Operating Instructions

Sartobind STIC® PA

Void Volume Optimized Capsules and Cassettes With 4 mm Bed Height

1000032238

SARTORIUS

Product Overview

These instructions are valid for the following products:

4 mm:	nano 1 mL	mini 10 mL	75 mL	200 mL	400 mL

600 mL

Jumbo 2.5 L

Cassette 0.8 L

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 disfonctionnement, 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。**

⚠ **当製品を該当しない用途、あるいは当製品取扱説明書に記載されていない応用分野において使用した場合、当製品の機能上の不具合や損傷、人体への危害、あるいは他の物品の損傷を招く恐れがあります。特に明記のない場合、当製品は滅菌処理されていません。当メンブレンはグリセリンを用いて乾燥させてあります。**

Intended use

The membrane chromatography products – also described as membrane adsorbers – should be used only once for flowthrough (negative) chromatography applications to avoid carryover as well as tedious and costly cleaning validation procedure.

Sartobind STIC® PA nano 1 mL has been developed as a scouting device for working with small sample volumes while retaining the cylindrical design of the large scale membrane adsorbers.

Sartobind® mini 10 capsules have been developed for first scale up trials and preclinical production. This device size closes the gap between the nano and the 75 mL size.

Sartobind STIC® PA 75 mL up to Jumbo 2.5 L have been developed for intermediate and pilot scale up to production scale in the biopharmaceutical industry.

Sartobind® 0.8 L cassettes are used in the Pilot Scale Filter Holder of up to 10.4 L membrane volume for the biopharmaceutical production.

Table of Contents

1 Storage Conditions

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

2 Introduction

The capsules and cassettes with 4 mm bed height are salt tolerant weak anion exchange chromatography devices based on macroporous membranes. They can be used for chromatographic separation in downstream processing of viruses and proteins. The ligand is coupled to the membrane which is fitted into a plastic housing ready to use. The devices are constructed with optimized fluid channels.

The capsules contain a central core and the cassettes a spacer element to minimize void volume. To set up and operate the Sartobind® Jumbo (2.5 liter membrane volume) we recommend the Jumbo trolley (see chapter "11.2 Accessories", page 50). These products are intended for single use to avoid carryover as well as tedious and costly cleaning validation procedures (see also section "7.12 Regeneration and Storage", page 34). They are applied for contaminant removal from proteins and viral | virus like particles (VLP) vaccines in flow-through mode (negative chromatography) to bind DNA, residual protein, host cell proteins (HCP), endotoxins and viruses.

