

Operating Instructions

Sartobind® Q and S

Void Volume Optimized Capsules and Cassettes
With 4 | 8 mm Bed Height



4 mm: nano 1 mL

75 mL

200 mL

400 mL

600 mL

Jumbo 2.5 L

Cassette 0.8 L

8 mm: nano 3 mL

150 mL

400 mL

800 mL

1.2 L

Jumbo 5L


Cassette 1.6 L


1000032237





SARTORIUS


Read operational instructions carefully before using Sartobind capsules.


 Use of the products 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 products are supplied as non-sterile unless otherwise expressly described. The membrane is dried from glycerol.

 Die Verwendung dieser Produkte für Anwendungen, für die sie nicht bestimmt oder nicht in dieser Anleitung beschrieben sind, können zu einer schlechteren Funktion, Zerstörung der Produkte oder sogar zu Verletzungen von Mensch und Material führen. Die Produkte sind nicht steril sofern dies nicht ausdrücklich anders beschrieben ist. Die enthaltene Membran wird aus Glycerin getrocknet.

 L'utilisation des produits pour des applications non-spécifiées ou décrites dans ce manuel peut causer un dysfonctionnement, une destruction du produit, des dommages matériels ou même corporels. Les produits sont fournis non-stériles, sauf indication contraire expressément mentionnée. La membrane est séchée avec de la Glycérine.

 La utilización de este producto en aplicaciones ajenas o no establecidas en el manual de operación, puede provocar un mal funcionamiento del producto, del material, así como daños personales. Los productos suministrados no son estériles a menos que se describa lo contrario. La membrana ha sido secada de glicerina.

 把产品用于手册中没指定或描述的应用, 可能导致产品失效, 人员受伤, 或使产品及物料受损。囊式膜柱以非灭菌的形式供应。膜用甘油脱水。

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Intended use

The membrane chromatography products also described as membrane adsorbers are intended and validated for single use to avoid carryover as well as tedious and costly cleaning validation procedure. However it is technically possible to reuse after cleaning in place depending on application, character of sample and process. Additional cleaning and validation steps will be needed to assure constant binding capacity and flow rate after each cycle.

The **4 mm** product line has been developed for the highest possible flow rate and is typically applied for flow-through polishing applications in which binding capacity is typically no limitation.

The **8 mm** product line is used when bind & elute applications or the highest possible binding capacity is needed. If you are not sure what to use, the 8 mm should be used as this bed height provides the best ratio of void volume to membrane volume and the maximum achievable dynamic binding capacity.

For **scale-up** we recommend to stay within one bed height size as this is the least complex procedure. Nevertheless you may change to a different bed height later by keeping residence time constant (see chapter "7.15 Scaling up", page 40).

Sartobind nano 1 and 3 mL capsules have been developed for working with small sample volumes. They are perfect for small scale applications, and also for screening purposes and laboratory-scale bind & elute and flow-through purifications.

Sartobind 75 and 150 mL capsules have been developed for intermediate and pilot scale.

Sartobind 200 mL up to Jumbo 5 L capsules have been developed for production purposes in the biopharmaceutical industry.

Sartobind 0.8 or 1.6 L cassettes are used in the Pilot Scale Filter Holder of up to 20.8 L membrane volume for the biopharmaceutical production.

Table of Contents

1	Storage Conditions	10
2	Introduction	10
3	Technical Data	16
3.1	Bed height 4 mm	16
3.2	Bed height 4 mm	18
4	Materials	20
5	Binding Capacity	21
6	Installation	22
7	Operation	26
7.1	Venting	26
7.2	Cleaning and equilibration	29
7.3	Autoclaving	29
7.4	Recommended flow rates and equilibration buffer volumes	33
7.5	Buffer conditions	34

7.6	Selection of pH conditions.....	35
7.7	Contaminant removal from therapeutic proteins and other sources in flow-through mode	36
7.8	Sample preparation.....	37
7.9	Washing	37
7.10	Elution	38
7.11	Draining	38
7.12	Regeneration and Storage.....	39
7.13	Chemical stability.....	39
7.14	Operation of the Sartobind nano with peristaltic pumps or liquid chromatography (LC) systems.....	39
7.15	Scaling up.....	40

8 Testing of Membrane Samples

	Enclosed with 5 L Capsules.....	44
8.1	Testing Sartobind Q 30 mm discs.....	44
8.2	Testing Sartobind S 30 mm discs	49

9	Integrity Test by Diffusion	54
9.1	Installation for test	54
9.2	Operation of test	55
10	Troubleshooting	60
11	Quality Assurance	65
12	Ordering Information	66
12.1	Products 4 mm bed height	66
12.2	Products 8 mm bed height	69
12.3	Accessories	72
13	Dimensions and Connections	74

1 Storage Conditions

Sartobind capsules should be stored clean, dry and away from direct sunlight in the closed bag and box at room temperature. Store membrane samples enclosed in Jumbo shipment in a safe place as they cannot be ordered again.

2 Introduction

The capsules and cassettes with 4 and 8 mm bed height are ion exchange chromatography devices based on macroporous membranes. They can be used for chromatographic separations in the downstream processing of viruses and proteins. 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. The devices are constructed with optimized fluid channels.

The capsules carry a central core and the cassettes a spacer element to minimize void volume. To set up and operate the Sartobind Jumbo we recommend the trolley (see chapter

"12.3 Accessories", page 72). Capsules and cassettes with strong basic and strong acidic ion exchange Membrane Adsorber (MA) are available. These products are intended for single use to avoid carryover as well as tedious and costly cleaning validation procedures. However, it is technically possible to reuse after cleaning in place (see also section "7.12 Regeneration and Storage", page 39).

Their major application is for single use as they are validated for contaminant removal from proteins in flow-through mode (negative chromatography) to bind DNA, residual protein, host cell proteins (HCP), endotoxins, viruses and aggregates. The devices can also be used to capture large proteins, viruses such as adeno-, lentiviruses and virus like particles (VLP).

