sartorius stedim biotech

**Operating Instructions**

# Sartobind® IEX SingleSep

pico 0.08 ml | nano 1 ml | mini 7 ml | 5" 70 ml | 10" 180 ml
20" 360 ml | 30" 540 ml | mega 1.62 l

A Separation Technology Based on
Macroporous Membranes,
4 mm Bed Height

85032-541-33

Read operational instructions carefully before using Sartobind capsules.

⚠ **Important**
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. The capsules are supplied as non-sterile. The membrane is dried from glycerol.

**Intended use**
The products are intended and validated for single use to avoid carryover as well as tedious and costly cleaning validation procedure.

**Sartobind pico 0.08 ml** is used for process development when smallest sample quantities are available only.

**Sartobind IEX nano 1 ml and mini 7 ml** have been developed for working with small sample volumes while retaining the cylindrical design of large scale Membrane Adsorber. They are perfect for small scale applications, and also for screening purposes and laboratory-scale flowthrough purifications.

**Sartobind IEX 5", 10", 20", 30" and mega** have been developed for intermediate and pilot scale up to production scale in the biopharmaceutical industry.

# Table of Contents

# 1. Storage conditions

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

# 2. Introduction

Sartobind capsules are ion exchange chromatography devices based on macroporous membranes. They can be used in the downstream processing of proteins for single-use chromatography. The ion exchange ligands are coupled to a membrane which is fitted into a plastic housing for quick handling, making ion exchange purification nearly as easy as filtration.

Eight sizes of capsules with a strong basic and six sizes with a strong acidic ion exchange Membrane Adsorber (MA) are available. They can be applied for contaminant removal from proteins in flowthrough mode (negative chromatography) to bind DNA, residual protein, host cell proteins, endotoxins and viruses.

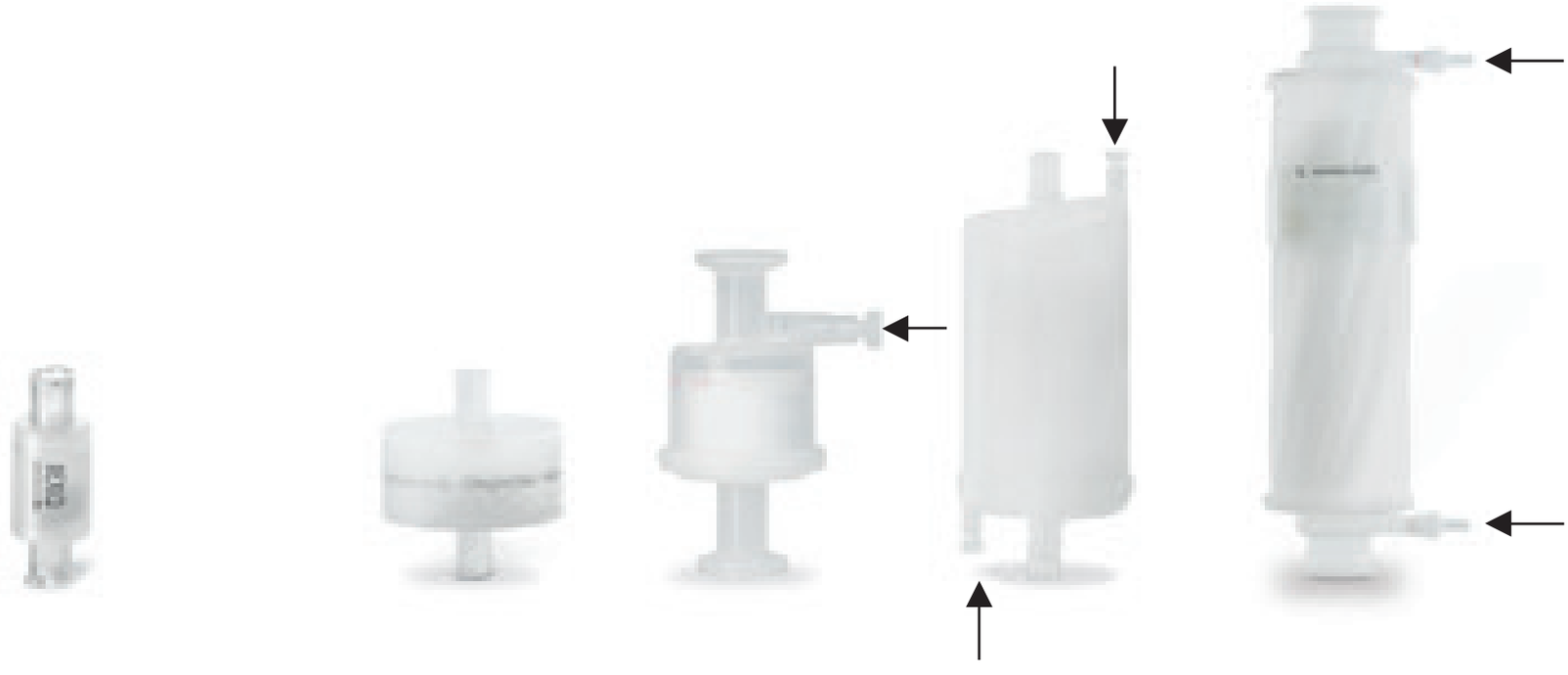


Fig. 1:
Sartobind pico 0.08 ml

Fig. 2:
Sartobind IEX nano, mini, 5” and 10” capsules. The arrows indicate the vent valves. For 10”, 20” and 30” an optional stainless steel holder is available (see fig. 3).