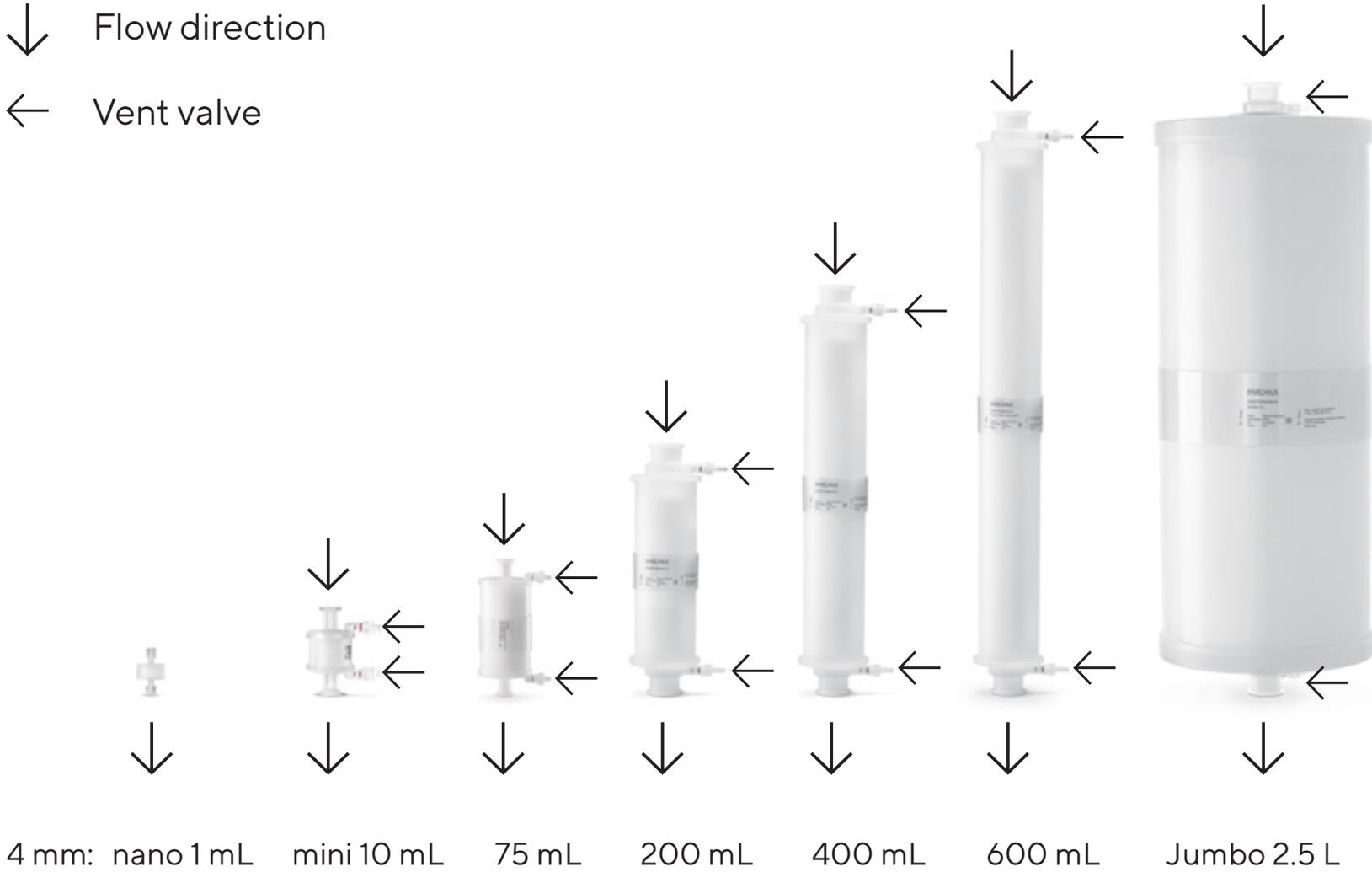
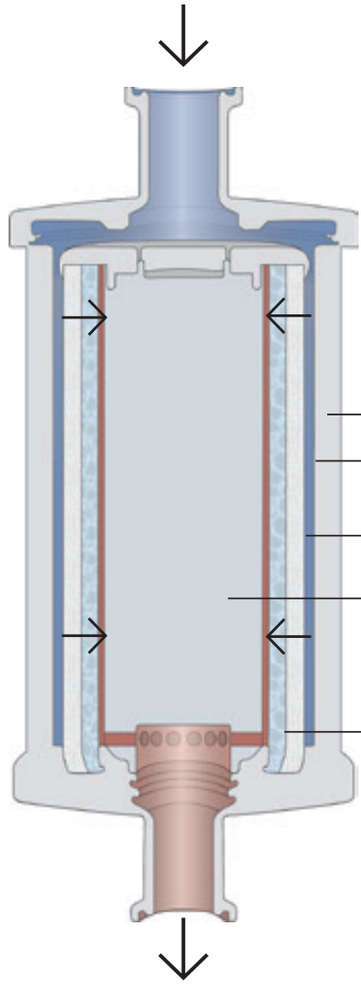


Fig. 1: Flow direction and position of vent valves of 4 mm capsules

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

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



The direction of flow ↓ is imprinted on the banderole of the unit and for the nano, the mini and cassettes on the capsule itself.

Upstream channel
Membrane (4 mm)
Fleece
Central core

Downstream channel

Fig. 3: Construction and flow path inside the capsules

For nano, mini and 75 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 inaccessable for gases and fluids. The two flat membrane stacks of the cassettes are separated by a central spacer element.

