Operating Instructions

Sartobind® IEX MA 15 | 75 | 100

A Separation Technology Based on Macroporous Membranes

85032-539-13

SARTORIUS

Read operational instructions carefully before using Sartobind capsules.

⚠ **Use of the product in applications not specified or not described in this manual, may result in improper function, personal injury, or damage of the product or material.**

⚠ **Membrane Adsorber (MA) units should be visually inspected before use.**

⚠ **The units are supplied as non-sterile.**

⚠ **The membrane is dried from glycerol and should be pre-washed with equilibration buffer before use.**

⚠ **If you plan to scale up later, preferentially use Sartobind capsules with 4 or 8 mm bed height: e.g. Sartobind Q nano with 1 or 3 mL as downscale.**

Intended use

The products are intended for ion exchange (IEX) chromatography work in a laboratory for research purposes only.

Sartobind MA 15 units with 3 membrane layers can be used for screening of buffer conditions and quantitative purifications at exceptional high flow rate.

Sartobind MA 75 units contain 15 membrane layers and have been developed for bind and elute and flowthrough applications at high flow rate.

Sartobind MA 100 units with 5 membrane layers can be used for screening of buffer conditions and quantitative work at exceptional high flow rate.

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1 Storage Conditions

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

2 Introduction

Traditional chromatography uses porous particles packed into columns. As liquid flows through the column and around the beads, bio-molecules in the liquid diffuse into the pores of the beads to binding sites on the inner surface of the pores. The limiting factor in low pressure column chromatography is the time required for the molecules to diffuse into and out of the pores. The various steps of equilibration, loading, washing, elution and regeneration can take hours.

Sartobind membranes are made from regenerated and stabilized cellulose. The stabilization and cross-linking generate high chemical stability. Conventional ion exchange ligands are covalently attached to the macroporous membrane support.

The membrane layers form chromatographic bed between 3 and 15 layers which is incorporated into housings.

Pressure forces the liquid through the pores of the membrane, bringing target substances to direct contact with the binding sites. This direct convection to the binding sites minimizes diffusion limitation of mass transfer and increases the speed of operation to 10 – 30 fold compared to traditional column chromatography.

The Luer Lock connectors on top and bottom allow you to run the units with a syringe and a prefilter or to connect several units to each other to get a larger bed volume or to run units with different chemistries.

3 Technical Data

	MA 15	MA 75	MA 100
Membrane Area [cm^2]	15	75	100
Number of layers	3	15	5
Bed height [mm]	0.8	4.0	1.4
Membrane volume [mL]	0.41	2.1	2.8
Membrane diameter [mm]	25	25	50
Typical dynamic binding capacity 10% for Q [mg/unit]*	12	60	80
Typical dynamic binding capacity 10% for S [mg/unit]*	10.5	52.5	70
Typical dynamic binding capacity 10% for D [mg/unit]**	–	60	–
Ligand density [µeq/cm^2]	2 – 5 (Q, S), 2 – 4 (D)**		
Maximum pressure at 20°C	6 bar | 0.6 MPa | 87 psi		

* See also chapter "5 Binding Capacity", page 11.

** D type is available as MA 75 only.

4 Materials

Housing and basic membrane

Housing MA 15, 75, 100	Polypropylene
Membrane matrix	Stabilized reinforced cellulose
Nominal pore size	> 3 µm
Membrane ligands	
Strong basic anion exchanger	Quaternary ammonium (Q) $R\text{-}CH_2\text{-}N^+(CH_3)_3$
Strong acidic cation exchanger	Sulfonic acid (S) $R\text{-}CH_2\text{-}SO_3^-$
Weak basic anion exchanger	Diethylamine (D) $R\text{-}CH_2\text{–}N(C_2H_5)_2$

5 Binding Capacity

Data are based on dynamic binding capacity measurements 10% using MA15 run at 10 mL/min.