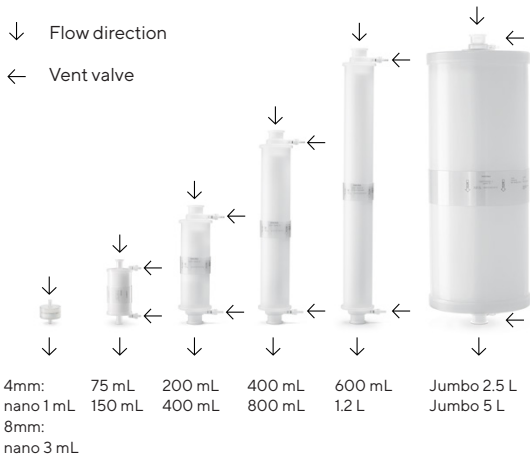



Fig. 1:
Flow direction and position of vent valves
of 4 and 8 mm capsules



Fig. 2:
Flow direction and position
of vent valve
connection of
4 and 8 mm
cassettes

 **Devices should be visually inspected before use. In case of visible damage, the module must be replaced. Close vent valves before use by screwing the valve clockwise. For the cassettes use the clamps at the manifold sets.**

For the nano and 75/150 mL devices the central core is made from a solid polypropylene cylinder. For the larger capsules it is made from a self-contained air filled polypropylene cylinder. The interior of the core is inaccessible for gases and fluids. The two flat membrane stacks of the cassettes are separated by a central spacer element.

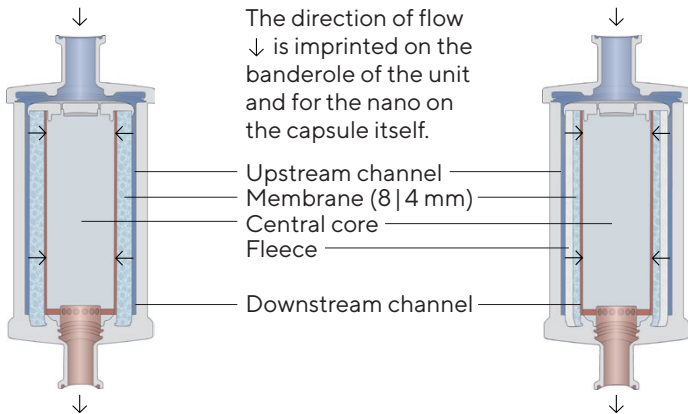


Fig. 3: Construction and flow path inside the 8 mm (left) and 4 mm (right) capsules.

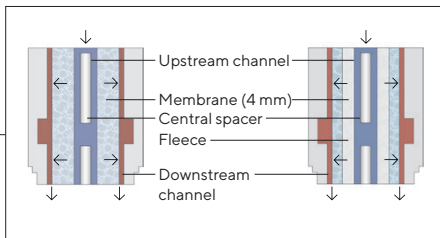


Fig. 4: Side view cassette;
highlighted section
see Fig. 5

Fig. 5: Construction and flow path inside
the 8 mm (left and 4 mm (right) cassettes

3 Technical Data

3.1 Bed height 4 mm

Membrane volume (MV)	1 mL	75 mL
Nominal membrane area	36.4 cm ²	2,700 cm ²
Bed height	4 mm	4 mm
Design	Cylindrical	Cylindrical
Sartobind Q typical 10% dynamic binding capacity*	29 mg	2.16 g
Sartobind S typical 10% dynamic binding capacity*	25 mg	1.89 g
Maximum pressure bar (MPa, psig) at 20°C	4 (0.4, 58)	4 (0.4, 58)
Maximum pressure during venting bar (MPa, psig) at 20°C	3.5 –	200 0.5 (0.05, 7)
Nominal void volume (mL)	3.5	200
Nominal void volume (MV)	3.5	2.7
Approximate weight	10 g	400 g

1 mL membrane = 36.4 cm² membrane

Ion capacity per cm² of membranes: 2–5 µeq

Short term pH stability Q | S: 1–14 | 3–14 refers to cleaning in place and regeneration procedures during operation

200 mL	400 mL	600 mL	2.5 L	800 mL
7,300 cm ²	14,600 cm ²	22,000 cm ²	91,000 cm ²	29,000 cm ²
4 mm	4 mm	4 mm	4 mm	4 mm
Cylindrical	Cylindrical	Cylindrical	Cylindrical	Flat sheet
5.8 g	11.7 g	17.6 g	73 g	23.2 g
5.1 g	10.2 g	15.4 g	–	20.3 g
4 (0.4, 58)	4 (0.4, 58)	4 (0.4, 58)	3 (0.3, 43.5)	2 (0.2, 29)
540	1,080	1,600	7,000	2500
0.5 (0.05, 7)	0.5 (0.05, 7)	0.5 (0.05, 7)	0.5 (0.05, 7)	0.5
540	1,080	1,600	7,000	2,500
2.7	2.7	2.7	2.8	3.1
750 g	1.3 kg	1.9 kg	16 kg 20 kg wet 23 kg filled	4.9 kg 6.0 kg wet

Long term storage pH stability Q|S: 2-12|4-13 refers to overnight storage and longer.
 Preferably store units in 20% ethanol|buffer

* See section “5 Binding Capacity”, page 21

3.2 Bed height 4 mm

Membrane volume (MV)	3 mL	150 mL
Nominal membrane area	110 cm ²	5,500 cm ²
Bed height	8 mm	8 mm
Design	Cylindrical	Cylindrical
Sartobind Q typical 10% dynamic binding capacity*	88 mg	4.4 g
Sartobind S typical 10% dynamic binding capacity*	77 mg	3.9 g
Maximum pressure bar (MPa, psig) at 20°C	4 (0.4, 58)	4 (0.4, 58)
Maximum pressure during venting bar (Mpa, psig) at 20°C	–	0.5 (0.05, 7)
Nominal void volume (mL)	4	200
Nominal void volume (MV)	1.3	1.3
Approximate weight	10 g	400 g

1 mL membrane = 36.4 cm² membrane

Ion capacity per cm² of membranes: 2–5 µeq

Short term pH stability Q | S: 1–14 | 3–14 refers to cleaning in place and regeneration procedures during operation