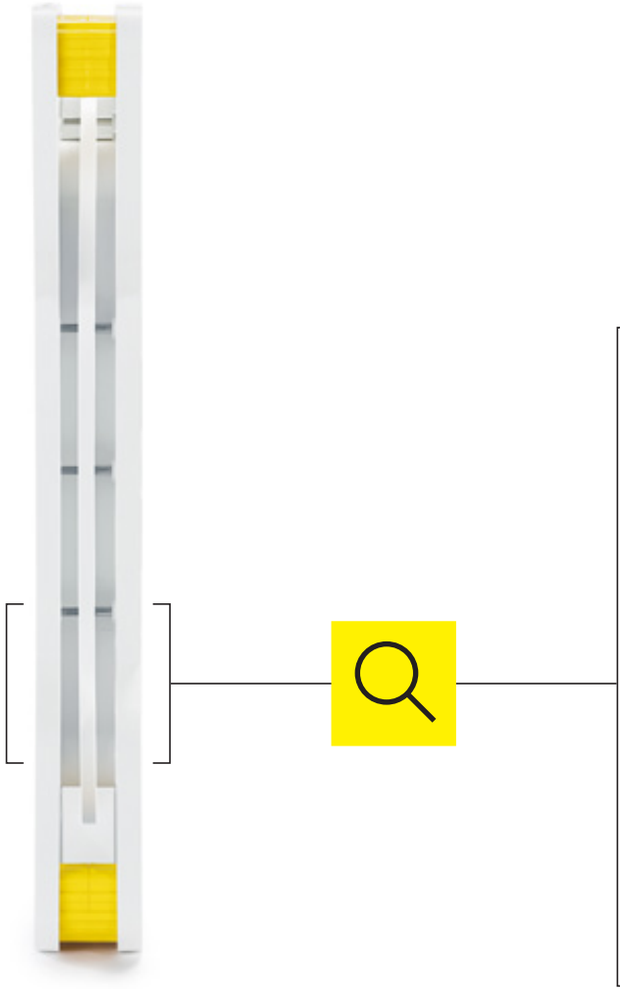


Fig. 4: Side view cassette;
highlighted section
see Fig. 5

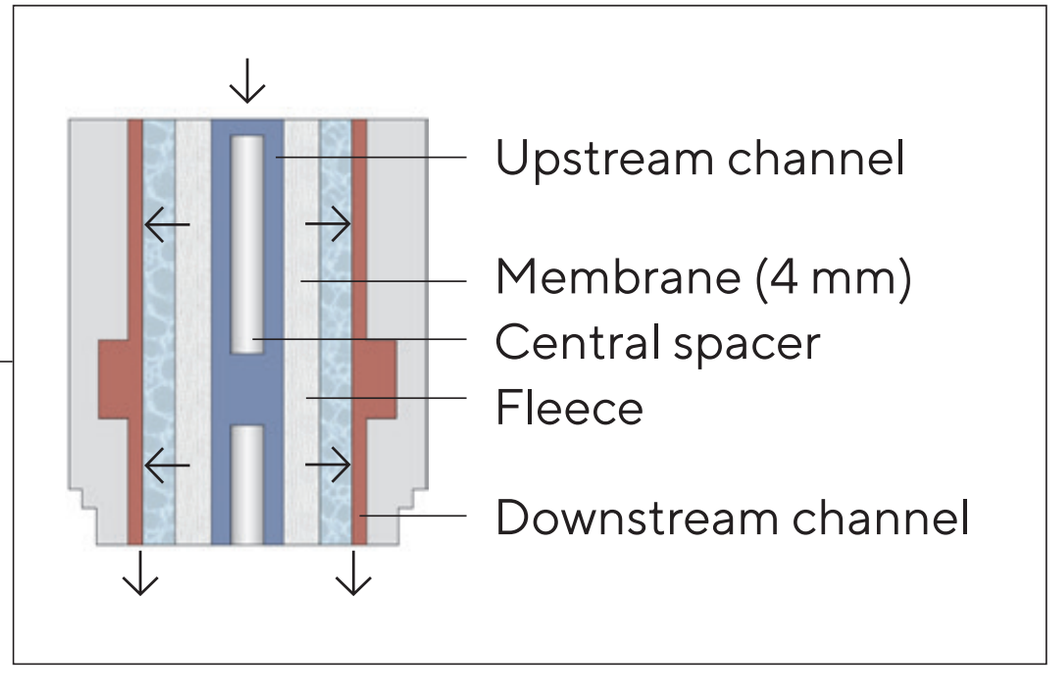


Fig. 5: Construction and flow path inside
the 4 mm cassette

3 Technical Data

Membrane volume (MV)	1 mL	10 mL	75 mL
Nominal membrane area	36.4 cm^2	364 cm^2	2,700 cm^2
Bed height	4 mm	4 mm	4 mm
Design	Cylindrical	Cylindrical	Cylindrical
Sartobind STIC® PA typical 10% dynamic binding capacity*	50 mg	500 mg	3.75 g
Maximum pressure bar (MPa, psig) at 20°C	4 (0.4, 58)	4 (0.4, 58)	4 (0.4, 58)
Maximum pressure during venting bar (MPa, psig) at 20°C	–	0.5 (0.05, 7)	0.5 (0.05, 7)
Nominal void volume (mL)	3.5	32	200
Nominal void volume (MV)	3.5	3.2	2.7
Approximate weight	10 g	65 g	400 g

1 mL membrane = 36.4 cm^2 membrane
Ion capacity per cm^2 of membranes: 18–22 µeq
Short term pH stability 2 – 14 refers to cleaning in place procedure described in section ”7.2 Cleaning and equilibration“, page 26

200 mL	400 mL	600 mL	2.5 L	0.8 L
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
10 g	20 g	30 g	127 g	40
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
540	1,080	1,600	7,000	2500
2.7	2.7	2.7	2.8	3.1
760 g	1.3 kg	1.9 kg	16 kg 20 kg wet 23 kg filled	4.9 kg 6.0 kg wet

* See section ”5 Binding Capacity“, page 19

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 STIC PA	Week anion STIC PA; primary amine (R-NH_2)
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

5 Binding Capacity

Data are based on dynamic binding capactiy measurements at 10% breakthrough using 3 layers of 5 cm^2 membrane discs (15 cm^2 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**
1.4 mg/cm^2 (50 mg/mL)	BSA (bovine serum albumin) in 20 mM Tris/HCl 150 mM NaCl, pH 7.5

6 Installation

The content of the package is described in chapter "11 Ordering Information", page 48. When unpacking capsules, protect the inlet and outlet connectors from damage. Do not keep or place the capsule with the connectors directly on the floor. This might damage the sanitary adapters.