Membrane	Typical dynamic binding capacity 10% [mg/cm^2]	Reference protein and buffer
Q	0.8	BSA (bovine serum albumin) in 20 mM Tris | HCl, pH 7.5
S	0.7	Lysozyme in 10 mM potassium phosphate, pH 7.0
D	0.8	BSA (bovine serum albumin) in 20 mM Bis Tris, pH 6.0

6 Installation

The contents of the packages are described in chapter 10.1, page 27.

The Sartobind Membrane Adsorber (MA) units are ready-to-use devices and can be used out of the box. They can be operated with a syringe (chapter 7.1 to 7.8, page 14), peristaltic pump or liquid chromatography (LC) system (follow chapter 7.11, page 22, first).

The unit should be installed in an upright position in the process flow. The female Luer connector is the inlet and the male Luer connection is the outlet of the unit. The flow is guided from top through the 3 to 15 membrane layer to the outlet. The units can also be connected in series to increase the binding capacity.

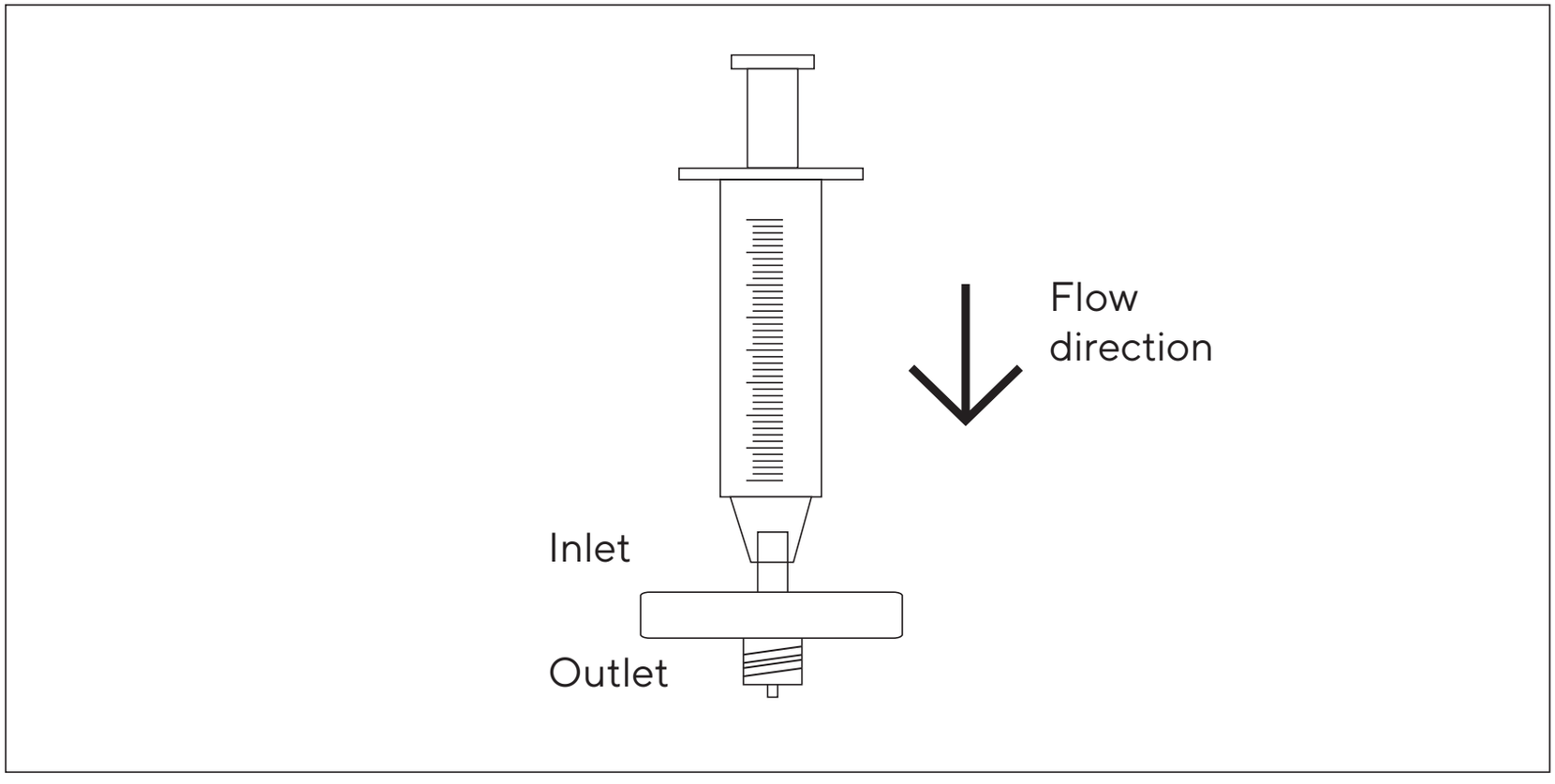

Fig. 1: Flow direction of buffer

7 Operation

7.1 Venting

It is important to remove air from the unit completely.

Fill a 10 – 20 mL Luer syringe with equilibration buffer and connect to Luer female inlet, then hold unit upright (outlet is up) and expel air until the first fluid is seen at the outlet. If you still detect any air in the filled unit, close it at the outlet with enclosed cap, flip the syringe and the MA so that the syringe is on top and the unit is on the bottom, hold the syringe up and move the plunger a few strokes up and down that air bubbles can ascend into the syringe. Very small air bubbles observed directly below the inlet do not disturb separations. The unit will function normally as long as the small air bubbles remain outside of the membrane bed.

7.2 Recommended buffer volumes and flow rates

Membranes are dried from glycerol to avoid shrinking. For pre-washing with e.g. equilibration buffer or 1 M NaCl the following flushing volumes are recommended. The range of the recommended flow rate is 5 – 30 membrane volumes per minute.

Tab. 1: Buffer consumption of Sartobind MA units [mL]

	MA 15	MA 75	MA 100
Equilibration	10	15	20
