Sartobind® Membrane Adsorbers A Separation Technology Based on Microporous Membrane Ion Exchangers

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

Sartorius has attached various functional groups covalently to the inner surface of synthetic microporous membranes. Pressure filtration forces the liquid through the micropores of the membrane, bringing target substances into direct contact with the binding sites. This direct convection to the binding sites minimizes diffusion limitation of mass transfer without sacrificing capacity. The micro-porous membrane ion exchangers, which are available, include the following benefits:

- O High dynamic binding capacity at high flow rates
- O Ready-to-use units
- O Rapid screening for scale-up
- O Easy handling
- O Reusable units can be utilized with syringes, peristaltic pumps or with existing workstations

1. Application Areas for Membrane Adsorbers (MA)

1.1. The membrane ion exchanger can be used for the ultrarapid determination of the optimal binding and elution conditions (pH range and ionic strength) of biomolecules.

1.2. Ultrarapid concentration of proteins from highly dilute solutions:
Depending on the protein concentration of the initial solution, up to several hundred-fold concentrations can be achieved within minutes.

1.3. Removal of contaminants
Contaminants (e.g. endotoxin, DNA-fragments) can be drastically reduced from target substances with Q membrane.

1.4. Biomolecule separation
from a mixture (e.g. proteins, peptides, nucleotides, DNA, plasmids, vituses)

a) By choosing the appropriate conditions, the target substance is selectively bound whereas contaminants pass through the membrane.
b) By choosing the appropriate conditions, contaminants are retained whereas the target substance passes through the membrane ("negative adsorbent").
c) Bound biomolecules can be separated by step or by gradient elution.

1.5 On account of the high speed provided by Sartorius membrane ion exchangers for purification processes, they are ideal for purification of labile proteins.

2. Functional groups

Sulfonic acid (S) $R\text{-}CH_2\text{-}SO_3^-$	Strongly acidic cation exchanger
Quaternary ammonium (Q) $R\text{-}CH_2\text{-}N^+\text{-}(CH_3)_3$	Strongly basic anion exchanger
Carboxyl (C) $R\text{-}COO^-$	Weakly acidic cation exchanger
Diethylamine (D) $R\text{-}CH_2\text{-}N\text{-}(C_2H_5)_2$	Weakly basic anion exchanger

All MA-types passed the USP Cytotoxicity test with MRC-5 and the USP Plastic Class VI test.

3. Technical Specifications

	MA15[1]	MA100[1]
Membrane material	Supported cross-linked regenerated Cellulose	Supported cross-linked regenerated Cellulose
Effective adsorption area[2]	15 cm^2	100 cm^2
Minimum protein binding capacity (depending on conditions)	S15: 12 mg Lysozyme C15: 9 mg Lysozyme Q15: 12 mg BSA D15: 9 mg BSA	S100: 80 mg Lysozyme C100: 60 mg Lysozyme Q100: 80 mg BSA D100: 60 mg BSA
Recovery	90–100%	90–100%
Flow rate	> 50 ml/min. unit. bar	> 75 ml/min. unit. bar
Housing material	Polysulfone	Polysulfone
Connector inlet	Female luer lock	Female luer lock
Connector outlet	Male luer lock	Male luer lock
Max. operating pressure	105 psi (7 bar)	90 psi (6 bar)
Regeneration	0.2 N NaOH, 1 h with 1 N NaOH or 1 N HCl or 70% ethanol	
Stability	pH 2–13	
Unstable	organic solvents (except lower alcohols); oxidative conditions (hypochlorite, H_2O_2)	
Storage	non-used units dry at room temperature; already used units wet in buffer, 20% ethanol in 1 M KCl or saline solution with bacteriostatic agents **Do not store units in water pure**	

[1] non-sterile
[2] 36.4 cm^2 ≙ 1 ml membrane

4. Operating procedure

The new membrane ion exchangers can be utilized with syringes, peristaltic pumps or existing workstations. When using syringes or peristaltic pumps follow steps 4.1 to 4.7. When using existing workstations follow steps 4.1 to 5.0.