400 mL	800 mL	1.2 L	5 L	1.6 L
14,600 cm ²	29,000 cm ²	44,000 cm ²	182,000 cm ²	58,000 cm ²
8 mm	8 mm	8 mm	8 mm	8 mm
Cylindrical	Cylindrical	Cylindrical	Cylindrical	Flat sheet
11.7 g	23.3 g	35 g	145 g	46 g
10.2 g	20.4 g	31 g	127 g	41 g
4 (0.4, 58)	4 (0.4, 58)	4 (0.4, 58)	3 (0.3, 43.5)	2 (0.2, 29)
0.5 (0.05, 7)	0.5 (0.05, 7)	0.5 (0.05, 7)	0.5 (0.05, 7)	0.5 (0.05, 7)
540	1,080	1,600	7,000	2,900
1.4	1.4	1.3	1.4	1.8
760 g	1.3 kg	1.9 kg	16 kg 20 kg wet 23 kg filled	4.9 kg 6.5 kg wet

Long term storage pH stability Q|S: 2-12|4-13 refers to overnight storage and longer.
 Preferably store units in 20% ethanol | buffer

* See section "5 Binding Capacity", page 21

4 Materials

Membrane materials

Matrix	Stabilized reinforced cellulose
Membrane thickness membrane volume = membrane area	275 μm 1 mL = 36.4 cm^2
Nominal pore size	> 3 μm
Ion exchanger ligand Q	Strong anion Q: quaternary ammonium ($\text{R-CH}_2\text{-N}^+(\text{CH}_3)_3$)
Ion exchanger ligand S	Strong cation S: sulfonic acid ($\text{R-CH}_2\text{-SO}_3^-$)

Capsule materials

Outer cage, inner core, end caps, capsule housing, nonwoven, fleece	Polypropylene
O-ring in vent valve (except nano)	EPDM (ethylene propylene diene monomer)

Cassette materials

Outer cage, seal, nonwoven, fleece	ABS, silicone, polyethylene, stable to gamma irradiation
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5 Binding Capacity

Data are based on dynamic binding capacity measurements 10% using 3 layers of 5 cm² membrane discs (15 cm² total area, membrane thickness of 275 µm) arranged in a holder and run at 10 mL/min.

	Typical dynamic binding capacity 10%	Reference protein and buffer
Q	0.8 mg/cm ² (29 mg/mL)	BSA (bovine serum albumin) in 20 mM Tris/HCl, pH 7.5
S	0.7 mg/cm ² (25 mg/mL)	Lysozyme in 10 mM potassium phosphate, pH 7.0

6 Installation

The content of the package is described in chapter “12 Ordering Information”, page 66. When unpacking capsule, protect inlet and outlet connectors from damage. Never keep or place the capsule directly on the floor on the connectors. This might damage the sanitary adapters.

For unpacking of Jumbo 2.5 or 5 L capsule, take the capsule including the styrene foam end protectors, out of the box and place it upright on the end protectors.

Move the Jumbo trolley (accessory) in place. Then remove upper foam protection and transparent bag. Lift the Jumbo directly onto the trolley (inlet is up and the arrow imprinted on the banderole is pointing down). We recommend to connect the Jumbo with the trolley by the three screws delivered with the trolley. To ensure safe unpacking, the protective caps on inlet and outlet should stay until you use the unit. Store the caps when you plan to autoclave (see chapter “7.3 Autoclaving”, page 29). The Jumbo 2.5 and 5 L carry protective caps on vent valves as well. Remove before venting.

The capsules and cassettes should be installed in an upright position in the process flow. In this position the inlet is up. The flow is guided to the upstream channel (i.e. the solution enters the device) passing through the membrane layers to the downstream channel and to the outlet of the device (see Fig. 3). Install the capsule and cassette(s) in-line with a prefilter (0.2 μm or 0.45 μm) in front of the device to prevent blockage or pressure build-up.

For using the cassettes you need an appropriate cassette holder and one Manifold Set (see chapter "12.3 Accessories", page 72). Before use you must read the Pilot Filter Holder manual, order no. 85037-547-72 or Process | Double Process Filter holder manual order no. 85037-553-19.

If you plan to use a different filter holder from other manufacturers, you have to contact your nearest Sartorius office for technical directions.

Unpack the Manifold Set containing one inlet and one outlet plate. Place the “INLET” marked plate at one end of the holder. “THIS SIDE UP” is readable on top. Place the “OUTLET” marked plate at the other end of the holder, again “THIS SIDE UP” is readable from the top. The fluid channels of both plates are oriented to each other.

**⚠ The cassettes must be oriented in the lowest possible position in the holder otherwise the system may leak.
The cassettes must have the same lot number.**

Put the desired number of Sartobind cassettes between the manifolds (see Fig. 6). Provided “THIS SIDE UP” is readable the cassettes are correctly installed.

The maximum clamping force for 1–13 cassettes with Pilot and Process holders before start of use is: 25 kilonewton or kN (200 bar, 2900 psig) for installed 12.6 cm² piston area. Now close all DRAIN and VENT valves of the manifold plates by the pinch clamp manually.

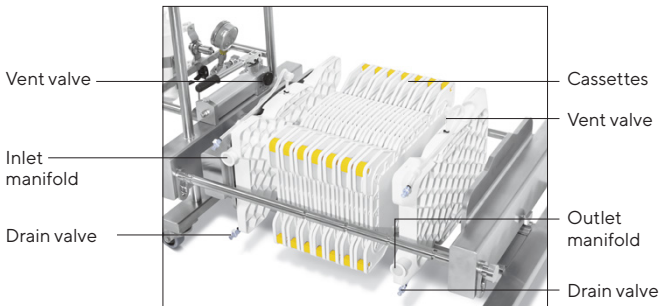


Fig. 6: Insert cassette(s) between the manifold inlet and outlet plates on the Pilot Filter Holder.

Connect the inlet and outlet plates with 1½ inch tri-clamp to the process solution. Maximum pressure for the set-up of 1 to 13 cassette(s) is 2 bar (0.2 MPa, 29 psig). Make sure that pump peak pressure caused by pulsation stays below this limit too.

