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A Geno Technology, Inc. (USA) brand name

G-Sep™ IDA Agarose Fast Flow

Nickel & Cobalt IDA IMAC Resins

(Cat. # 786-973, 786-974, 786-975, 786-976, 786-977, 786-978, 786-979, 786-980)

INTRODUCTION

Immobilized Metal Ion Affinity Chromatography (IMAC), developed by Porath (1975), is based on the interaction of certain protein residues (histidines, cysteines, and to some extent tryptophans) with cations of transition metals. G-Sep™ Ni IDA Agarose Fast Flow and G-Sep™ Co IDA Agarose Fast Flow are specifically designed for the purification of recombinant proteins fused to the 6x histidine (6XHis) tag.

The resins are specifically designed for the purification of recombinant proteins fused to the 6x histidine (6XHis) tag expressed in bacteria, insects, and mammalian cells. The resin is high affinity and selectivity for recombinant fusion proteins that are tagged with six tandem histidine residues. Both nickel and cobalt IDA are offered as nickel has a higher binding affinity, but lower specificity compared to cobalt. This means that although cobalt IDA has a slightly lower efficiency compared to nickel IDA resin there is a significant reduction in non-specific binding.

G-Sep™ IDA Agarose Fast Flow (FF) resins are available with cobalt or nickel ions immobilized onto highly cross-linked 6% agarose beads using iminodiacetic acid groups (IDA). The G-Sep™ IDA Agarose Fast Flow (FF) resins have high chemical stability, allowing well proven cleaning-in-place (CIP) and sanitization protocols.

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-973	G-Sep™ Ni IDA Agarose Fast Flow	5ml
786-974	G-Sep™ Ni IDA Agarose Fast Flow	25ml
786-975	G-Sep™ Ni IDA Agarose Fast Flow	100ml
786-976	G-Sep™ Ni IDA Agarose Fast Flow	500ml
786-977	G-Sep™ Co IDA Agarose Fast Flow	5ml
786-978	G-Sep™ Co IDA Agarose Fast Flow	25ml
786-979	G-Sep™ Co IDA Agarose Fast Flow	100ml
786-980	G-Sep™ Co IDA Agarose Fast Flow	500ml

STORAGE CONDITIONS

It is shipped at ambient temperature. Upon arrival, store at 4°C. DO NOT FREEZE.

SPECIFICATIONS

	G-Sep™ Ni IDA Agarose	G-Sep™ Co IDA Agarose
Matrix	Cross-linked agarose beads, 6%	
Bead form	Spherical, diameter 50-160µm	
Spacer	Epichlorohydrin	
Chelating Agent	Iminodiacetic acid	
Active group	Ni^{2+}	Co^{2+}
Metal ion density	20-40µmol /ml	
Binding Capacity	5-10mg His-tagged protein/ml medium	
pH stability Working Range	3-12	
pH stability Cleaning-in-Place (CIP)	2-14	
Maximum Flow Velocity	450cm/h	
Exclusion limit(globular proteins)	4×10^6	
Physical Stability	Negligible volume variation due to changes in pH or ionic strength	
Chemical Stability	Stable to all commonly used aqueous buffers: 6M urea, 8M guanidine hydrochloride,	
Autoclavable	121°C, pH 7, for 30 min	
Storage Conditions	2 to 8°C, 20% Ethanol	

PREPARING THE MEDIUM

G-Sep™ IDA Agarose Fast Flow (FF) resins are supplied in a solution containing 20% ethanol.

Prepare a 75% slurry using a binding/ eluent buffer of choice. The slurry will be 75% settled resin and 25% buffer. It is recommended to degas the slurry before packing.

PACKING G-SEP™ IDA AGAROSE FAST FLOW

1. Equilibrate all material to room temperature.

2. De-gas the slurry

3. Eliminate air from the column dead spaces by flushing the end pieces with buffer. Make sure no air has been trapped under the column net. Close the column outlet with a few centimeters of buffer remaining in the column.

4. Pour the gel slurry into the column in one continuous motion. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles.

5. Fill the remainder of the column with buffer, mount the column top piece onto the column and connect the column to a pump.

6. Open the bottom outlet of the column and set the pump to run at the desired flow rate. This should be at least 133% of the flow rate to be used during subsequent chromatographic procedures. However, the maximum flow rate is typically employed during packing.

7. Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached.

Using an adapter

1. After the medium have been packed as described above, close the column outlet and remove the top piece from the column. Carefully fill the rest of the column with buffer to form an upward meniscus at the top.

2. Insert the adaptor into the top of the column at an angle, taking care not to trap air under the net.

3. Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the pump.

4. Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by eluent. Valves on the inlet side of the column should be turned in all directions during this procedure to ensure that air is removed.

5. Lock the adapter in position on the medium surface, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the bed is stable. Re-position the adapter on the medium surface as necessary.