Fig. 3: 30 inch capsule on stainless steel holder (accessory)

Fig. 4: Mega with stainless steel legs (accessory)

# 3. Technical Data

| | |
|---|---|
| Bed height (all capsules) | 4 mm |
| Membrane volume \| area pico capsule | 0.08 ml \| 2.9 $cm^2$ |
| Membrane volume \| area nano capsule | 1 ml \| 36 $cm^2$ |
| Membrane volume \| area mini capsule | 7 ml \| 250 $cm^2$ |
| Membrane volume \| area 5” capsule | 70 ml \| 0.25 $m^2$ |
| Membrane volume \| area 10” capsule | 180 ml \| 0.66 $m^2$ |
| Membrane volume \| area 20” capsule | 360 ml \| 1.3 $m^2$ |
| Membrane volume \| area 30” capsule | 540 ml \| 2 $m^2$ |
| Membrane volume \| area mega capsule | 1620 ml \| 6 $m^2$ |
| Typical dynamic binding capacity*<br>10% for bovine serum albumin on Q<br>for lysozyme on S | <br>0.8 mg/$cm^2$<br>0.7 mg/$cm^2$ |

| | |
|---|---|
| Ion capacity per $cm^2$ of membranes | 2–5 µeq |
| Pico | 6 bar (0.6 MPa, 88 psi) |
| Nano to 30” units | 4 bar (0.4 MPa, 58 psi) |
| Mega | 3 bar (0.3 MPa, 44 psi) |
| Maximum pressure during venting | 0.5 bar, 0.05 MPa, 7 psi |
| Short term** pH stability Q \| S | 2–14 \| 3–14 |

* See also section 5. binding capacity

** Short term refers to cleaning in place and regeneration procedures during operation of units, e.g. with 1 N NaOH or 0.1 N HCl for 1 hour

# 4. Materials

| | |
|---|---|
| Housing | Polypropylene |
| Membrane matrix | Stabilized reinforced cellulose, nominal pore size >3 µm |
| Ion exchange ligand Sartobind Q | Strong basic anion exchanger: quaternary ammonium ($R\text{-}CH_2\text{–}N^+(CH_3)_3$) |
| Ion exchange ligand Sartobind S | Strong acidic cation exchanger:<br>– Sulfonic acid ($R\text{-}CH_2\text{–}SO_3^-$) |
| Gaskets in valves | – Silicone in 5” up to mega sizes<br>– Polytetrafluoroethylene (PTFE), only mini size |

# 5. Binding capacity

Data are based on dynamic binding capacity measurements 10% using 3 layers of 5 $cm^2$ membrane discs (15 $cm^2$ total area) arranged in a holder and run at 10 ml/min.

| | 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 |

| Unit | Area | Typical dynamic binding capacity 10% |
|---|---|---|
| Sartobind Q SingleSep pico 0.08 ml | 2.9 $cm^2$ | 2.3 mg |
| Sartobind S SingleSep pico 0.08 ml | 2.9 $cm^2$ | 2.0 mg |
| Sartobind Q SingleSep nano 1 ml | 36 $cm^2$ | 29 mg |
| Sartobind S SingleSep nano 1 ml | 36 $cm^2$ | 25 mg |
| Sartobind Q SingleSep mini 7 ml | 250 $cm^2$ | 200 mg |
| Sartobind S SingleSep mini 7 ml | 250 $cm^2$ | 175 mg |
| Sartobind Q SingleSep 5” 70 ml | 2500 $cm^2$ | 2.0 g |
| Sartobind S SingleSep 5” 70 ml | 2500 $cm^2$ | 1.75 g |
| Sartobind Q SingleSep 10” 180 ml | 6600 $cm^2$ | 5.3 g |
| Sartobind S SingleSep 10” 180 ml | 6600 $cm^2$ | 4.6 g |
| Sartobind Q SingleSep 20” 360 ml | 1.3 $m^2$ | 10.6 g |
| Sartobind Q SingleSep 30” 540 ml | 2 $m^2$ | 16 g |
| Sartobind S SingleSep 30” 540 ml | 2 $m^2$ | 14 g |
| Sartobind Q SingleSep mega 1.62 l | 6 $m^2$ | 48 g |

# 6. Installation

The contents of the package are described in chapter 11.1. When unpacking capsules, protect inlet and outlet connectors from damage. Lay down the mega capsule first and connect stainless steel legs before set up. Never keep or place capsules on connectors. Store items securely and never in upright position. The mega carries protective caps on inlet, outlet and vent valves. Remove before venting.

The Sartobind SingleSep capsules should be installed in an upright position in the process flow as indicated by an arrow (pico, nano, mini, 10”, 20”, 30” or mega) or IN and OUT signs (5”) on the capsule. In this position the inlet is up. See also Chapter 11.
For the 10, 20 or 30 inch capsules, stainless steel holders for one (see figure 1) or three capsules are available (see chapter 10. Accessories). Stainless steel legs are available for the mega (see figure 2).