7 Operation

7.1 Venting

It is important to remove air from the unit completely.

All capsules except nano carry vent valves (see Fig. 1). The vent valves are equipped with hose barb connectors for the fluid spilled out during venting. After unpacking check vent valve position. When turning anticlockwise, the valve is open, when turning clockwise, the valve is closed. Before opening the vent valve, please connect the valves with flexible tubing (inner diameter 6 mm) to waste. During venting of capsules please do not exceed 0.05 MPa (0.5 bar | 7.3 psi) 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 $\frac{1}{3}$ turn to left until all air is replaced by fluid. For venting the cassettes, tubes with quick connectors are attached to the inlet and outlet manifolds and closed by a pinch clamp.

For the nano capsule 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. Another method is to connect a second empty syringe to the top of the nano and expel air and buffer into that syringe, disconnect the upper syringe to push out air and reconnect to the nano, turn it and purge the solvent back and forth.

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.

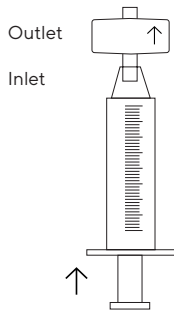


Fig. 7: Filling the Sartobind nano with a Luer syringe for air removal

7.2 Cleaning and equilibration

The devices have to be cleaned in place directly before use with 1 N NaOH, 30 min at 20°C.

1. For sanitization use 30 membrane volumes (MV) of 1 N NaOH solution at the flow rate of 1 MV/min.
Preferentially work at room temperature as low temperature increases viscosity of solvents. Also cold NaOH can induce swelling of the cellulose matrix and significantly reduce flow rate.
2. Flush with 10 MV of 1 N NaCl at 5 MV/min
3. Flush with 10 MV of equilibration buffer at 5 MV/min

7.3 Autoclaving

The cassette material is not compatible to autoclaving but to gamma irradiation. If you need sterile cassettes, please contact your nearest Sartorius office.

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.** The protective caps enclosed in the Jumbo delivery must be reinstalled on inlet and outlet connectors of the Jumbo. Close valves immediately after sterilisation. For autoclaving Sartobind Jumbo refer to separate autoclaving instructions enclosed in delivery.







Membrane adsorbers can be run at much higher flow rate per volume than resin columns. As a rule of thumb, flow rates of 5 membrane volumes per minute are recommended for 8 mm bed height and 10 – 30 membrane volumes for 4 mm bed height. This recommendation is only a guideline since buffers and samples have different compositions and viscosities. Please test your planned flow rates using a small scale device to ensure that they fit into your pump capacities and device pressure limits.

Lower flow rates than the recommended ones can also be used but will typically not improve binding capacity or overall performance. Cold room temperature increases buffer viscosity and possibly back pressure.

The equilibration buffer volume is typically 10 membrane volumes depending on the type of buffer.









For the cassettes, flow rate and equilibration volumes have to be multiplied with the number of cassettes in use.

7.4 Recommended flow rates and equilibration buffer volumes

						
Membrane volume (MV)	1 mL	3 mL	75 mL	150 mL	200 mL	400 mL
Bed height (mm)	4	8	4	8	4	8
Rec. flow rate (L/min)	0.02	0.015	1.5	0.75	4	2
Rec. equilibration volume* (L)	0.1	0.03	0.75	1.5	2	4

* Refer to 7.2 Cleaning and equilibration


** Multiply with number of used cassettes

							
400 mL	800 mL	600 mL	1.2 L	2.5 L	5 L	0.8 L	1.6 L
4	8	4	8	4	8	4	8
8	4	12	6	50	25	16**	8**
4	8	6	12	25	50	8**	16**

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 or 0.45 μm filters before use and the quality of water and chemicals should be of high purity.

 **Do not apply pure water as it may lead to a reversible swelling and decrease of the flow rate of the membrane.**

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 . If the pH of the buffer is 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). If the pH of the buffer is 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 in flow-through mode

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 with an anion exchanger. The product of interest, the monoclonal antibody with isoelectric points (pI) of 8 – 9.5 for example, will not bind and pass through Sartobind Q. The influence of the flow rate is very low.

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

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. Sartopore® 2 capsule. For small volumes in the mL range use a 0.2 µm Minisart® filter with Luer outlet (order number 16532-K for polyethersulfone or 16534-K for cellulose acetate membrane).

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

7.9 Washing


When using capsules in bind & elute mode, wash with equilibration buffer after sample loading.

7.10 Elution

To elute target protein or the virus, virus like particle (VLP) use buffers with appropriate salt concentration.

7.11 Draining

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

 **A dual air regulator system is recommended to prevent over-pressurization of the Sartobind devices. The first regulator should reduce line air pressure to 2 bar. The second regulator, positioned immediately upstream of the Sartobind, should reduce the 2 bar regulated supply pressure to the <1 bar (14.5 psi) for a capsule and 0.5 bar (7.3 psi) for 1 up to 13 cassettes draining pressure.**

7.12 Regeneration and Storage

After elution wash with equilibration buffer. If necessary, use 1 N NaOH, 1 N HCl or 70% ethanol for 1 hour for regeneration and store in 20% ethanol in equilibration buffer.

7.13 Chemical stability

The devices are stable against all commonly used buffers in chromatography. Do not use oxidizing agents.

7.14 Operation of the Sartobind nano with peristaltic pumps or liquid chromatography (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.

7.15 Scaling up

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

Recommendations:

Maintain

- Bed height (automatically kept constant when using capsules with same bed height)
- Linear flow (automatically kept constant when using capsules with same bed height)
- Sample concentration

Increase (see scaling factors in the following table)

- Sample loading volumes
- Volumetric flow rate
- Membrane volume

Scale up calculations are done preferably by keeping the bed height and changing the membrane volume as the calculation is most simple. Other methods for scale up via residence time will lead to same result. The residence time is the membrane volume divided by the flow rate. You may change from 4 mm to 8 mm by keeping the residence time constant which is equal to the flow rate expressed in membrane volumes per minute but not above 5 MV/min.