6. The column is now packed and equilibrated and ready for use.

OPERATION

Equilibration

The choice of buffer depends on the particular properties of the protein as well as of the type of chelate used. The buffers used most frequently are acetate (50mM) or phosphate (10-150mM). The pH of binding buffers generally leads to neutrality (pH 7.0-8.0), but can vary over the range 5.5-8.5. To avoid ionic interchange, add 0.15-0.5M of NaCl. In some cases to increase selectivity of the binding of target protein it is necessary to add to the Equilibration Buffer a small concentration of imidazole (10-40mM). Equilibrate the column with the starting buffer when the pH and/or conductivity of the effluent is the same as the starting buffer.

Sample preparation

It is important to determine whether the His-tagged protein is soluble in the cytoplasm, or is present in inclusion bodies. If the latter, the protein must be solubilized with denaturants such as urea of guanidine hydrochloride before purification. Denaturants ensure that otherwise insoluble His-tagged proteins can be purified, and will cause a conformational change that exposes the His-tag, enabling more efficient binding to the Ni-IDA Agarose beads. Denaturing conditions, however, generally result in the complete loss of biological and biochemical activity of the proteins being purified. Purification under native conditions may result in the co-purification of unrelated, untagged proteins than when denaturing conditions are employed. Nonspecific binding is minimized by adding a low concentration of imidazole to the native lysis buffer and native equilibration buffer.

Operating flow rates

The typical linear flow rate of G-Sep™ IDA Agarose Fast Flow (FF) resins is 300-400cm/h through 15 cm bed height at a pressure of 0.1MPa.

Binding

A slight increase of contact time may facilitate binding. The pH of binding buffers generally leads to neutrality (pH 7.0-8.0), but can vary over the range 5.5-8.5. To avoid ionic interchange, add 0.15-0.5M of NaCl. In some cases to increase the selectivity of the binding of target protein it is necessary to add to the Equilibration Buffer a small concentration of imidazole (10-40mM).

Washing

Wash the column 3 times with 10 bed volumes of the Equilibration Buffer. This volume may be adjusted as needed. In some cases good results are obtainable with less washing, while in others, additional washing may be required.

Elution

Elution is possible by several techniques, including lowering the pH, or with the use of a metal complexing agent such as imidazole or EDTA in the buffer. There is a balance between the concentration of imidazole required to elute the His-tagged protein, and the amount needed to avoid non-specific binding of contaminants.

The recommended Elution Buffer contains 0.5M of the complexing agent imidazole as starting concentration. It is also possible to use concentration gradients of this reagent (0–0.5M). Most proteins are eluted in concentrations around 250mM. In all cases it is important to check the pH.

Decreasing the pH of the Elution Buffer by 0.5pH units (generally at least between pH 3.0 and 4.0) also allows the elution of the desired protein.

For His-tagged proteins that are unstable at a pH<7.0: You can elute with a stepped imidazole gradient at a neutral pH (pH 7.0–7.5). Use 10mM imidazole, then 50mM, 75mM and so on until the protein elutes.

Regeneration

It is recommended to do regeneration when an appreciable diminution in the yield is observed. The frequency of these stages varies with the protein and the conditions used.

1. Wash G-Sep™ IDA Agarose Fast Flow (FF) resins with 5 volumes of Stripping Buffer, which strips the metal ions from the agarose.
   Stripping Buffer: 20mM NaH_2PO_4 pH7.0, 50mM EDTA,0.5M NaCl

2. Wash the agarose with 5 volumes dH_2O.

3. Elimination of ionic interactions: Wash in batch for approximately 20 minutes in a solution with 1.5M NaCl. Later wash with 10 column volumes of distilled water to eliminate ions.

4. Elimination of precipitated proteins (may be responsible for column pressure changes). Wash in batch at least 2 hours with a solution 1.0M NaOH. Eliminate the NaOH with 10 column volumes of distilled water.

5. Elimination of strong hydrophobic interactions: resuspend the resin in batch with a solution of isopropanol 30 % for approximately 20 min. Then wash with 10 column volumes of distilled water to eliminate the isopropanol.

6. Wash in batch for 2 h with a solution 0.5 % of non-ionic acetic acid detergent 0.1M. Rinse the detergent with ethanol 70 % (approximately 10 column volumes).

7. Finally wash with 10 column volumes of distilled water to rinse out the ethanol.

8. To recharge the agarose with Ni^{2+}, wash with 5 volumes 0.1M $NiSO_4 \times 6\ H_2O$ or 0.1M cobalt(II) sulfate heptahydrate.

9. Wash out excess metal ions with 5 volumes dH_2O.

10. Preparation of the column by adding 5 column volumes of the binding buffer.

11. If G-Sep™ IDA Agarose Fast Flow (FF) resins should be stored, add unbuffered 20% ethanol and store at 2 - 8°C.Do not freeze.

Storage

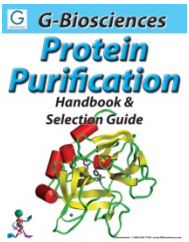
G-Sep™ IDA Agarose Fast Flow resins should be stored in the salt form in a buffer containing 20% ethanol. Recommended storage at 4 to 30°C. Do not freeze.

Shelf life

3 year

RELATED PRODUCTS

Download our Sample Preparation and Protein Purification Handbooks.



http://info2.gbiosciences.com/complete-protein-purification-handbook

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