Flow direction
In Sartobind pico the flow is from top through 4 mm membrane bed to the outlet.

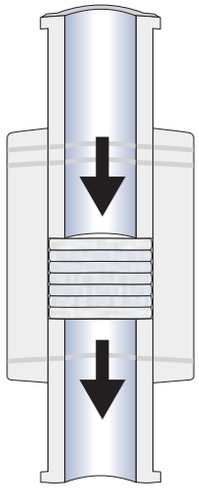


Fig. 5: Flow pattern inside Sartobind pico capsule

The flow pattern inside the capsule is from outside of the membrane cylinder through the membrane bed to the inside and to outlet.

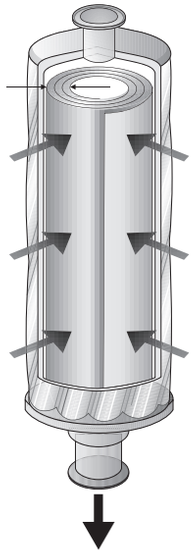


Fig. 6: Flow direction inside the nano to mega capsules

⚠ **Important**
Capsules should be visually inspected before use. In case of damage, the capsule has to be replaced. Close vent valves before use by screwing the valve in clockwise.

# 7. Operation

## 7.1 Venting

It is important to remove air from the unit completely. For the nano and pico fill a 10–20 ml Luer syringe with equilibration buffer and connect to the capsule. Hold capsule upright (outlet is up) and expel air as shown in Fig. 7. If you still detect any air in the filled unit, close the outlet, hold the syringe up and move the plunger slightly up and down that air bubbles can ascend into the syringe.

Very small air bubbles observed directly below the inlet of the nano do not disturb separations. The capsule will function normally as long as the small air bubbles remain outside of the membrane bed.

For the pico removal of air is critical. Expel air as in Fig. 7 and connect syringe at outlet and purge again. The 5, 10, 20, 30 inch and mega capsules carry vent valves. Before opening the vent valve, please connect the valves by flexible tubes (inner diameter 6 mm) to waste. During venting please do not exceed 0.05 MPa pressure as the vent valve O-ring could change its position which will result in insufficient closing of the valve. For appropriate venting, open the vent valve screw 1/3 turn to left until all air is replaced by fluid.

To vent the 5 inch capsules use paper tissue to absorb spilled fluid when opening. The vent valve of the mini capsules contain a hydrophobic membrane to prevent the passage of fluid.

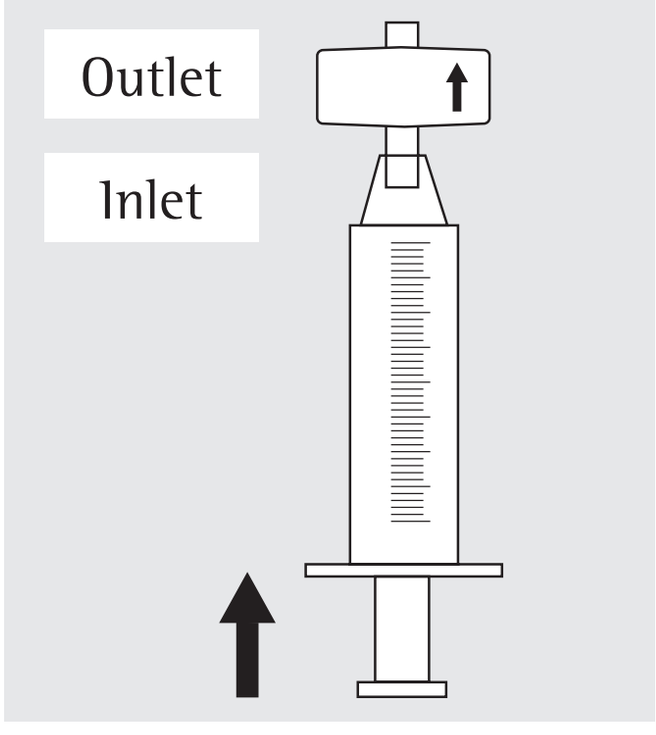



Fig. 7: Filling the Sartobind nano with a Luer syringe

## 7.2 Cleaning and preconditioning

The modules can be cleaned in place directly after unpacking with 1 N NaOH, 1 hour at 20°C.

Example: Cleaning a 10 inch capsule with 180 ml bed volume (BV)
1. 1 N NaOH at a flow rate of 1 BV/min for 1 h (in total 10.8 l)
2. 10 BV 1 N NaCl (in total 1.8 l)
3. 10 BV equilibration buffer (in total 1.8 l)

Specifically for the pico a sufficient flushing with equilibration buffer is required to stabilize the pH value. Due to the void volume of the FPLC system, which is much larger than the bed volume of the pico device, NaOH residue could lead to a pH shift. In that case more flushing volume after a sanitization is needed.

## 7.3 Autoclaving

The capsules can be autoclaved once at 121°C for 30 minutes at 1 bar (0.1 MPa | 14.5 psi). Prewet the capsule with equilibration buffer. Do not use pure water. Close valves immediately after sterilisation. For autoclaving Sartobind mega refer to separate autoclaving instructions enclosed in delivery.