Using the Sartobind nano 1 or 3 mL the scale up factor for flow rate and binding capacity is equal to a multiplication factor of membrane volumes of the listed scale up devices:

Size	Membrane volume [mL]	Factor to increase* (from nano)	Bed height (mm)	Size	Factor to increase* (from nano)	Bed height (mm)
nano	1 mL	–	4 mm	3 mL	–	8 mm
5"	75 mL	75	4 mm	150 mL	50	8 mm
10"	200 mL	200	4 mm	400 mL	133	8 mm
20"	400 mL	400	4 mm	800 mL	266	8 mm
30"	600 mL	600	4 mm	1.2 L	400	8 mm
Jumbo	2.5 L	2500	4 mm	5 L	1667	8 mm
Cassette	800 mL	800	4 mm	1.6 L	533	8 mm
Cassettes**	10.4 L	10400	4 mm	20.8 L	6933	8 mm

* Flow rate and binding capacity; ** 13 Cassettes as example

Example: After breakthrough experiments with the nano 3 mL, you realize that a 1500-fold binding capacity is needed for a large scale run. You will choose the 5 liter Jumbo capsule. To determine the running conditions of the Jumbo and to keep consistent upscaling, increase flow rate by a factor of ~1670. To assure the scale up, additional experiments with the 150 mL (increase by a factor of 50) support this scale up calculation.

In the example above one and 13 cassettes are used, but any number of cassettes between one or 13 which is the maximum in the pilot filter holder can be applied. Then the factor to increase is adapted proportionally. For a larger scale above 20 L, a process and a double process filter holder is available as accessory (see chapter "12.3 Accessories", page 72). To assure the scale, intermediate sizes are recommended to assure the design.

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

8 Testing of Membrane Samples Enclosed with 5 L Capsules

Membrane quality is always tested as described in chapter "10 Troubleshooting", page 60. Additionally the 5 L Jumbo is delivered with membrane samples of same membrane lot that was used in the capsule. This allows again for testing of membrane with user specific tests before use of capsule. Static Binding capacity tests can be performed as follows:

8.1 Testing Sartobind Q 30 mm discs

Using the appropriate conditions, bovine serum albumin can be selectively bound to membrane adsorber 30 mm discs. By increasing the salt concentration of the buffer solution, the target substance can be recovered for subsequent quantification. BSA in excess is loaded at a batch experiment on Sartobind Q membrane disc. The total capacity is obtained after eluting the bound BSA by increasing salt concentration. The amount of BSA is determined by spectrophotometer.

8.1.1 Materials for test of Q membrane discs

Membrane sample:

Sartobind Q membrane, 30 mm diameter

(membrane area: 7.1 cm^2)

Test protein: bovine serum albumin (BSA)

Cat.No. 11930.03 SERVA

Buffers:

Equilibration buffer: 20 mM Tris|HCl,

(pH 7.4, conductivity 1.80 mS/cm)

Test solution: 0.2% BSA in equilibration buffer (2 mg/mL)

Elution buffer: 1 M NaCl in equilibration buffer

(Temperature of buffers: $20\text{--}25^\circ\text{C}$)

Equipments:

Glass or plastic dish

pH meter

Conductivity meter (temperature coefficient 2.00%/°C at 25°C reference temperature)

Spectrophotometer set at 280 nm

Orbital or reciprocal shaker set at approx. 80 rpm

Operation temperature: 20–25°C

8.1.2 Method for test of Q membrane discs

Equilibration

Apply 2 mL/cm² equilibration buffer (=14.2 mL per 30 mm membrane disc) into the glass dish.

Place a membrane sample into the equilibration buffer (make sure that the membrane is wetted completely) and shake three times for 5 min each at 80 rpm.

Remove the equilibration buffer after each step by vacuum pump.

Loading

Apply 2 mL/cm² test solution into the dish. Close the dish and shake for 12–18 h at 80 rpm.

Remove excess test solution by vacuum pump as much as possible.

Washing

Apply 2 mL/cm² of equilibration buffer and shake for 15 min.

Remove equilibration buffer by vacuum pump.

Repeat this step once.

Elution

Place each membrane disc in a separate Petri dish. Apply 10 mL elution buffer onto each membrane disc with a pipet. Shake for 1 h at 80 rpm (= elution solution). The volume of the elution solution = V_{elute} [mL].

Extinction measurements

Zero the instrument with equilibration buffer.

Measure the extinction of the test solution = E_{test} .

Zero the instrument with elution buffer.

Measure extinction of the elution solution = E_{elute} .

8.1.3 Calculation of membrane binding capacity

Binding capacity [mg/cm²]

$$= \frac{E_{\text{elute}} + C_{\text{test}} + V_{\text{elute}}}{E_{\text{test}} + \text{MA}}$$

$$= \frac{E_{\text{elute}} + 2 + 10}{1.25 + 7.1}$$

$$= E_{\text{elute}} + 2.25$$

E_{elute} : extinction 280 nm of the elution solution

C_{test} : concentration of the test solution (2 mg/mL)

V_{elute} : volume of the elution solution (20 mL)

E_{test} : extinction 280 nm of the test solution (= 1.25),
d (cell width) = 1 cm

MA: membrane area (= 7.1 cm²)

8.2 Testing Sartobind S 30 mm discs

Using the appropriate conditions, lysozyme can be selectively bound to membrane adsorber 30 mm discs. By increasing the salt concentration of the buffer solution, the target substance can be recovered for subsequent quantification. Lysozyme in excess is loaded at a batch experiment on Sartobind S membrane disc. The total capacity is obtained by eluting the bound protein by increasing salt concentration. The amount of lysozyme is determined by spectrophotometer.