Wash	10 – 20	10 – 20	10 – 20
Elution	5 – 20	5 – 20	5 – 20
Regeneration	5 – 10	10 – 20	10 – 20

Tab. 2: Flow rate recommendation (5 – 30 membrane volumes/min)

	MA 15	MA 75	MA 100
Recommended flow rate [mL/min]	2 – 12	10 – 63	14 – 84

7.3 Buffer conditions

In the majority of applications an equilibration buffer concentration of 10 mM provides sufficient buffering capacity and prevents the protein of interest from precipitation. The ionic strength should be kept as low as possible to avoid reduction of binding capacity. The buffer should have a pKa within 0.5 pH units of the pH used. It should be filtered with 0.2 µm filters before use and the quality of water and chemicals should be of high purity.

⚠ **Do not apply pure water through the units as it may lead to a reversible swelling and decrease of the flow rate of the membrane. The buffering ion should carry the same charge as the ion exchange ligand.**

Binding | equilibration buffer examples	
To bind BSA on Sartobind Q	20 mM Tris | HCl pH 7.5
To bind BSA on Sartobind D	20 mM Bis Tris pH 6.0
To bind lysozyme on Sartobind S	10 mM Potassium phosphate buffer pH 7.0

7.4 Selection of pH conditions

In ion exchange chromatography a charged molecule is bound to oppositely charged groups attached to the insoluble matrix. This binding is reversible by application of salt ions to the elution buffer. The pH value at which a biomolecule has no net charge is the isoelectric point (pI). In buffers below the pI (at least 1 pH unit), a protein, for example, carries a positive net charge and will bind to a cation exchanger (Sartobind S). In buffers above its pI (at least 1 pH unit), it will bind to an anion exchanger (Sartobind Q or D).

7.5 Contaminant removal from proteins in flow through operation

For contaminant removal from proteins such as monoclonal antibodies, pH conditions in the range of pH 6 to 8 are used in order to bind highly negatively charged DNA, endotoxins, contaminating proteins, some host cell proteins and viruses. The product of interest, the monoclonal antibody with pI of 8 – 9.5 for example, will not bind and pass through Sartobind Q or D.