## 7.4 Recommended buffer volumes and flow rates

Membranes are dried from 20% glycerol to avoid shrinking.
For pre-washing with e.g. equilibration buffer or buffer plus 1 M NaCl the following flushing volumes are recommended

| Capsule Size | Flushing Volume (l) |
|---|---|
| pico | 0.004 |
| Nano | 0.01 |
| Mini | 0.25 |
| 5” | 2.5 |
| 10” | 5.0 |
| 20” | 10 |
| 30” | 15 |
| Mega | 25 |

**⚠ Important**

The minimum buffer volume for equilibration is the same as the flushing volume. Check pH for small scale devices (especially pico). For pico, the flushing volume is  proportionally higher to enable thorough flushing when using with a LC system.

| Capsule Size | Recommended Flow Rates (l/min) |
|---|---|
| pico | 0.0024 |
| Nano | 0.03 |
| Mini | 0.2 |
| 5” | 1.9 |
| 10” | 5.0 |
| 20” | 10 |
| 30” | 15 |
| Mega | 50 |

Lower flow rates than recommended can also be used.
As a rule of thumb 30 bed volumes per minute flow rate are recommended.

## 7.5 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.

### ⚠ Important

Do not apply pure water 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. The minimum buffer volume for equilibration is the same as the flushing volume (see 7.2).

## 7.6 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 buffer eluting the molecule. The pH value at which a biomolecule has no net charge is the isoelectric point: pI. Below the isoelectric point (rule of the thumb at least 1 pH unit) a protein for example carries a positive net charge and will bind to a cation exchanger (Sartobind S). Above its isoelectric point (at least 1 pH unit) it will bind to an anion exchanger (Sartobind Q).

## 7.7 Contaminant removal from therapeutic proteins and other sources with Sartobind Q

For contaminant removal from products 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.

### 7.7.1 DNA removal

Working with a neutral pH buffer all DNA displays a highly negative charge due to the large number of phosphate groups present. The longer the DNA strains present in the sample the more they will bind strongly to the positively charged anion exchanger.

**Recommended conditions for protein solutions**

pH: 4–8
buffer containing 50 up to 800 mM NaCl
DNA binding capacity of Sartobind Q: ~5.6 mg/ml[1]
Device recommendation at start of experiments:
Sartobind Q SingleSep nano 1 ml
Flow rate: 30 membrane volumes/min

[1] Sartorius Stedim Biotech Application Note 85030-511-29 DNA Removal at the Process Scale Purification of Protein. Ion Exchange Chromatography with Sartobind® Membrane Adsorbers Sartorius Stedim Biotech Application note 85030-511-29

### Influence of pH
DNA removal can be achieved in a broad range of buffer pH.

### Influence of conductivity
Low conductivity supports the DNA removal process achieving log reduction values (LRVs) of up to 6. But also at higher salt concentrations (e.g. 0.8 M NaCl) a DNA reduction of 1 log can be achieved.

### Influence of flow rate
Very low. Use recommended flow rate whenever possible.
Lower flow rates do not affect the removal rate.

## 7.7.2 Host cell protein removal
During cell culture of chinese hamster ovary (CHO) cells the proteins present in the cell culture broth represent the proteom of this cell line with tens of thousands of proteins having different isoelectric points. A complete removal of host cell proteins with only anion exchange cannot be achieved. As described for most proteins, the isoelectric point is predominantly below 7. That is the reason why 70 to 80% of all proteins – including the host cell proteins – will be bound at a pH between 7 and 8.[2]

[2] Gianazza, E. and Righetti, P.G. J. Chromatography 193 (1980) 1-8

**Recommended conditions for protein solutions (e.g. monoclonal antibodies)**
pH: 6.5 – 8.5
Buffer with low conductivity: <50 mM NaCl
Host cell protein binding capacity: up to 40 mg/ml
Device recommendation at start of experiments:
Sartobind Q SingleSep nano 1 ml
Flow rate: 30 membrane volumes/min

**Influence of pH**
The pH of the buffer has a great influence on the binding of CHO proteins as a slight change towards alkaline pH can improve the binding capacity drastically. Below 6.5 the binding of host cell proteins drops 10–20% with each 0.5 pH step.

**Influence of conductivity**
Sartobind Q is a pure ion exchanger and conductivity conditions are very important and should be kept as low as possible. Conductivity is also dependent on temperature. Increased salt concentration or temperature will lead to stronger elution and will reduce the binding capacity quickly.
Elution conditions will start already with e.g. 50 mM NaCl.

**Influence of flow rate**
Very low. Use recommended flow rate whenever possible.
Lower flow rates can be used without any loss of performance.

### 7.7.3 Virus removal

Anion exchange chromatography is a method for the removal of adventitious and endogenous viruses during the downstream processing of pharmaceutical proteins. A number of model viruses behave similar to proteins and can be charged negatively to bind onto an anion exchanger. The removal performance is tested by spiking of DNA/RNA viruses into the protein solutions.

