SARTORIUS

85030-517-46

Instructions for Use

Sartobind® IDA Lab

A Separation Technology Based on Metal Chelate Membrane Adsorbers

1. Precautions

If the unit is damaged or incorrectly installed, liquids can leak from the unit. There is a risk of skin irritation.

- Wear the appropriate personal protective equipment, e.g., safety gloves and safety glasses.
- Perform a visual inspection before use and ensure all connections are fluid-tight.
- Ensure correct installation of the Luer Lock adapters, if applicable.

If too much pressure is applied to the unit, the unit housing may burst. There is a risk of cutting or irritation of the skin.
The microfilter for inline prefiltration might have a lower operating pressure than the unit.

- Do not exceed the maximum pressure of the unit.
- If operating with inline prefiltration, do not exceed the maximum pressure of the microfilter used.

2. Storage conditions

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

3. Introduction

Sartobind® Metal Chelate adsorbers represent a new generation of Immobilized Metal Affinity Chromatography (IMAC) purification units. They can be used with a peristaltic pump, liquid chromatography (LC) system, 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.

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 Lab pack contents

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
Quick start guide	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

4. 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.

5. 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

6. 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

7. Sample preparation

Prefiltration can be performed in a separate step prior to sample loading or during sample loading via inline prefiltration.

- Prepare the cell lysates according to your standard protocol (sodium phosphate is recommended as buffer system for cell lysis).
- If performing sample prefiltration as a separate step, prefilter the sample through a 0.2 µm membrane filter, e.g. Sartorius Minisart® S6534. Store the clarified sample on ice or under appropriate conditions.
- If performing inline prefiltration, proceed as described in chapter „11. Sample loading with inline prefiltration“.

8. Venting

- 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.
- Invert the unit so that the outlet is pointing up and depress the syringe plunger until the air is removed. Make sure that the unit is completely filled with fluid.
- Return the unit to an upright position and 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.
- The unit is ready for loading.

The upper part of the unit must always be filled completely with fluid to ensure even flow and distribution of the feed stream.

9. Pre-loading with metal ions

- Fill the syringe with 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.

10. Sample loading without inline prefiltration

- Apply the sample. Take care that no air enters the unit.
- Remove the lower cap.
- Let the sample 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.
- Close the outlet with the cap.

11. Sample loading with inline prefiltration

- Fill the syringe with sample and connect it to the top of a microfilter, e.g. Sartorius Minisart® S6534.
- Fill the microfilter with sample.
- Connect the bottom of the microfilter to the top of the unit. Take care that no air enters the unit.
- Remove the lower cap.
- Load the sample through the microfilter and the unit by applying gentle pressure with the syringe plunger.
- Close the outlet with the cap.

12. Washing

Wash with 10 mL of loading buffer.

13. Elution

Elute with 5 mL of elution buffer.

14. 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.

15. 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%.

16. Use of a peristaltic pump or LC system

- For the operation of the units with a peristaltic pump, connect a length of pump tubing to the Luer inlet.
- For the operation of the units with a LC system, use the Luer Lock adapters included in the scope of delivery. Replacement Luer Lock adapters can be ordered separately.
- Proceed as described until the unit is filled completely with equilibration buffer, the outlet is closed and the syringe is removed.
- Start your LC system or peristaltic pump at a very low flow rate. When fluid emerges, stop the pump.

When operating without inline prefiltration:

- Connect the tubing to the inlet of the unit (via the Luer Lock adapter, if required). Make sure that no air is introduced.
- Remove the lower cap. Run the pump until fluid emerges from the outlet of the unit.
- When using a LC system, connect the outlet of the unit via Luer Lock adapter to the UV flow cell.
- Load the sample.

When operating with inline prefiltration:

- Connect the tubing to the inlet of the microfilter (via the Luer Lock adapter, if required).
- Fill the microfilter with buffer until fluid emerges from the outlet.
- Connect the outlet of the microfilter to the inlet of the unit. Make sure that no air is introduced.
- When using a LC system, connect the the outlet of the unit via the Luer Lock adapter to the UV flow cell.
- Load the sample.

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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