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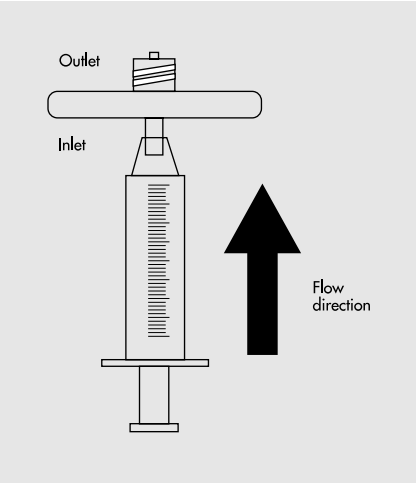
4.1. Equilibration

Equilibration buffer: Use buffer with an ionic strength that is as low as possible, so that the stability of the target protein is maintained. The pH of the equilibration buffer should be 1–2 units above the isoelectric point of the target protein for the Q- and D-type and 1–2 units below the isoelectric point for the S- and C-type.
Using a syringe, rinse approx. 10 ml (for MA15-units) or 30 ml (for MA100-units) of equilibration buffer. Rinse the buffer in an upward direction by applying moderate pressure to the syringe plunger (see picture). Avoid bubbles by pumping the plunger of the syringe a few times.



4.2. Loading

If the solutions contain particles, it is advisable to prefilter through a Minisart order no. 175 97. This type of Minisart, designed for single-use only, is surfactant-free, has negligible protein adsorption, and a pore size of 0.2 µm. Following prefiltration it is possible to establish the maximum binding capacity of the unit for the target protein. Using a syringe or a peristaltic pump, filter the loading solution at a flow rate of 10–50 ml/min for MA15-units and 30–200 ml/min for MA100-units. Continue sample filtration until breakthrough of target protein is detected in the filtrate.

4.3. Washing

To remove nonbound proteins pass sufficient buffer e.g. 10–20 ml (MA15-units) or 20–30 ml (MA100-units) of equilibration buffer through the unit by using a syringe or peristaltic pump until the effuent is free of protein.

4.4. Elution

To elute the target protein(s), filter 3–10 ml (MA15-units) or 20–40 ml (MA100-units) elution solution (i.e. increased salt concentration or pH-shift).

4.5. Regeneration

After elution, equilibrate the unit with equilibration buffer. Remove impurities, such as precipitated proteins or adsorbed substances, from the membrane by filtering 5–10 ml (MA15-units) or 20–30 ml (MA100-units) 0.2 N NaOH through it. If necessary, use for 1 hour 1 N NaOH or 1 N HCl or 70% ethanol. Afterwards, equilibrate the unit with equilibration buffer.

4.6. Storage

Store non-used units dry at room temperature. Units already used should be filled with 20% ethanol in 1 M KCl or saline solution with bacteriostatic agents, such as 0.002% chlorohexidine for anion (D, Q), or 0.5% chloretone for cation exchangers (S; C). **Do not store units in water**, use buffer or saline solution. Do not freeze the units.

4.7. Stability

The units are stable in a pH range 2–13. They are not compatible with organic solvents, except lower alcohols. Do not use oxidative conditions, such as hypochlorite or H_2O_2.

5. Use of Peristaltic Pumps or Workstations, such as FPLC® or Biopilot® Systems

For the operation of membrane adsorbers units with workstations, such as FPLC or Biopilot systems, specific connectors can be ordered. To properly vent the membrane adsorber unit attach a syringe filled with starting buffer to the outlet side of the unit using a short piece of tubing.

Flush the unit with the inlet side facing upwards until all air has been removed. Connect the inlet side to the workstation, remove the syringe and vent the outlet side. Connect the outlet to the workstation and proceed as described in steps 4.2 to 4.7.

6. Order Numbers

Membrane Adsorber-units (reusable*)	
S15X	Pack of 2 strongly acidic cation exchangers
Q15X	Pack of 2 strongly basic anion exchangers
C15X	Pack of 2 weakly acidic cation exchangers
D15X	Pack of 2 weakly basic anion exchangers
SQ15X	Pack of 1 strongly acidic cation exchanger and 1 strongly basic anion exchanger
CD15X	Pack of 1 weakly acidic cation exchanger and 1 weakly basic anion exchanger
S100X	Pack of 1 strongly acidic cation exchanger
Q100X	Pack of 1 strongly basic anion exchanger
C100X	Pack of 1 weakly acidic cation exchanger
D100X	Pack of 1 weakly basic anion exchanger

All packages include 1 Minisart syringe filter 0.2 µm for prefiltration and caps.*

* All MA-types are also available with 5 cm^2 effective adsorption area for disposable use.

Membrane Adsorber-units (disposable)	
S5F	Pack of 15 strongly acidic cation exchangers
Q5F	Pack of 15 strongly basic anion exchangers
D5F	Pack of 15 weakly basic anion exchangers
C5F	Pack of 15 weakly acidic cation exchangers

Accessories

Code	Quantity/ Package	Order Number
Minisart, surfactant free 0.2 µm, presterilzed	50	175 97K

For more information about applications, scale-up, connectors for workstations or other membrane ion exchanger types please contact your nearest Sartorius office or visit our web-page: http://www.sartorius.com.

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