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

Move the Jumbo trolley (see chapter "11.2 Accessories", page 50) 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 with 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 27). Remove before venting.

The capsules and cassettes should be installed in an upright position according to the process flow. In this position the inlet is up. The flow is guided to an external channel passing through the membrane layers to an internal channel and to the outlet of the capsule (see Fig. 3). Install the capsule 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 Sartobind® cassettes you need an appropriate cassette holder and one Manifold Set (see chapter "11.2 Accessories", page 50). Before use please 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, please contact your Sartorius office for technical advice.

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” mark on the manifold should be readable on the top. Place the manifold marked with “OUTLET” at the other end of the holder, so that “THIS SIDE UP” is readable from the top. The fluid channels of both plates are oriented in the same direction.

⚠ **The cassettes must be placed in the lowest possible position in the holder otherwise the system may leak.**
The cassettes used for chromatographic separation must originate from the same lot.

Put the desired number of Sartobind® cassettes between the manifolds (see Fig. 6). Correct orientation is given, when the mark “THIS SIDE UP” is readable on the top.

The clamping force for cassettes in Pilot and Process holders has to be adjusted to a minimum of 25 kN (optimal range: 25 – 30 kN) before use. In the Pilot holder up to 13 cassettes and a manifold set can be installed. Then close all DRAIN and VENT valves of the manifold plates manually using the pinch clamp.

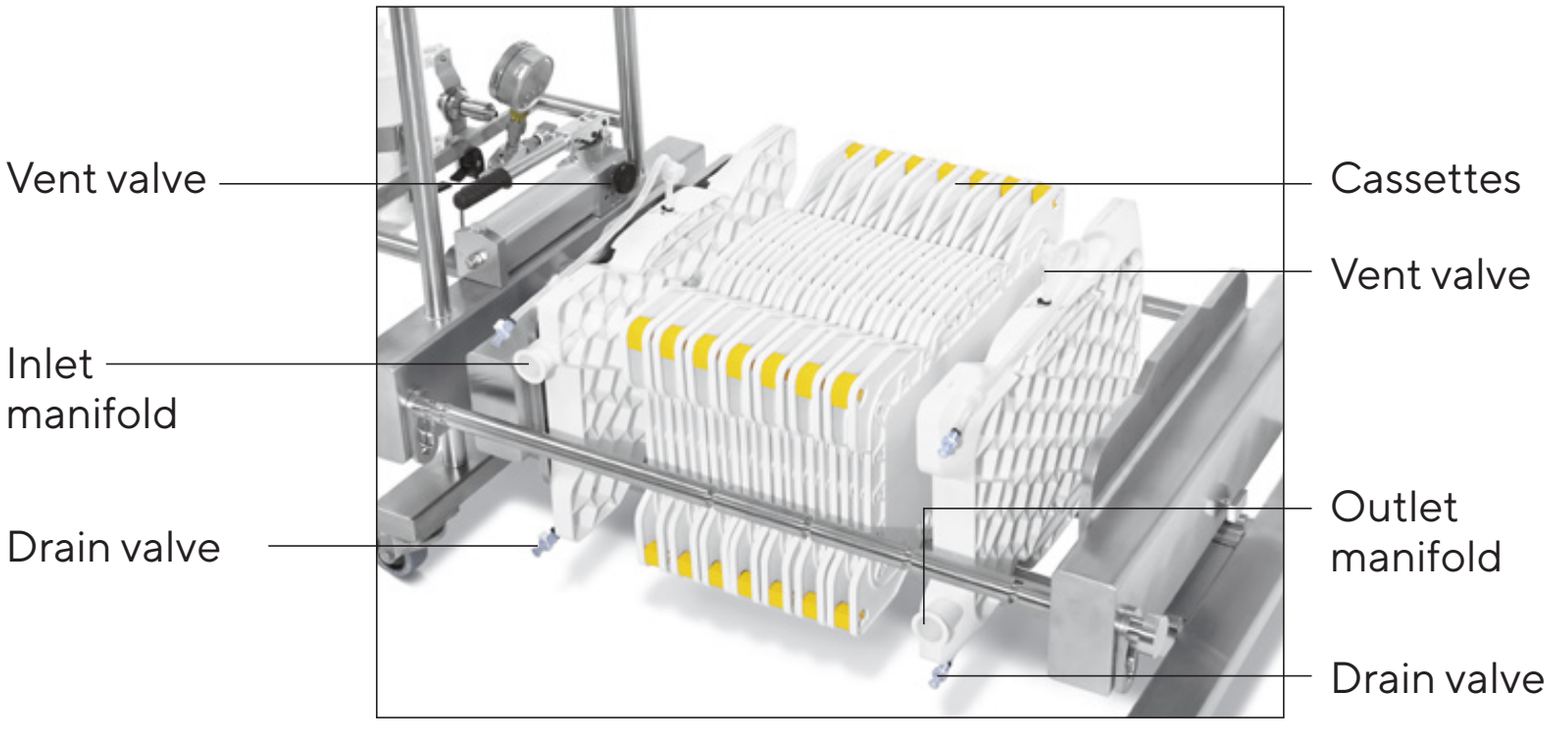


Fig. 6: Insert cassette(s) between the manifold inlet and outlet plates on the Pilot Filter Holder.