To remove contaminating proteins and aggregates with Sartobind S in flow through mode, process impurities have to be charged positively to bind while the target protein stays negative. At the pH of the buffer above the pI, the protein product flows through without binding.

7.6 Sample preparation and equilibration

The sample should be adjusted to the starting buffer and be prefiltered through a 0.2 µm membrane filter (not included). For small volumes in the mL range use a Minisart® with Luer outlet (order no. 16534-K).

The Minisart® shall be coupled directly to inlet of the MA unit during use as shown in Fig. 2 to avoid clogging of the adsorber. This is recommended also for usage of MA units with LC systems.

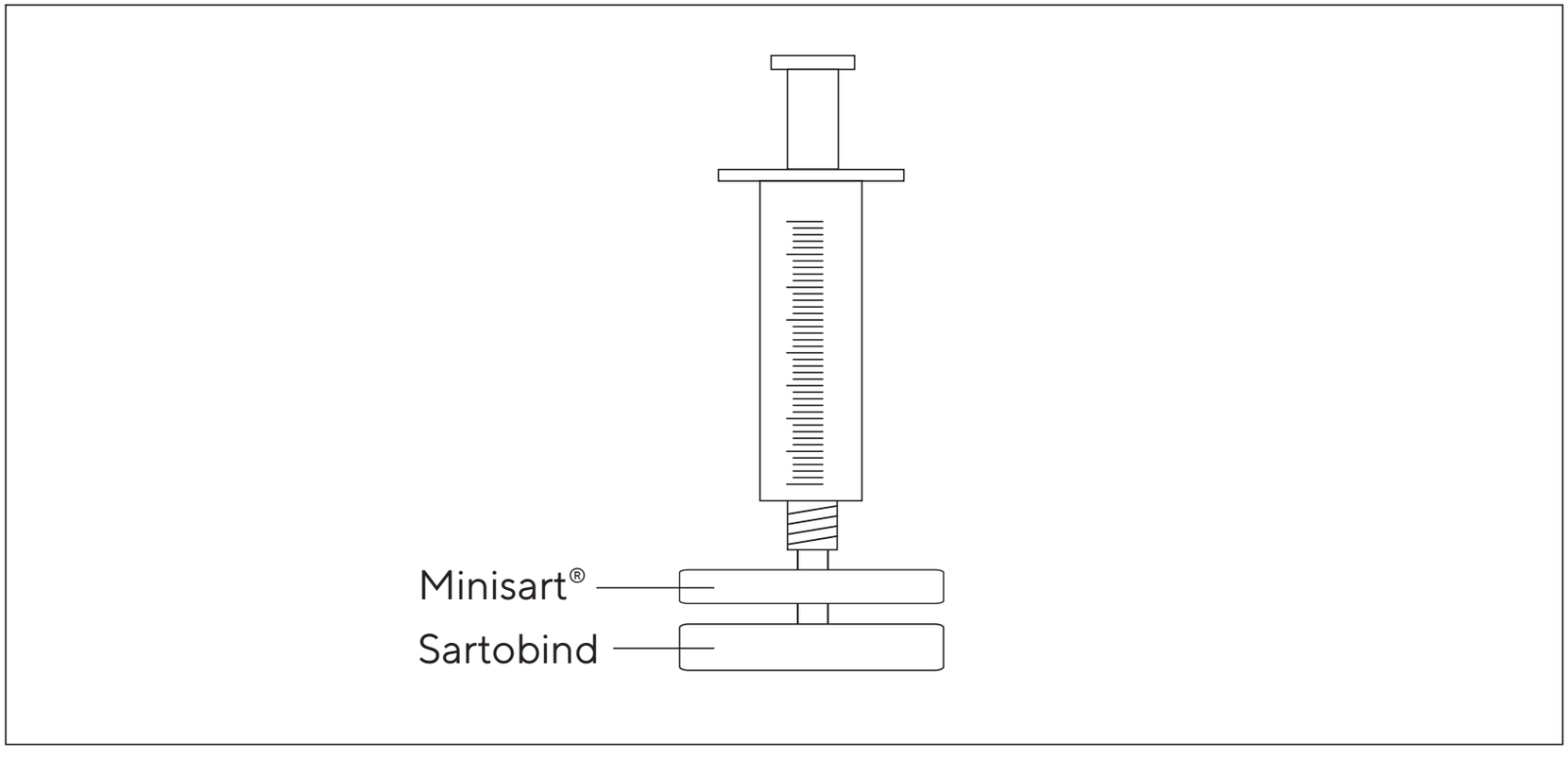

Fig. 2: Inline filtration with a Minisart® coupled to the inlet of Sartobind MA unit

⚠ **Unfiltered feed will block the Membrane Adsorber and lead to capacity loss and increased back pressure. We recommend inline filtering during operation. With increase of pressure replace filter and restart.**

7.7 Washing

When using MA units in bind and elute mode, wash with equilibration buffer (see Tab. 1 and 2) after the sample has been loaded.

7.8 Elution

Elute the target protein with elution buffer (Tab. 1 and 2), with increased salt concentration or shifted pH.

Elution buffer examples	
To elute BSA from Sartobind Q or D	1 M NaCl in binding buffer
To elute lysozyme from Sartobind S	1 M NaCl in binding buffer

7.9 Regeneration, cleaning and storage

After elution, wash the unit with equilibration buffer. If necessary, use 1 N NaOH, 1 N HCl or 70% ethanol for 1 hour and store in 20% ethanol in equilibration buffer. Do not store units in water. The MA 15, 75 or 100 can be cleaned in place directly after unpacking with 1 N NaOH, 1 hour at 20°C.

Example for cleaning of a Sartobind MA 75, bed volume 2.1 mL

1. 1 N NaOH at the flow rate of 1 bed volume (BV)/min at 20°C for 1 h (in total ~130 mL)
2. 10 BV (~20 mL) 1 M NaCl
3. 10 BV (~20 mL) equilibration buffer

7.10 Stability