The binding depends strongly on binding conditions. For a sufficient removal of viruses, the binding conditions for the target protein have to be determined first to avoid binding of protein. Otherwise wrong conditions can lead to product loss and less virus safety. Ideal binding conditions are typically found out by running a number of bind and elute cycles at 10% breakthrough capacity while varying the pH (e.g. 6.5–8.0) and changing the conductivity (3.0–9.0 mS/cm).[3]

[3] Sartorius Stedim Biotech Application Note 85030-522-22 Virus Purification and Removal. Ion Exchange Chromatography with Sartobind Membrane Adsorbers

**Recommended conditions for protein solutions (non binding condition for target protein)**
pH of buffer: ~7 (or slightly below pI of target protein)
Conductivity: 1–10 mS/cm
Device recommendation at start of experiments:
Sartobind Q SingleSep nano 1 ml
Flow rate: 20–30 membrane volumes/min.
Lower flow rate does not affect the removal rate.

**Influence of pH**
Binding conditions for virus spikes in antibody solutions improve with higher pH (typically chosen around 7).

**Influence of conductivity**
Binding condition for viruses improve with decreasing conductivity (typically below 4 mS/cm).

**Influence of protein concentration**
Effective virus removal has been achieved with 10 kg protein loaded onto 1 liter membrane adsorber. The proteins flow through and the virus is bound.

**Influence of flow rate**
Very low. Use recommended flow rate whenever possible.

### 7.7.4 Endotoxin removal

Endotoxins are lipopolysaccharides from cell walls of gram negative bacteria and make up the majority of pyrogens in pharma products. They must be removed to typically <0.25 EU/ml.

0.1 ng = 1 EU/ml
Test: LAL (Limulus amoebocyte lysate) test is a clotting test achieved by mixing the lysate with the sample.
LAL is capable of detecting 8 picograms of endotoxin per ml which is equivalent to approximately 200 gram negative microorganisms/ml.

Endotoxins are present in a number of different forms of micelles and vesicles. They are negatively charged at pH 3–9.

**Recommended conditions for protein solutions**
pH: 0.5 to 1.0 pH unit below the isoelectric point of the target protein (flowthrough)
Buffer with low conductivity: up to 200 mM NaCl
Endotoxin removal can be achieved at 50,000 to 30,000 EU/ml: up to 1.5 mg endotoxin (15,000,000 EU) per ml Sartobind Q membrane
Device recommendation at start of experiments:
Sartobind Q SingleSep nano 1 ml
Flow rate: 30 membrane volumes/min or higher

**Influence of the endotoxin concentration**
Large influence due to the linear binding of endotoxins at increased concentrations. High endotoxin concentrations much above 50,000 EU/ml may lead to high binding but also to quick breakthrough. Very low endotoxin concentrations will lead to lower binding capacity on the matrix.

## Influence of buffer choice

There is a big influence of buffer choice. Measurements with phosphate buffers achieved log reduction value (LRV) of up to 3, with Tris/HCl buffers LRV of 5 was reached.[4]

## Influence of other charged products

Large influence if they bind to the matrix and decrease binding capacity for endotoxins. If there is any other contaminant negatively charged at the working pH, e.g. DNA, the binding of endotoxins will be reduced. If you observe protein binding you can reduce it by adding NaCl (e.g. 150 mM).

## Influence of pH

Relatively low influence as the charge of the endotoxins keeps always negative.

## Influence of conductivity

It is important to work under non binding conditions for proteins. Increase conductivity to up to 20 mS/cm if necessary.

## Influence of flow rate

Very low. Use recommended flow rate whenever possible.

[4] Sartorius Stedim Application Note Endotoxin Removal. 85030-531-53 Ion Exchange Chromatography with Sartobind® Membrane Adsorbers

## 7.8 Sample preparation

The sample should be adjusted to the starting buffer conditions and be prefiltered through a 0.2 µm membrane filter e.g. Sartobran P capsule (5231307H...). For small volumes in the ml range use a 0.2 µm Minisart filter with Luer Lok outlet (order no 16534-K).

### ⚠ Important

Unfiltered feed will block the Membrane Adsorber and lead to capacity loss and increased back pressure.

## 7.9 Stability

The capsules are stable against all commonly used buffers in chromatography. Avoid oxidizing agents. Discard after one use.

## 7.10 Operation of the Sartobind nano and mini capsules with peristaltic pumps or LC systems

After the unit is filled completely with equilibration buffer, close the outlet of the Sartobind nano and remove the syringe. Start your LC system or peristaltic pump at a low flow rate. When fluid emerges, stop the pump, connect the tubing to the inlet of the Sartobind nano. 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.

Operation of Sartobind mini is done accordingly. To connect it to a LC system connectors, UNF 10–32 to 25 mm sanitary (Order no.: 1ZAGV0003) are available as accessory.

## 7.11 Draining

You may drain the capsules by application of air or nitrogen pressure (<1 bar | 14.5 psi) to the inlet of the capsule.

## 7.12 Scaling up

Complete break through experiments for the target compound to be bound on the membrane matrix. After optimisation of binding conditions of the contaminants, the purification step can be scaled up to a larger capsule.