⚠ **During flushing the clamping force may reduce. In order to avoid dripping during operation, it is recommended to re-adjust the clamping force to a minimum of 25 kN before you continue with equilibration.**

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 the entire air from the unit before use. All capsules except nano have 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 ⅓ 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 with a pinch clamp.

For nano 1 mL, 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 performance. The capsule function will not be influenced as long as the small air bubbles remain outside of the membrane bed.

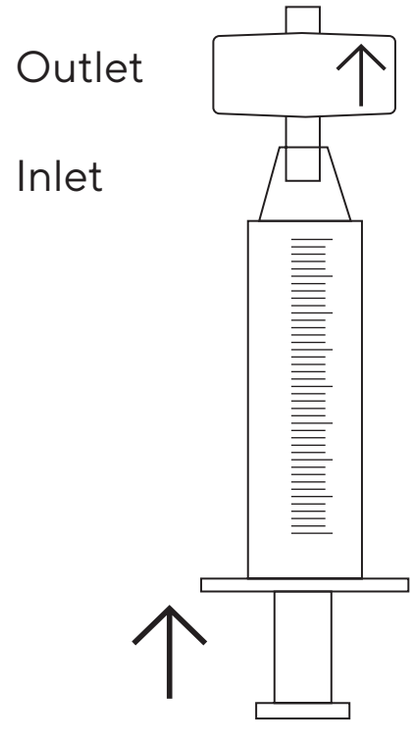


Fig. 7: Filling the Sartobind® nano with a Luer syringe for air removal

7.2 Cleaning and equilibration

The devices have to be cleaned in place directly before use with 1 N NaOH for 30 min at 20°C. Preferentially, work at room temperature as low temperature increases viscosity of solvents. Also cold NaOH can cause swelling of the cellulose matrix significantly which will result in pressure increase.

1. For sanitization use 30 membrane volumes (MV) of 1 N NaOH solution at a flow rate of 1 MV/min.
2. Flush with 100 MV of equilibration buffer (e.g. 20 mM Tris/HCl, 150 mM NaCl, pH 7.5) at 5 MV/min.

7.3 Autoclaving

⚠ **CAUTION!**
The cassette material is not compatible to autoclaving.

The capsules can be autoclaved once at 121°C for 30 minutes at 1 bar (0.1 MPa | 14.5 psi). Pre-wet the capsule with equilibration buffer. When using water you may detect a lower flow rate which is due to swelling.

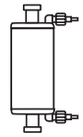
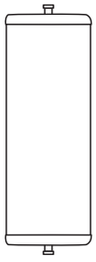
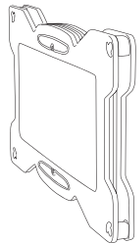
The protective caps enclosed in the Jumbo delivery must be reinistalled on inlet and outlet connectors of the Jumbo. Close valves immediately after sterilisation. For autoclaving of Sartobind® Jumbo refer to separate autoclaving instructions enclosed in delivery.

7.4 Recommended flow rates and equilibration volumes

Membrane adsorbers can be run at much higher flow rate per volume than resin columns. The recommended flow rates for membrane adsorbers with 4 mm bed height are between 10 to 30 membrane volumes per minute. This recommendation is only a guideline as buffers and samples have different compositions and viscosities. Please test your respective flow rates with a small scale device to ensure that the flow rate fits with your pump capacities and the 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 volume is 100 membrane volumes.

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

								
Membrane volume (MV)	1 mL	10 mL	75 mL	200 mL	400 mL	600 mL	2.5 L	800 mL
Rec. flow rate (L/min)	0.02	0.2	1.5	4	8	12	50	16**
Rec. equilibration volume* (L)	0.1	1	7.5	20	40	60	250	80**

* Refer to 7.2 Cleaning and equilibration
** Multiply with number of used cassettes

7.5 Buffer conditions

The ionic strength of buffers used during loading on Sartobind STIC® PA can be higher than for conventional anion exchange membrane adsorbers. Up to 20 mS/cm are possible for Sartobind STIC® PA. The pKa of the chosen buffer should not exceed ± 0.5 pH units of the operation pH. 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.