8.2.1 Materials for test of S membrane discs

Membrane sample:

Sartobind S membrane, 30 mm diameter
(membrane area: 7.1 cm²)

Test protein: lysozyme Cat.No. L-6776 Sigma

Buffers:

Equilibration buffer: 10 mM potassium phosphate,
(pH 7.0, conductivity 1.75 mS/cm)

Test solution: 0.2% lysozyme in equilibration buffer
(2 mg/mL)

Elution buffer: 1 M NaCl in equilibration buffer
(Temperature of buffers: 20–25°C)

Equipments:

Plastic or glass dish

pH meter

Conductivity meter (temperature coefficient 2.00%/°C
at 25°C reference temperature)

Spectrophotometer set at 280 nm

Orbital or reciprocal shaker set at approx. 80 rpm

Operation temperature: 20–25°C

8.2.2 Method for test of S membrane discs

Equilibration

Apply 2 mL/cm² equilibration buffer into the dish. Place a membrane sample into the equilibration buffer (make sure that the membrane is wetted completely) and shake three times for 5 min each at 80 rpm. Remove the equilibration buffer after each step by vacuum pump.

Loading

Apply 2 mL/cm² test solution into the dish. Close the dish and shake for 12–18 h at 80 rpm.
Remove excess test solution by vacuum pump as much as possible.

Washing

Apply 2 mL/cm² of equilibration buffer and shake for 15 min.
Remove equilibration buffer by vacuum pump.
Repeat this step once.

Elution

Place each membrane disc in a separate Petri dish. Apply 10 mL elution buffer onto each membrane disc with a pipet. Shake for 1 h at 80 rpm (= elution solution). The volume of the elution solution = V_{elute} [mL].

Extinction measurements

Zero the instrument with equilibration buffer. Measure the extinction of the test solution = E_{test} .

Zero the instrument with elution buffer. Measure extinction of the elution solution = E_{elute} .

8.2.3 Calculation of membrane binding capacity

Binding capacity [mg/cm²]

$$= \frac{E_{\text{elute}} + C_{\text{test}} + V_{\text{elute}}}{E_{\text{test}} + \text{MA}}$$

$$= \frac{E_{\text{elute}} + 2 + 10}{5.0 + 7.1}$$

$$= E_{\text{elute}} + 0.56$$

E_{elute} : extinction 280 nm of the elution solution

C_{test} : concentration of the test solution (2 mg/mL)

V_{elute} : volume of the elution solution (20 mL)

E_{test} : extinction 280 nm of the test solution (= 5.0)

MA: membrane area (= 7.1 cm²)

9 Integrity Test by Diffusion

The integrity of a membrane adsorber 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 devices and to detect major bypasses, large holes and faulty assembly.

9.1 Installation for test

Test procedure has been generated with current Sartocheck instrument family e.g. Sartocheck® 4 Plus (26288), 4 (16288) or 3 plus (16290).


Use of earlier Sartocheck instruments will generate faulty data.
Install adsorber as shown in Fig. 8

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

9.2 Operation of test

9.2.1 Pre-washing of device

Pre-wash with 30 membrane volumes (MV) of buffer or 0.9% NaCl in water at recommended flow rate.

 Do not use pure water as this may lead to reversible swelling of the membrane strongly affecting the flow rate. Prior to integrity testing, the capsule needs to be prewashed, 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 device until all air is replaced by testing solvent.

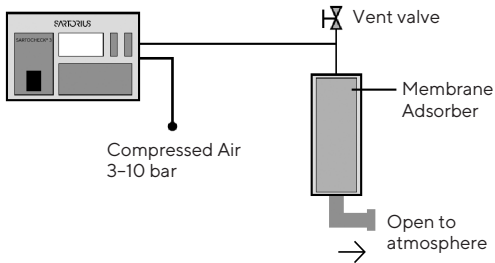


Fig. 8: Set up of diffusion test with Sartocheck® 4 Plus, 4 or 3 Plus

9.2.2 Diffusion measurement with Sartocheck® 4 Plus

- Choose **Programming** in the main menu
- Choose **Diffusion Test**

Choose the test pressure, stabilization and testing time for your device from the table. If you set the Net Volume to zero, Sartocheck® automatically measures the upstream void volume including tubing.

Test parameters

Size	Bed height (mm)	Membrane volume (mL)	Test pressure mbar (psi)	Stabilisation time (min)	Testing time (min)	Diffusion max. mL/min
nano	4 mm	1 mL	200 (2.9)	2	1	15
5"	4 mm	75 mL	200 (2.9)	2	1	15
10"	4 mm	200 mL	200 (2.9)	3	1	15
20"	4 mm	400 mL	200 (2.9)	3	1	15
30"	4 mm	600 mL	200 (2.9)	3	1	15
Jumbo	4 mm	2.5 L	200 (2.9)	3	1	15
Cassette(s)*	4 mm	0.8–10.4 L	200 (2.9)	5	1	15–195

* Diffusion max. per 4 or 8 mm cassette is 15 mL/min multiplied by number of cassettes

Test parameters

Size	Bed height (mm)	Membrane volume (MV)	Test pressure mbar (psi)	Stabilisation time (min)	Testing time (min)	Diffusion max. mL/min
nano	8 mm	3 mL	200 (2.9)	2	1	15
5"	8 mm	150 mL	200 (2.9)	2	1	15
10"	8 mm	400 mL	200 (2.9)	3	1	15
20"	8 mm	800 mL	200 (2.9)	3	1	15
30"	8 mm	1.2 L	200 (2.9)	3	1	15
Jumbo	8 mm	5 L	200 (2.9)	5	1	15
Cassette(s)*	8 mm	1.6 – 20.8 L	200 (2.9)	5	1	15 – 195

* Diffusion max. per 4 or 8 mm cassette is 15 mL/min multiplied by number of cassettes

9.2.3 Results and evaluation for one device

- Diffusion \leq Diffusion max.:
Test passed (diffusion value on the print out)
- Diffusion $>$ Diffusion max.:
Diffusion max.: Test failed (red text on the print out)

The maximum allowed diffusion values are per device.

If you set up for example 10 cassettes in a holder the values have to be added up and the max diffusion value will be 150 mL.

