SARTORIUS

85030-517-46

Instructions for Use

Sartobind® IDA 75

A Separation Technology Based on Metal Chelate Membrane Adsorbers

1. Storage conditions

Sartobind IDA 75 should be stored clean, dry and away from direct sunlight in the box at room temperature.

2. Introduction

Sartobind Metal Chelate adsorbers represent a new generation of Immobilized Metal Affinity Chromatography (IMAC) purification devices. They can simply be used in an HPLC, FPLC or operated by hand with a syringe connected via Luer Lock. The iminodiacetic acid (IDA) ligand is attached to the inner surface of a membrane which is fitted into a filter holder for easy and quick handling, making His-tagged protein purification nearly as easy as filtration.

IMAC is a common and effective tool for the purification of polyhistidine tagged proteins. The method is based on the ability of histidine containing proteins to bind to immobilized metal ions. Especially strong interactions take place with the commonly used polyhistidine (His 6-tag)* with six consecutive histidine residues. Using the IMAC principle, polyhistidine tagged proteins can be concentrated to a high degree of purity even from cell lysates or culture supernatants. The IDA groups can be loaded with different metal ions depending on the particular application. We suggest using nickel (Ni^{2+}), cobalt (Co^{2+}), copper (Cu^{2+}) or zinc (Zn^{2+}) ions, but other metal ions can easily be immobilized on the membrane. Proteins engineered with polyhistidine tags passing through the prepared membrane are preferentially bound. These bound proteins can be easily eluted from the membrane using buffers with varying concentrations of imidazole. Sartobind Metal Chelate units are designed to simplify the chromatographic steps normally associated with IMAC. This makes them also a convenient and quick tool for screening purposes. The 15 layer device Sartobind IDA 75 is perfectly designed as a down scale unit for Sartobind large scale modules and capsules.

There are a number of various expression systems for polyhistidine tagged proteins available. This protocol addresses protein purification from bacterial expression systems. The procedure may be adapted to other related expression systems, e.g. yeast or eukaryotic cells.

⚠ **Please note that loading of the membrane with metal ions should take place immediately before sample purification.**

Sartobind IDA 75 pack contains

Order number	93IDA-42DB-12--V
No. of units	2
Adapter Luer male to UNF 10–32 female, PEEK	1
Adapter Luer female to UNF 10–32 female, PEEK	1
Instruction manual	1

Technical Data

Matrix	Stabilized reinforced cellulose, nominal pore size > 3 µm
Membrane area	75 cm² (2.1 mL membrane volume)
Number of layers | bed height	15 | 4 mm
Ligand	Iminodiacetic acid
Ligand density	approx. 5 µeq/cm²
Approx. binding capacity for 6× His-tagged protein	
per cm²	100 µg
per unit	7.5 mg
Min. binding capacity	3.0 mg cytochrome c (horse heart)
Recommended flow rate	10 – 63 mL/min
pH stability	
long term	3 - 9
short term	1 - 12
Maximum pressure	0.6 MPa|87 psi | 6 bar
Housing	Polypropylene

3. Materials needed

No further hardware than a 10 mL syringe with Luer Lock connector and beakers are required: A syringe can be used to push the fluids through the unit at velocity up to approximately 10 mL/min.

4. Selection of metal ions for pre-loading

Sartobind Metal Chelate adsorbers were specifically designed to allow you the choice of the metal ions to be immobilized on the membrane. If the conditions of optimal binding of the target protein are unknown, we recommend to start with nickel (Ni^{2+}), cobalt (Co^{2+}), copper (Cu^{2+}) or zinc (Zn^{2+}) ions. For the initial run, you can either use one of the metal ion solutions described in table 1, or screen all four metal ion solutions in parallel to find the best performance for your application. In this case, please use one Sartobind Metal Chelate unit for each aqueous metal ion solution. For certain proteins or applications, the use of different metal ions apart from nickel (Ni^{2+}), cobalt (Co^{2+}), copper (Cu^{2+}) or zinc (Zn^{2+}) may increase the degree of purity.

