.
7. Load the sample.
8. Wash with at least 5 CV of running buffer or until the OD₂₈₀ reading returns to baseline level.
9. Elute with 5-10 CV chosen elution buffer, stepwise, or gradient. Collect eluate in appropriately sized fractions.
10. Strip with 5-10 CV of elution buffer.
11. Re-equilibrate with 5 CV loading buffer.

Instructions for Use – Manual Use with Syringe

Materials Required

- Syringes (5-30 mL) with luer lock fittings
- Filtered buffers

Syringe Protocol

Note: *It is important to avoid introducing air into the column. Remove air bubbles from fluid filled syringe before attachment to the column each time the syringe is changed.*

When pushing fluid through the syringe, maintain a relatively constant flow rate with minimal backpressure, typically 0.2 to 2.0 mL/min.

1. Fill a syringe with ultrapure water. To avoid getting air into the column, load syringe with excess water.
2. Wash the column with 5-10 CV of ultrapure water by securing the filled syringe to the column luer connector. Check that there are no air bubbles at the site of attachment. Apply gentle pressure to push water through the column.
3. Fill a syringe with 100 mM of chosen metal ion solution (30 to 150 mM is acceptable) .
4. Load 4-6 CV of 100 mM chosen metal solution (more volume for lower concentration).
5. Wash with 2-3 CV of 1M NaCl.
6. Wash the column with 10-20 CV of ultrapure water.
7. Fill a syringe with loading buffer.
8. Equilibrate the column with 5-10 CV of loading buffer.
9. Fill a syringe with sample.

Instructions for Use – Manual Use with Syringe *(continued)*

10. Load sample onto the column, avoiding the introduction of air bubbles.
 - Collect flow through fraction to assess possible target protein breakthrough if capacity is exceeded.
11. Wash column with 5-10 CV of loading buffer to remove unbound proteins.
 - Collect wash fraction for analysis if desired.
 - Additional washes with low concentrations of imidazole (5-50 mM) can be used to elute weakly binding proteins as necessary.
12. Fill a syringe with elution buffer. Secure this syringe to the column luer connector. Check that there are no air bubbles at the site of attachment.
13. Run 5-10 CV of elution buffer through the column to elute bound proteins.
 - Collect all effluent containing eluted protein(s) in appropriately sized fractions.
14. If the column will be reused, strip residual protein with 5-10 CV of elution buffer.
15. Fill the syringe with loading buffer. Equilibrate the column with 5-10 CV of loading buffer.

Procedure for the Determination of Target Protein Dynamic Binding Capacity (DBC) Using an Automated Chromatography System

For the most accurate prediction of DBC during a purification scheme, chose conditions that closely match conditions to be used during target protein purification.

System Parameters

- Metal Loading Step
 - Flow rate: 3-6 mL/min (or intended flow rate)
 - Wash with 5-10 CV of ultrapure water
 - Load 10 CV of 30 mM of chosen metal solution
 - Wash column with 20 CV ultrapure water
- Protein Loading Step
 - Flow rate: 0.2 to 2.0 mL/min (or intended flow rate)
 - Equilibrate: 10-20 CV loading buffer
 - Sample load: inject sufficient quantity of protein-containing sample to exceed column capacity
- Wash and Elute Steps
 - Wash: 10-20 CV loading buffer
 - Elute: 10 CV elution buffer
 - Re-equilibration: 10-20 CV loading buffer

Calculation of the DBC of the AcroSep column (1.04 mL resin)

- Formula: $DBC = C \times (V_L - V_0)$

C = Concentration of load

V_L = Volume at 10% or 50% breakthrough

V_0 = From the void volume determination described above, V_0 is the total volume passing through the system from the time of injection (0% deflection of OD₂₈₀) until protein is detected (increase in OD₂₈₀).

Cleaning

General Cleaning

Column performance may decline over time due to incomplete removal of proteins or contaminants. They are usually removed with the following procedure:

1. Wash column with 10 CV of loading buffer.
2. Wash column with 5 CV of 1-2 M NaCl in buffer with a pH 4-8.5.
3. Wash column with 10 CV of loading buffer.

Metal ions can be stripped from the column as follows:

1. Wash column with 10 CV of 25 mM EDTA.
2. Wash column with 10 CV of loading buffer.

Storage Recommendations

- The column must be stored at 2-8 °C (36-46 °F) and cannot be frozen.
- Between runs, store the column at 2-8 °C (36-46 °F) in loading buffer.
- The storage buffer may also contain bacteriostatic agents such as 20% (v/v) ethanol and/or 1 M NaCl.

Adapter Recommendations

AcroSep pre-packed columns are made with a luer inlet and outlet for easy connection to syringes. The following table lists recommendations if adapters are needed to connect the columns to other types of tubing.

Connection To	Adapters (Upchurch Scientific*)
1/16" OD Teflon* and Tefzel* Tubing	1 kit (P-837) <i>Instructions provided with kit</i>
1/8" OD Teflon and Tefzel Tubing	1 kit (P-838) <i>Instructions provided with kit</i>
1/16" Stainless Steel Tubing	1 inlet fitting (P-658), 1 outlet fitting (P-655), 2 ferrules (P-259), 2 nuts (LT-115) <i>Instructions provided with fittings</i>
1/32" Stainless Steel Tubing	1 inlet fitting (P-658), 1 outlet fitting (P-655), 2 ferrules (P-248), 2 nuts (LT-115) <i>Instructions provided with fittings</i>

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