⚠ **It is recommended to use monovalent buffers e.g. TRIS or Acetate. Multivalent buffers like phosphate or citrate will reduce the binding capacity for proteins. Contaminants such as DNA and endotoxin can still bind depending on character of multivalent buffer. Up to ~150 mM salt can be used to achieve better separation of target molecules from contaminants. Application of pure water may lead to a reversible swelling of the membrane and may reduce permeability.**

7.6 Selection of pH and salt conditions

In ion exchange chromatography a charged molecule is bound to oppositely charged groups attached to the insoluble matrix. This binding is reversible and induced by an increase of the salt concentration in the elution buffer. 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 has a positive net charge and will bind to a cation exchanger (e.g. Sartobind® S). If the pH of the buffer is above its isoelectric point (at least 1 pH unit), it will bind to anion exchangers (e.g. Sartobind® Q or Sartobind STIC® PA).

The amine ligand used for Sartobind STIC® PA is a weak anion exchanger. This means the positive charge is reduced at higher pH. To optimize the binding capacity and load volume, multiple pH values should be tested (e.g. on 96 well plates).

Conventional ion exchangers are loaded at low conductivity. Proteins are easily eluted by adding e.g. 1 M NaCl. Sartobind STIC® PA binding is also influenced by salt but higher levels of salt are needed to elute the molecules. At a level of e.g. 150 mM NaCl where conventional anion exchanges do not bind, the salt tolerant membrane shows good binding capacity. To remove protein from the membrane, higher salt concentration than for conventional ion exchanger are required.

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 should be used. Contaminants include highly negatively charged DNA, endotoxins, protein contaminants, some host cell proteins and viruses. The product of interest, the monoclonal antibody with

isoelectric points (pI) of 8 – 9.5 for example, will not bind and pass through the Sartobind STIC® PA. The influence of the flow rate on the performance is very low.

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 XLG 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 Membrane Adsorber and lead to capacity loss and increased back pressure. We recommend inline filtering during operation. When the pressure increases replace the prefilter.**

7.9 Washing

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

7.10 Elution

To elute the target protein use buffer with appropriate salt concentration. Take into account that Sartobind STIC® PA has been developed for single-use and that the original binding capacity typically cannot be restored due to strong binding of the primary amine ligand to the negatively charged species.

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 device.

⚠ **A dual air regulator system is recommended to prevent over-pressure of the Sartobind® device. The first regulator should reduce line air pressure to 2 bar.**
The second regulator, positioned immediately upstream of the Sartobind® capsule, 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 to 13 cassettes draining pressure.

7.12 Regeneration and Storage

After elution and wash with equilibration buffer a regeneration step with 1 N NaOH for 1 hour can be used to clean Sartobind STIC® PA. However, binding capacity cannot be completely restored. Sartobind STIC® PA can be stored in equilibration buffer with 20% ethanol.

7.13 Chemical stability

The devices are stable for 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 the 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 are typically run at much higher flow rates than columns, there is no risk of bubble formation in the UV cell when removing the flow restrictor.

7.15 Scaling up

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

Recommendations:
Maintain
– Bed height (stay within the same bed height when scaling up)
– Linear flow (when using capsules with same bed height, the flow rate will scale up linear when keeping MV/min constant)
– Sample concentration

Increase (see scaling factors in the following table)
– Sample load volume
– Volumetric flow rate
– Membrane volume

Scale up is done preferably by keeping the bed height constant and adjusting the membrane volume. This will make the calculation simple. Other methods for scale up via residence time will lead to same results. Residence time is calculated by the membrane volume divided by the flow rate.

When using Sartobind® nano 1 mL, the scale up factor for flow rate and binding capacity is equal to the multiplication factor of the membrane volumes for the listed scale up devices:

Size	Membrane volume [mL]	Factor to increase* (from nano)
nano	1 mL	-
mini	10 mL	10
5"	75 mL	75
10"	200 mL	200
20"	400 mL	400
30"	600 mL	600
Jumbo	2.5 L	2500
Cassette	800 mL	800
Cassettes**	10.4 L	10,400

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

Example: After breakthrough experiments with the nano you determined that a 500 fold higher binding capacity is needed. Then you choose the 600 mL capsule. Then adjust the flow rate by a factor of 600.

⚠ **Keep sample concentration constant in lab and production scale. Adjustments might be required due to additional volumes from tubing and the system.**

8 Integrity Test by Diffusion

The integrity of the membrane adsorber can be tested 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.

8.1 Installation

Install capsule as shown in Fig. 8.

The test procedure has been developed and checked with the Sartocheck® instrument family e.g. Sartocheck® 4 Plus (26288) or 4 (16288). The use of Sartocheck® instruments older than Sartocheck® 4 will generate faulty data.