10 Troubleshooting

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", page 26.
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 in the described flow direction (Feed enters capsule on top and leaves it on bottom).
I deviated from the CIP and flushing equilibration procedure		The capsules have been qualified and validated according the given procedure. If a deviation is necessary, the results may also deviate from the given validation data.
High back pressure	Material has not been filtered	Prefilter with 0.2 µm or 0.45 µm filter before processing through the unit (preferentially inline).

Problem	Possible cause	Action
High back pressure	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 adsorber is clogged membrane fouling	Replace unit. Perform a regeneration cycle. You may backflush within given flow and pressure limits, perform a regeneration cycle.
	Viscosity swelling effects	Work at room temperature, avoid lower temperatures
	Pure water leads to swelling of membrane	Add sodium chloride or use ionic buffers
Target molecule is not bound	Conditions for binding are insufficient	Decrease conductivity, control other process parameters as type of buffer and pH.

Problem	Possible cause	Action
Binding capacity is not sufficient	Process conditions not optimized	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.
Reuse is needed	For economic or practical reasons	The major application of Sartobind capsules is the single use and they are constructed in plastic housing for this. Also they are validated and certified only for one use. 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. Plastic materials and membranes allow CIP and long term storage if carefully treated. For reuse validation we assist you with our Validation Service. Please ask your local representative.

Problem	Possible cause	Action
Binding capacity decreases after several uses	Improper filtration	Prefilter with 0.2 µm filter before processing through the unit.
	Some molecule species binds tightly (e.g. DNA on Sartobind Q) and cannot be removed with 1 N NaOH 1 h	Use capsule only once or use DNA endonucleases such as Denarase®.
	Protein 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).
	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

Problem	Possible cause	Action
A vertical line is seen on one capsule side when filled	Membrane edge visible	No action necessary. It can be visible the edge of the fleece touching the inner tube.
I purged with air or nitrogen and lost flow and binding capability.	Air has entered into the pores	See troubleshooting "Applied bubble point instead of diffusion test" below.
Accidentally a bubble point test instead of diffusion test has been run	Operation error	The membrane has then to be purged extensively to remove all the air which has been pressed into the pores. If properly purged, the diffusion test can be run successfully and the device works as expected.
Cassettes system leaks or fails at integrity test	Wrong assembly	Position manifolds and cassettes at the lowest position in the holder otherwise seals are not perfectly aligned.

11 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 evenness. Capsules, cassettes 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. A validation and an extractables guide are available on request.

12 Ordering Information

12.1 Products 4 mm bed height

Order number	Description	Quantity
96IEXQ42DN-11	Sartobind Q nano 1 mL, 4 mm, Luer female connectors, 2 PEEK adapters Luer male to UNF 10-32 female, manual, certificate	1
96IEXQ42DN-11--A	Sartobind Q nano 1 mL, 4 mm, Luer female connectors, 2 PEEK adapters Luer male to UNF 10-32 female, manual, certificate	4
96IEXS42DN-11	Sartobind S nano 1 mL, 4 mm, Luer female connectors, 2 PEEK adapters Luer male to UNF 10-32 female, manual, certificate	1
96IEXS42DN-11--A	Sartobind S nano 1 mL, 4 mm, Luer female connectors, 2 PEEK adapters Luer male to UNF 10-32 female, manual, certificate	4
96IEXQ42D9MOO--A	Sartobind Q 75 mL, 4 mm, hose barb connectors, manual, certificate	4

Order number	Description	Quantity
96IEXQ42D9MFF--A	Sartobind Q 75 mL, 4 mm, 3/4" sanitary clamp, manual, certificate	4
96IEXS42D9MOO--A	Sartobind S 75 mL, 4 mm, hose barb connectors, manual, certificate	4
96IEXS42D9MFF--A	Sartobind S 75 mL, 4 mm, 3/4" sanitary clamp, manual, certificate	4
96IEXQ42D1GSS	Sartobind Q 200 mL, 4 mm, 1½" sanitary clamp, manual, certificate	1
96IEXS42D1GSS	Sartobind S 200 mL, 4 mm, 1½" sanitary clamp, manual, certificate	1
96IEXQ42D2HSS	Sartobind Q 400 mL, 4 mm, 1½" sanitary clamp, manual, certificate	1
96IEXS42D2HSS	Sartobind S 400 mL, 4 mm, 1½" sanitary clamp, manual, certificate	1
96IEXQ42D3KSS	Sartobind Q 600 mL, 4 mm, 1½" sanitary clamp, manual, certificate	1

Order number	Description	Quantity
96IEXS42D3KSS	Sartobind S 600 mL, 4 mm, 1½" sanitary clamp, manual, certificate	1
96IEXQ42D3NSS	Sartobind Q Jumbo 2.5 l, 4 mm, 1½" sanitary clamp, 2 protective caps, manual, autoclaving instructions, certificate	1
98IEXQ42D-L	Sartobind Q Cassette 0.8 L, 4 mm, 1½" sanitary clamp via manifold set (accessory), manual, certificate	1
98IEXS42D-L	Sartobind S Cassette 0.8 L, 4 mm, 1½" sanitary clamp via manifold set (accessory), manual, certificate	1
98IEXQ42DGL	Sartobind Q Cassette 0.8 L, 4 mm, gamma irradiated, 1½" sanitary clamp via manifold set (accessory), manual, certificate	1

12.2 Products 8 mm bed height

Order number	Description	Quantity
96IEXQ42EUC11--A	Sartobind Q nano 3 mL, 8 mm, Luer female connectors, 2 PEEK adapters Luer male to UNF 10-32 female, manual, certificate	4
96IEXS42EUC11--A	Sartobind S nano 3 mL, 8 mm, Luer female connectors, 2 PEEK adapters Luer male to UNF 10-32 female, manual, certificate	4
96IEXQ42E9BFF	Sartobind Q 150 mL, 8 mm, ¾" sanitary clamp, manual, certificate	1
96IEXS42E9BFF	Sartobind S 150 mL, 8 mm, ¾" sanitary clamp, manual, certificate	1
96IEXQ42E1HSS	Sartobind Q 400 mL, 8 mm, 1½" sanitary clamp, manual, certificate	1
96IEXS42E1HSS	Sartobind S 400 mL, 8 mm, 1½" sanitary clamp, manual, certificate	1
96IEXQ42E2LSS	Sartobind Q 800 mL, 8 mm, 1½" sanitary clamp, manual, certificate	1