**Recommendations**

Maintain:

– Bed height (automatically kept constant when using SingleSep with 4 mm bed height)
– Linear flow (automatically kept constant when scaling with 4 mm bed height)
– Sample concentration

Increase (see scaling factors in the following table):

– Sample loading
– Volumetric flow rate
– Membrane volume

Scale up calculations are done preferably by the membrane volume as the calculation is most simple. Other methods for scale up via residence time will lead to same result. Using the Sartobind nano 1 ml the scale up factor for flow rate and binding capacity is equal to the membrane volumes of the target scale up device as listed:

| Device | Bed volume [ml] | Factor to increase flow rate (from nano) | Factor to increase binding capacity (from nano) |
|---|---|---|---|
| pico | 0.08 | – | – |
| Nano | 1 | 1 | 1 |
| Mini | 7 | 7 | 7 |
| 5 inch | 70 | 70 | 70 |
| 10 inch | 180 | 180 | 180 |
| 20 inch | 360 | 360 | 360 |
| 30 inch | 540 | 540 | 540 |
| Mega | 1620 | 1620 | 1620 |

Example: After breakthrough experiments with the nano you found 500 fold binding capacity is needed. You will choose the 30 inch capsule. Then increase flow rate by a factor of 540.

⚠ **Important**
Keep sample concentration constant in lab and production scale.
Watch out for volumes in the piping and flow rate in whole system.

When using the Sartobind pico for scale up calculations smallest deviation in flow rate or incorrectly given void volume of the FPLC System may have a severe impact on the scale up calculation.
Use the nano as intermediate scale up control.

# 8. Diffusion test of 10", 20", 30" and mega capsules

The integrity of the capsule can be controlled by a diffusion test. The testing procedure describes the diffusion test for pre and post use. The test is intended to discriminate between defective and intact capsules and to detect major bypasses, large holes and faulty assembly. Smaller capsules than 10” cannot be tested due to technical reasons.

## 8.1 Installation for test

Test procedure has been generated with current Sartocheck instrument family e.g. Sartocheck 4 Plus (26288) or 3 Plus (16290). Use of earlier Sartocheck instruments will generate faulty data. Install capsule as shown in Fig. 8.

Please note that the test procedure with other vendor’s integrity testers can require a different set up.

## 8.2 Operation of test

### 8.2.1 Pre-washing of capsule

Pre-wash with 30 membrane volumes (MV) of equilibration buffer at flow rate of 10 MV/min.

## ⚠ Important

The capsule needs to be pre-washed with the testing solvent, to remove any glycerol. The washing solution should be at room temperature. Keep the unit in an upright position for proper venting and open the vent screw on top of the unit until all air is replaced by testing solvent.

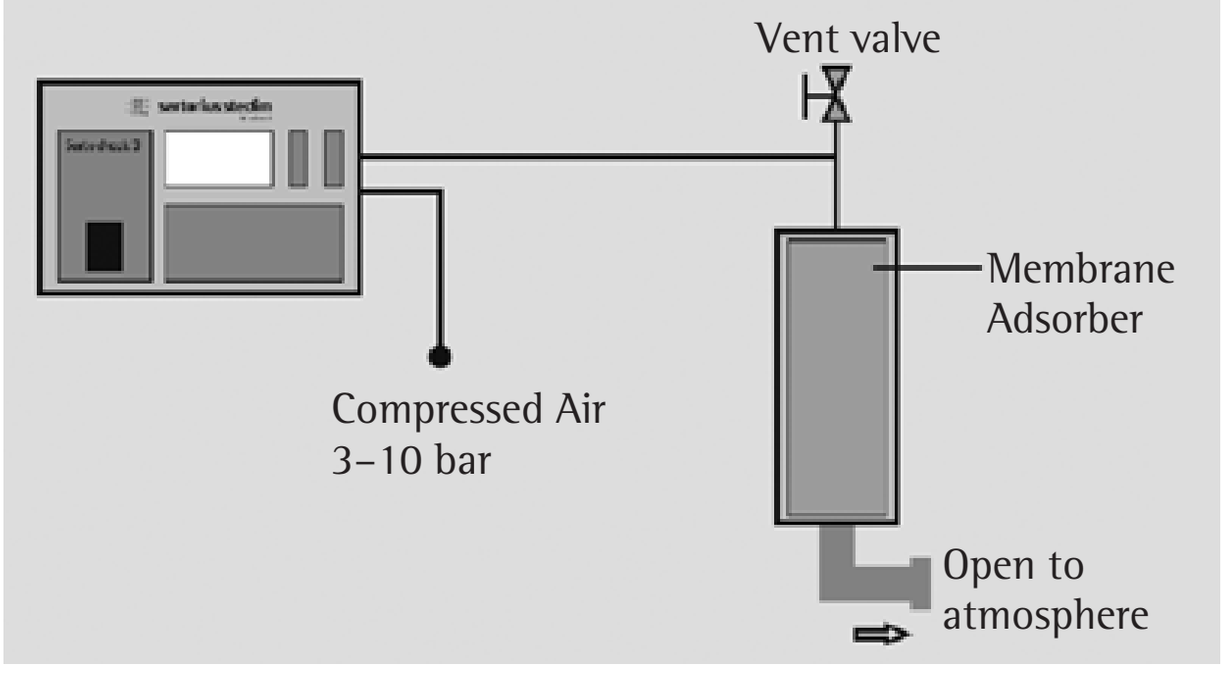



Fig. 8: Set up of diffusion test with Sartocheck 4 Plus or 3 Plus

### 8.2.2 Diffusion measurement with Sartocheck®

Choose PROGRAMMING in the main menu
Choose DIFFUSION TEST

Test parameters
Test pressure: 200 mbar (2.9 psi)
Stabilisation time: 5 min for mega, 3 min for other capsules
Testing time: 1 min
Diffusion max.: 15 ml/min
Net volume: 0
If net volume is set to zero, Sartocheck automatically measures the void volume.