The MA 15, 75 and 100 units are stable against all commonly used buffers in chromatography, e.g. 8 M urea, 8 M guanidine hydrochloride, ethanol and acetone. They can be sanitized after prewashing with 1 N NaOH, 1 hour at room temperature (20°C). Do **not** use oxidizing agents such as hypochlorite or H_2O_2.

pH Stability

	Q	S	D
Short term*	2 – 14	3 – 14	2 – 14
Long term**	2 – 12	4 – 13	4 – 13

* Short term refers to cleaning in place and regeneration procedures during operation of units.

** Long term: Storage of units e.g. overnight and longer. Preferably store units in 20% ethanol in buffer within pH limits.

7.11 Operation with peristaltic pump or LC system

After the unit is filled completely with equilibration buffer, close the outlet of the MA 15, 75 or 100 and remove the syringe. Start your LC system or peristaltic pump at a low flow rate. When fluid emerges, stop the pump and connect the tubing to the inlet of the unit. Make sure that no air is introduced. Remove the cap from outlet. Run the pump until fluid emerges from the outlet of the unit and stop it. Then connect the outlet of the unit via Luer adapter to the LC detector and proceed with loading. If your system pressure is too high, refer to your LC system manual to remove any flow restrictor after the UV cell, as the system may generate a pressure above the allowed maximum pressure. As Membrane Adsorbers run typically at much higher flow rates than columns, there is no risk of bubble formation in the UV cell when removing the restrictor.

8 Troubleshooting

Problem	Possible cause	Action
High back pressure during sample loading.	Material has not been filtered.	Prefilter with 0.2 µm filter before processing through the unit.
	Material has been filtered but was stored before purification.	Proteins can form aggregates within hours or during operation. Thus we recommend to prefilter inline by attaching a 0.2 µm filter in front of the adsorber. When you observe again pressure built up, replace the filter.
	LC system generates high pressure.	Remove restrictor after the UV cell.
	The MA 15, 75 or 100 adsorber is clogged.	Perform a regeneration cycle.