Order number	Description	Quantity
96IEXS42E2LSS	Sartobind S 800 mL, 8 mm, 1½" sanitary clamp, manual, certificate	1
96IEXQ42E3FSS	Sartobind Q 1.2 L, 8 mm, 1½" sanitary clamp, manual, certificate	1
96IEXS42E3FSS	Sartobind S 1.2 L, 8 mm, 1½" sanitary clamp, manual, certificate	1
96IEXQ42E3ESS	Sartobind Q Jumbo 5 L, 8 mm, 1½" sanitary clamp, 2 protective caps, 15 membrane discs 30 mm, manual, autoclaving instructions, certificate	1
96IEXS42E3ESS	Sartobind S Jumbo 5 L, 8 mm, 1½" sanitary clamp, 2 protective caps, 15 membrane discs 30 mm, manual, autoclaving instructions, certificate	1
98IEXQ42E-P	Sartobind Q Cassette 1.6 L, 8 mm, 1½" sanitary clamp via manifold set (accessory), manual, certificate	1




Order number	Description	Quantity
98IEXS42E-P	Sartobind S Cassette 1.6 L, 8 mm, 1½" sanitary clamp via manifold set (accessory), manual, certificate	1
98IEXQ42EGP	Sartobind Q Cassette 1.6 L, 8 mm, gamma irradiated, 1½" sanitary clamp via manifold set (accessory), manual, certificate	1





12.3 Accessories

Order number	Description	Quantity
1ZA---0004	Adapter Luer male to UNF 10–32 female, PEEK	1
1ZAOGV0003	Adapter UNF 10–32 female to sanitary $\frac{3}{4}$ ", 25 mm, polyoxymethylene	2
5ZGI--0001	Holder for 1 × 200 to 1,200 mL (10–30") capsule, stainless steel, 3 legs	1
5ZALB-0002	Distribution adapter for 3 × 200 (10–30") to 1200 mL capsules, 1 × 2", 3 × $1\frac{1}{2}$ ", sanitary, stainless steel	1
7ZAL-V0013	Reducing adapter $1\frac{1}{2}$ " (50.5 mm) to $\frac{3}{4}$ " (25 mm), sanitary	1
7ZAL-V0010	Reducing adapter 2" (64 mm) to $1\frac{1}{2}$ " (50.5 mm), sanitary	1
9ZGL--0102	Trolley for Jumbo 2.5 or 5 L, stainless steel	1
16290	Sartocheck 3 Plus Integrity Tester	1
26288---FT	Sartocheck 4 Plus Filter Integrity Tester	1

Order number	Description	Quantity
29Z-S00001	Manifold set for Sartoclear® Sartobind®, 1½" sanitary clamp	2
29Z-S00003	Manifold set for Sartobind®, gamma irradiated, 1½" sanitary clamp	2
2ZGL--0005	Pilot filter holder for Sartoclear® Sartobind®	1
2ZGL--0006	Process filter holder for Sartoclear® Sartobind®	1
2ZGL--0007	Double process filter holder for Sartoclear® Sartobind®	1
2ZGL--0008	Drip pan for Pilot Filter holder	1
2ZGL--0015	Drip pan for Process and double Process Filter Holder	1

13 Dimensions and Connections

			
Membrane volume 4 mm	1 mL	75 mL	200 mL
Membrane volume 8 mm	3 mL	150 mL	400 mL
Size	nano	5"	10"
Dimensions in mm	37×33 H×Ø	190×77 H×Ø	350×100 H×Ø
Connectors	Luer female	Sanitary ¾" 25 mm outer, 14 mm inner diameter"	Sanitary 1½" 50.5 mm outer, 36 mm inner diameter
Hose barb version (H × d) mm	n.a.	203×77	n.a.
Size of hose barb connectors mm	n.a.	12.7–15.7	n.a.
Recommended internal diameter of flexible tube mm	n.a.	12.7 mm	n.a.
Gaskets	n.a.	¾", inner diameter 16 mm	1½", inner diameter 35.8 mm

			
400 mL 800 mL	600 mL 1.2 L	2.5 L 5 L	0.8 L 1.6 L
20"	30"	Jumbo	Cassette
570×100 H×Ø	810×100 H×Ø	850×302 H×Ø	634×387×49 W×L×Ø
Sanitary 1½" 50.5 mm outer, 36 mm inner diameter	Sanitary 1½" 50.5 mm outer, 36 mm inner diameter	Sanitary 1½" 50.5 mm outer, 36 mm inner diameter	Via manifold: Sanitary 1½" 50.5 mm outer, 36 mm inner diameter
n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	n.a.	n.a.
1½", inner diameter 35.8 mm	1½", inner diameter 35.8 mm	1½", inner diameter 35.8 mm	For manifold: 1½", inner diameter 35.8 mm"

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List of Sartorius material numbers applying to EPA-FIFRA

96IEXQ42D1GSS	96IEXS42E3FSS
96IEXQ42D2HSS	96IEXS42E9BFF
96IEXQ42D3KSS	96IEXS42EUC11-A
96IEXQ42D3NSS	98IEXQ42D-L
96IEXQ42D9MFF-A	98IEXQ42DGL
96IEXQ42D9MOO-A	98IEXQ42E-P
96IEXQ42DN-11	98IEXQ42EGP
96IEXQ42DN-11-A	98IEXS42D-L
96IEXQ42E1HSS	98IEXS42E-P
96IEXQ42E2LSS	
96IEXQ42E3ESS	
96IEXQ42E3FSS	
96IEXQ42E9BFF	
96IEXQ42EUC11-A	
96IEXS42D1GSS	
96IEXS42D2HSS	
96IEXS42D3KSS	
96IEXS42D9MFF-A	
96IEXS42D9MOO-A	
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96IEXS42E1HSS	
96IEXS42E2LSS	
96IEXS42E3ESS	