### 8.2.3 Results and evaluation

– Diffusion < Diffusion max
Test passed (diffusion value on the print out)
– Diffusion > Diffusion max
Test failed (red text on the print out)

# 9. Troubleshooting

| Problem | Possible cause | Action |
|---|---|---|
| Break through data of Sartobind pico do not fit to larger capsules | LC pump delivers different flow rate than indicated or given void volume of the LC system is incorrect. | Control flow rate of chromatography pump with a graduated cylinder and correct the system to desired flow rate.<br>Check system void volume and enter the correct value. |
| Reuse is needed | Laboratory work is eased, economic or practical reasons | The major application of capsules is the single use and they are constructed in plastic housing for this. Also they are validated and certified only for one use.<br>Technically they can be reused. The durability of the unit depends on the nature of sample and sample preparation, prefiltration as well as proper regeneration and application. |

| Problem | Possible cause | Action |
|---|---|---|
| Air bubbles can be seen | Incomplete air removal | Small air bubbles seen in the top of the unit do not interfere with the purification as long as they do not touch the membrane bed. If too much air is enclosed, repeat removal as described in chapter 7.1 Venting. |
| I installed the capsule upside down | Installation of capsule may be easier in the process flow | Validation has been done with a process flow from top to bottom. Thus it is clearly recommended to use capsules (including pico device) in the described flow direction (Feed enters capsule on top and leaves on the bottom). |

| 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 (only for nano units). |

| Problem | Possible cause | Action |
|---|---|---|
| Target molecule is not bound | Conditions for binding are insufficient | Decrease salt concentration, control other process parameters as pH and keep temperature constant (ph change). |
| Binding capacity is not sufficient | Process optimization | 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. |

# 10. Quality assurance

This product is tested for protein dynamic binding capacity and flow rate. Sartobind membranes have been tested for protein dynamic binding capacity, flow rate, thickness, and charge density. Capsules and membranes are manufactured in a controlled environment. The product meets all Sartorius Stedim Biotech standards for traceability, production and specifications as given here or exceeded them as certified in the quality assurance certificate enclosed. The validation guide is available on request.

# 11. Ordering information

| Order number | Description and type of connectors | Quantity |
|---|---|---|
| Pico capsules | 0.08 ml, 2.9 $cm^2$ | |
| 92IEXQ42DD-11--D | Sartobind Q pico 0.08 ml<br>Luer female, 2 adapters Luer male | 10 |
| 92IEXS42DD-11--D | Sartobind S pico 0.08 ml<br>Luer female, 2 adapters Luer male | 10 |
| | Each pico package contains<br>2 × Adapter Luer male to<br>UNF 10 - 32 female, PEEK | |

| Order number | Description and type of connectors | Quantity |
|---|---|---|
| Nano capsules | 1 ml, 36 $cm^2$ | |
| 92IEXQ42DN-11 | Sartobind Q SingleSep nano capsule, Luer female | 1 |
| 92IEXQ42DN-11--A | Sartobind Q SingleSep nano capsule, Luer female | 4 |
| 92IEXS42DN-11 | Sartobind S SingleSep nano capsule, Luer female | 1 |
| 92IEXS42DN-11--A | Sartobind S SingleSep nano capsule, Luer female | 4 |
| | Each nano package contains 2 × Adapter Luer male to UNF 10 – 32 female, PEEK | |

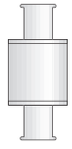
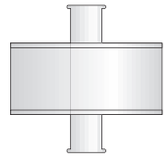
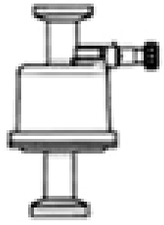
| Order number | Description and type of connectors | Quantity |
|---|---|---|
| Mini capsules | 7 ml, 250 $cm^2$ | |
| 92lEXQ42D4-SS--A | Sartobind Q SingleSep mini capsule, ¾” sanitary clamp | 4 |
| 92lEXQ42D4-OO--A | Sartobind Q SingleSep mini capsule, hose barb | 4 |
| 92lEXS42D4-SS--A | Sartobind S SingleSep mini capsule, ¾” sanitary clamp | 4 |
| 92lEXS42D4-OO--A | Sartobind S SingleSep mini capsule, hose barb | 4 |