Problem	Possible cause	Action
Target molecule is not bound.	Conditions for binding are insufficient.	Decrease conductivity, control other process parameters as type of buffer and pH.
	Proteins or contaminants are still bound from last cycle.	Perform a regeneration cycle.
Binding capacity decreases after several uses.	Improper filtration	Prefilter with 0.2 µm filter before processing through the unit.
	Some molecule species binds tightly	The binding capacity of the unit depends on the nature of sample and sample preparation, prefiltration as well as proper regeneration and application. Tightly bound molecules may be removable by specific enzymes, or use unit only once.
	Proteins or contaminants are still bound from last cycle	Run a 1 M NaCl buffer step to elute tightly bound proteins quantitatively. Then regenerate adsorber by loading with 1 N NaOH and keep it for 1 hour at room temperature (20°C).

Problem	Possible cause	Action
Binding capacity decreases after several uses.	Wrong storage	Do **not** store in sodium hydroxide containing buffers. Store long term* in 20% ethanol-buffer (e.g. equilibration buffer) solution and do **not** use oxidative chemicals in buffers.
Early break-through of protein.	Binding capacity is not sufficient.	Use larger adsorber device, or connect two adsorbers (same size) in series (i.e. connect outlet of first adsorber to inlet of second) to achieve higher binding capacity. As a rule of thumb the pressure doubles when the flow rate is kept constant and the number of membrane layers is doubled. We do not recommend to run two adsorbers in parallel.

* Long term storage is defined as storage of units when not in operation, e.g. storage overnight and longer.

9 Quality Assurance

Sartobind membranes have been tested for protein binding capacity and flow rate.

Sartobind MA units are manufactured in a controlled environment and have been tested for protein binding capacity and flow rate.

The product meets all Sartorius Stedim Biotech standards for traceability, production and specifications as given here or exceeded them.

10 Ordering Information

10.1 Sartobind MA units (reusable)

Order number	Description	Quantity
93IEXQ42GB-12--A	Sartobind Q 15, Luer female and male connectors	4
93IEXS42GB-12--A	Sartobind S 15 Luer female and male connectors	4
93IEXQ42DB-12--V	Sartobind Q 75, Luer female and male connectors	2
93IEXS42DB-12--V	Sartobind S 75, Luer female and male connectors	2
93IEXD42DB-12--V	Sartobind D 75, Luer female and male connectors	2
93IEXQ42BC-12	Sartobind Q 100, Luer female and male connectors	1
93IEXS42BC-12	Sartobind S 100, Luer female and male connectors	1

10.2 Accessories

Order number	Description	Quantity
1ZA- - -0004	Luer male adapter to UNF 10 – 32 female, PEEK	1
1ZA- - -0005	Luer female adapter to UNF 10 – 32 female, PEEK	1
17002---140	2 × M6 female to female Luer and 2 × M6 female to male Luer Lock Adapter, black Tefzel	1

11 Dimensions and Connections

	MA 15	MA 75	MA 100
			
Membrane Area	15 cm^2	75 cm^2	100 cm^2
Membrane volume	0.41 mL	2.1 mL	2.8 mL
Dimensions (height × diameter)	25 × 36 mm	28 × 36 mm	31 × 66 mm
Frontal surface area	5 cm^2	5 cm^2	10 cm^2
Connector inlet		Luer female	
Connector outlet		Luer male	
Approximate weight	9 g	10 g	35 g

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The information and figures contained in these instructions correspond to the version date specified below.
Sartorius reserves the right to make changes to the technology, features, specifications and design of the equipment without notice.
Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote the other gender as well.

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93IEXD42DB-12–V
93IEXQ42BC-12
93IEXQ42DB-12–V
93IEXQ42GB-12–A
93IEXS42BC-12
93IEXS42DB-12–V
93IEXS42GB-12–A