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

8.2 Operation procedure

8.2.1 Pre-washing of device

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

⚠ **The capsule needs to be pre-washed with the testing solvent, to remove any glycerol. The washing solution should have 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 the testing solvent.**

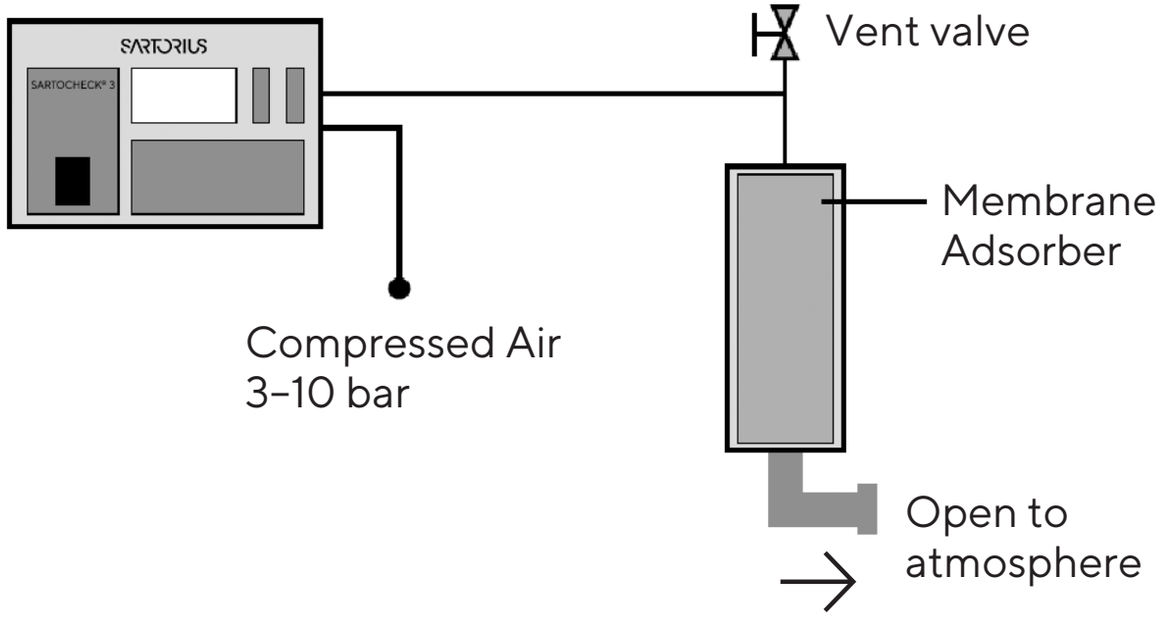


Fig. 8: Set up of diffusion test with Sartocheck®.

8.2.2 Diffusion measurement with Sartocheck®

– Choose “Programming” in the main menu
– Choose “Diffusion Test”

Choose the test pressure, stabilization and testing time for your device from the table (next page). 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 (MV)	Test pressure mbar (psi)	Stabili-sation time (min)	Testing time (min)	Diffusion max. mL/min
nano	4 mm	1 mL	100 (1.45)	3	1	15
mini	4 mm	10 mL	100 (1.45)	2	1	15
5"	4 mm	75 mL	100 (1.45)	2	1	75
10"	4 mm	200 mL	100 (1.45)	3	1	75
20"	4 mm	400 mL	100 (1.45)	3	1	75
30"	4 mm	600 mL	100 (1.45)	3	1	75
Jumbo	4 mm	2.5 L	100 (1.45)	3	1	150
Cassette(s)*	4 mm	0.8 – 10.4 L	100 (1.45)	5	1	15 – 195

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

8.2.3 Results and evaluation for one device

– 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
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 24.
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 pre-filter 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 | mem-brane fouling	Replace unit. You may backflush within given flow and pressure limits, perform a regeneration cycle.
	Viscosity | swelling effects	Work at room temperature, avoid lower tem-peratures
	Pure water leads to swelling of membrane	Add sodium chloride or use ionic buffers
I have to use water for wetting the capsules	Economic consideratons; easier usage	You may use pure water but you have to expect a decreased flow rate which may lead to a higher backpressure. The use of water will not change diffusion testing values. The flow rate returns to normal after using buffer again.

Problem	Possible cause	Action
Target molecule is not bound	Conditions for binding are insufficient	Process conditions, e.g. prefiltration, pH, conductivity, multivalent buffers etc. have to be checked and optimized. Sartobind STIC® membrane is salt tolerant.
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.
Incomplete elution	Strong binding	Use capsule only for polishing in flowthrough mode.
I want to reuse the STIC capsules	Economic reasons	The ligand strongly binds the contaminants and cleaning with 1 N NaOH does typcially not restore 100 % of the binding capacity depending on the character of your sample and the contaminants. Sartobind STIC® has been developed for mainly single-use application to avoid the revalidation.

Problem	Possible cause	Action
I need to remove DNA | endotoxins but I have to use PBS	I heard PBS is a multivalent buffer which reduces binding capacity	Go ahead with the PBS as DNA is a high negatively charged species and will bind to STIC. The same accounts for endotoxins. You may even work at pH conditions which normally would not be accessible as target protein could be bound.
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.

10 Quality Assurance

The final Sartobind® products are tested for protein dynamic binding capacity and flow rate. Sartobind® membranes are tested for protein dynamic binding capacity, flow rate, thickness, and eveness.

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.