Table 1: Recommended aqueous solutions of Ni^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+} ions for pre-loading

Ni^{2+}	0.1 M nickel sulphate (e.g. $NiSO_4 \cdot 6\ H_2O$) in equilibration buffer
Co^{2+}	0.1 M cobalt chloride (e.g. $CoCl_2 \cdot 6\ H_2O$) in equilibration buffer
Cu^{2+}	0.1 M copper sulphate (e.g. $CuSO_4$ or $CuSO_4 \cdot 5\ H_2O$) in equilibration buffer
Zn^{2+}	0.1 M zinc chloride (e.g. $ZnCl_2$) in equilibration buffer

5. Recommended buffers

As mentioned above, expression systems for proteins with polyhistidine tags are highly diverse. Therefore, the described purification buffers should be considered only as guidelines. For the best performance and recovery, we recommend optimization of conditions for the individual target protein. Contaminating proteins in the eluate can be reduced by varying the imidazole concentration in the washing buffer. If the polyhistidine tagged protein cannot be detected after elution with 250 mM imidazole, the imidazole concentration of the elution buffer should be increased. Depending on the elution conditions, leaching of the metal ions from the membrane may occur. We recommend the following buffers for purifying the protein of interest under native conditions.

Table 2: Buffer recommendations for purification under native conditions

Equilibration buffer	0.1 M sodium acetate, 0.5 M NaCl pH 4.5
Metal solution	Equilibration buffer + 0.1 M metal solution
Loading buffer	50 mM sodium phosphate, 0.5 M NaCl pH 8.0
Elution buffer	Equilibration buffer + 0.1 M imidazole
Metal stripping	1 M H_2SO_4

6. Sample preparation

Prepare the cell lysates according to your standard protocol (sodium phosphate is recommended as buffer system for cell lysis). Prefilter the sample through a 0.2 µm membrane filter e.g. Sartorius Minisart® 16534. Store the clarified sample on ice or under appropriate conditions.

7. Pre-loading with metal ions

- Hold the unit in a vertical position. Remove the upper cap.
- Fill a 10 mL syringe with 10 mL of equilibration buffer and connect to the top.
- Remove the lower cap. Fill the upper part of the unit and remove any remaining air by moving the plunger up and down with short strokes. Make sure that the unit is completely filled with fluid.
- Close the outlet of the unit with the cap, remove the syringe from the unit, remove the plunger from the syringe and re-connect the syringe to the unit. It is now ready for loading.

⚠ **The upper part of the filter holder has to be filled always completely with fluid to ensure even flow and distribution of the feed stream.**

- Fill into the syringe 10 mL of the 0.1 M metal solution. Take care that no air enters the unit.
- Remove the lower cap and let it flow by gravity. The fluid may be pushed through the unit by applying gentle pressure with the syringe plunger.
- Then fill in 10 mL equilibration buffer to remove unbound metal ions.
- Repeat this washing step.
- Equilibrate the unit with 10 mL of loading buffer.
- Repeat this step.
- Close the outlet with the cap.
- Now the Sartobind Metal Chelate adsorber unit is ready for sample loading.

8. Sample loading

- Apply the sample and remove the lower cap. Take care that no air enters the unit.
- Remove the lower cap and let it flow by gravity until the fluid level has reached the bottom of the syringe again. The fluid can be pushed through the unit by applying gentle pressure with the plunger.

9. Washing

Wash with 10 mL of loading buffer.

10. Elution

Elute with 5 mL of elution buffer.

11. Regeneration

Regenerate the Membrane Adsorber by passing 10 mL of equilibration buffer. Strip the chelated metal ions by passing 10 mL of 1 M sulfuric acid through the unit. Attention: Wear safety goggles and protective clothes when handling concentrated acids. Pass 10 mL of equilibration buffer through the unit, repeat this step twice. Ensure that the pH has reached the value of the equilibration buffer. Load the unit with metal ions as described above.

12. Storage after use

Keep the used unit filled with equilibration buffer in the presence of an antimicrobial agent such as sodium azide at a concentration of 0.02%.

13. Use of a peristaltic pump or HPLC

- For the operation of the adsorber units in a HPLC system or with a peristalic pump, specific Luer Lock adapters are needed. They can be ordered as an accessory. Proceed as described until the unit is filled completely with equilibration buffer, the outlet is closed and the syringe is removed.
- Start your HPLC or peristaltic pump at a very low flow rate. When fluid emerges, stop the pump, connect the tubing via a Luer Lock adapter to the inlet of the unit. Make sure that no air is introduced.
- Remove the lower cap. Run the pump until fluid emerges from the outlet of the unit and stop it. Then connect outlet of the unit via Luer Lock adapter to the HPLC detector.

14. Accessory

Order number	17002---140
Description	2 pairs of Luer Lock adapters for inlet and outlet to M6 female

For more information about applications, scale-up or other membrane types please contact your nearest Sartorius office or visit our homepage: www.sartorius.com.

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