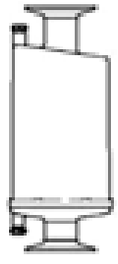
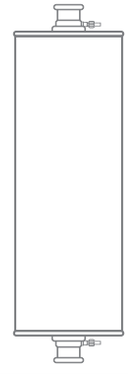
| Order number | Description and type of connectors | Quantity |
|---|---|---|
| 5” capsules | 70 ml, 2500 $cm^2$ | |
| 92IEXQ42D9-SS--A | Sartobind Q SingleSep 5” capsule, 1½” sanitary clamp | 4 |
| 92IEXQ42D9-OO--A | Sartobind Q SingleSep 5” capsule, hose barb | 4 |
| 92IEXS42D9-SS--A | Sartobind S SingleSep 5” capsule, 1½” sanitary clamp | 4 |
| 92IEXS42D9-OO--A | Sartobind S SingleSep 5” capsule, hose barb | 4 |
| 10” capsules | 180 ml, 6600 $cm^2$ | |
| 92IEXQ42D1-SS | Sartobind Q SingleSep 10” capsule, 1½” sanitary clamp | 1 |
| 92IEXS42D1-SS | Sartobind S SingleSep 10” capsule, 1½” sanitary clamp | 1 |
| 20” capsules | 360 ml, 1.3 $m^2$ | |
| 92IEXQ42D2-SS | Sartobind Q SingleSep 20” capsule, 1½” sanitary clamp | 1 |

| Order number | Description and type of connectors | Quantity |
|---|---|---|
| 30” capsules | 540 ml, 2 $m^2$ | |
| 92lEXQ42D3-SS | Sartobind Q SingleSep 30” capsule, 1½” sanitary clamp | 1 |
| 92lEXS42D3-SS | Sartobind S SingleSep 30” capsule, 1½” sanitary clamp | 1 |
| Mega capsule | 1620 ml, 6 $m^2$ | |
| q92lEXQ42DC3SS | Sartobind Q SingleSep mega capsule, 1½” sanitary clamp | 1 |
| | Sartobind pico up to mega packages contain 1 manual and 1 certificate. | |

| Order number | Description | Quantity |
|---|---|---|
| Accessories | | |
| 1ZA---0004 | Adapter Luer male to UNF-10 – 32 female, PEEK | 1 |
| 1ZA0GV0003 | Adapter UNF 10–32 female to 25 mm, sanitary, polyoxymethylene | 2 |
| 9ZA1AM0001 | Stainless steel legs for mega | 3 |
| 5ZG1--0001 | Holder for 1× 10”, 20” or 30” capsule, stainless steel, 3 legs | 1 |
| 5ZALB-0002 | Distribution adapter for 3 capsules, stainless steel | 1 |
| 5ZGLG-0004 | Holder for 3×10”, 20” and 30” capsules, stainless steel, 3 legs | 1 |
| 7ZAL-V0013 | Reducing adapter 1½”–¾”; 50.5/25 mm, sanitary | 2 |
| 7ZAL-V0010 | Reducing adapter 2”–1½”; 64/50.5 mm, sanitary | 2 |

# 12. Dimensions and Connections

| **Sartobind SingleSep** | **pico** | **nano** | **mini** |
|---|---|---|---|
| Direction of flow ↓ | This side up  |  |  |
| Connector | Luer Lok | Luer Lok | Sanitary ¾” |
| Dimensions (height × diameter) | 31 × 11 mm | 37 × 33 mm | 87 × 45 mm |
| Approximate capsule weight | 1.5 g | 10 g | 30 g |
| Frontal surface area ($cm^2$) | 0.19 | 2.4 | 20 |
| Inlet connector | female | female | 25 mm outer 10 mm inner diameter |

| 5" | 10", 20", 30" | mega |
|---|---|---|
|  |  |  |
| Sanitary 1 ½” | Sanitary 1 ½” | Sanitary 1 ½” |
| 210 × 70 mm | 365 × 100 mm (10”)<br>620 × 100 mm (20”)<br>870 × 100 mm (30”) | 910 × 190 mm |
| 0.2 kg | 0.8 \| 1.4 \| 2 kg | 5.6 kg |
| 160 | 450 \| 900 \| 1350 | 4050 |
| 50.5 mm outer<br>15 mm inner<br>diameter | 50.5 mm outer<br>36 mm inner<br>diameter | 50.5 mm outer<br>36 mm inner<br>diameter |

| Sartobind SingleSep | pico | nano | mini |
|---|---|---|---|
| Outlet connector | as above | as above | as above outer 19 mm inner diameter |
| Inlet gasket | – | – | ¾”, internal diameter 16 mm |
| Outlet gasket | – | – | as above |

| Hose barb version | | | |
|---|---|---|---|
| Size of capsule (height × diameter) | – | – | 103 × 45 mm |
| Size of hose barb connectors | – | – | 6–12 mm |
| Recommended internal diameter of flexible tube | – | – | 6–10 mm |

For more information about validation service and other Membrane Adsorber types please contact your local Sartorius Stedim Biotech representative or visit our homepage.

| 5" | 10", 20", 30" | mega |
|---|---|---|
| 50.5 mm<br>19 mm inner diameter | as above | as above |
| 1 ½", internal diameter<br>35.8 mm | 1 ½", internal diameter<br>35.8 mm | 1 ½", internal diameter<br>35.8 mm |
| as above | as above | as above |
| | | |
| 190 × 70 mm | – | – |
| | | |
| 14.5 mm | – | – |
| 12 mm | – | – |

Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen, Germany

Phone +49.551.308.0
Fax +49.551.308.3289
www.sartorius-stedim.com



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| |
|---|
| 92IEXQ42D4-OO-A |
| 92IEXQ42D4-SS-A |
| 92IEXS42D4-OO-A |
| 92IEXS42D4-SS-A |