11 Ordering Information

11.1 Products

Order number	Description and type of connectors	Quantity
96STPA42DN-11—A	Sartobind STIC® PA nano 1 mL, 4 mm, Luer female connectors, 2 PEEK adapters Luer male to UNF 10 – 32 female, manual, certificate	4
96STPA42D4R11--A	Sartobind STIC® PA mini 10 mL, 4 mm, Luer female connectors, 8 PEEK adapters Luer male to UNF 10–32 female, manual, certificate	4
96STPA42D4RFF--A	Sartobind STIC® PA mini 10 mL, 4 mm, ¾" sanitary clamp, manual, certificate	4
96STPA42D4ROO--A	Sartobind STIC® PA mini 10 mL, 4mm, hose barb connectors, manual, certificate	4
96STPA42D9MFF--A	Sartobind STIC® PA 75 mL, 4 mm, ¾" sanitary clamp, manual, certificate	4
96STPA42D1GSS	Sartobind STIC® PA 200 mL, 4 mm, 1½" sanitary clamp, manual, certificate	1

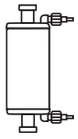
Order number	Description and type of connectors	Quantity
96STPA42D2HSS	Sartobind STIC® PA 400 mL, 4 mm 1½" sanitary clamp, manual, certificate	1
96STPA42D3KSS	Sartobind STIC® PA 600 mL, 4 mm, 1½" sanitary clamp, manual, certificate	1
96STPA42D3NSS	Sartobind STIC® PA Jumbo 2.5 L, 4 mm 1½" sanitary clamp, 2 protective caps, manual, autoclaving instructions, certificate	1
98STPA42D-L	Sartobind STIC® PA Cassette 0.8 L, 4 mm, 1½" sanitary clamp via manifold set (accessory), manual, certificate	1

11.2 Accessories

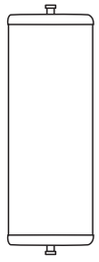
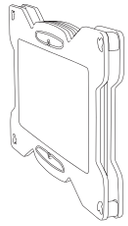
Order number	Description	Quantity
1ZA---0004	Adapter Luer male to UNF 10 – 32 female, PEEK	1
1ZAOGV0003	Adapter UNF 10 – 32 female to sanitary ¾“, 25 mm, polyoxymethylene	2
5ZGI--0001	Holder for 1 × 200 to 1,200 mL (10 – 30“) capsule, stainless steel, 3 legs	1
5ZALB-0002	Distribution adapter for 3 × 200 (10 – 30") to 1200 mL capsules, 1 × 2“, 3 × 1½“, sanitary, stainless steel	1
7ZAL-V0013	Reducing adapter 1½" (50.5 mm) to ¾“ (25 mm), sanitary	1
7ZAL-V0010	Reducing adapter 2" (64 mm) to 1½" (50.5 mm), sanitary	1
9ZGL--0102	Trolley for Jumbo 2.5 or 5 L, stainless steel	1
16288	Sartocheck® 4 Plus Integrity Tester	1

Order number	Description	Quantity
26288---FT	Sartocheck® 4 Plus Filter Integrity Tester	1
29Z-S00001	Manifold set for Sartoclear® | Sartobind®, 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

12 Dimensions and Connections

Membrane volume 4 mm bed height	 1 mL	 10 mL	 75 mL
Size	nano	mini	5″
Dimensions in mm	37 × 31 H × Ø	Luer: 70 × 54.5 Sanitary: 100 × 54.5 Hose barb: 110 × 54.5 H × Ø	190 × 77 H × Ø
Connectors	Luer female	– Luer female – Sanitary ¾″, 25 mm outer, 14 mm inner Ø – Hose barb ½″, 12.7 mm*	Sanitary ¾″ 25 mm outer, 14 mm inner Ø
Gaskets	n.a.	¾″, inner Ø 16 mm	¾″, inner Ø 16 mm

n.a.=not available | * Recommended internal diameter of flexible tube: ½″, 12.7 mm

				
200 mL	400 mL	600 mL	2.5 L	0.8 L
10″	20″	30″	Jumbo	Cassette
350 × 100 H × Ø	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 Ø	Sanitary 1½″ 50.5 mm outer, 36 mm inner Ø	Sanitary 1½″ 50.5 mm outer, 36 mm inner Ø	Sanitary 1½″ 50.5 mm outer, 36 mm inner Ø	Via manifold: Sanitary 1½″ 50.5 mm outer, 36 mm inner Ø
1½″, inner Ø 35.8 mm	1½″, inner Ø 35.8 mm	1½″, inner Ø 35.8 mm	1½″, inner Ø 35.8 mm	For manifold: 1½″, inner Ø 35.8 mm

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

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List of Sartorius material numbers applying to EPA-FIFRA

96STPA42D1GSS
96STPA42D2HSS
96STPA42D3KSS
96STPA42D3NSS
96STPA42D9MFF--A
96STPA42DN-11--A
98STPA42D-L