sartorius stedim biotech

Operating Instructions

Sartobind® Selection Kit

pico 0.08 mL with Q, S and STIC PA membranes

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

85037-545-67

Read operational instructions carefully before using Sartobind capsules.

⚠ Warning

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 capsules are supplied as non-sterile. The membrane is dried from glycerol. For in vitro use only.

⚠ Achtung

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 Kapsulen sind nicht steril. Die enthaltene Membran wird aus Glycerin getrocknet. Nur für den In-vitro-Einsatz.

⚠ Attention

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 capsules sont fournies non-stériles. La membrane est séchée avec de la Glycérine. Pour usage in vitro uniquement.

⚠ Advertencia

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. Las cápsulas suministradas en este producto no son estériles. La membrana es de secado de Glicerina. Solo para su uso in vitro.

⚠ Attenzione

L'utilizzo dei prodotti per applicazioni non specificate o non descritte in questo manuale, può comportare un malfunzionamento, un danno al prodotto stesso o a persone o cose.
Le capsule sono fornite non-sterilizzate. La membrana è asciugata da glicerina. Solo per uso in vitro.

⚠ 주의 사항

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⚠ 警告

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。

⚠ 警告

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Intended use

The products are intended for single use to avoid Carry-over as well as tedious and costly cleaning validation procedure. Sartobind pico 0.08 ml is used for process development when only Small sample quantities are available.

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1. Storage conditions

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

2. Introduction

Sartobind Membrane Adsorbers are ion exchange chromatography devices based on macroporous membranes. They can be used in downstream processing for protein purification as single-use chromatography capsules. The ion exchange ligands are coupled to a membrane and fit into a plastic housing for quick handling, making ion exchange purification nearly as easy as filtration.

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, or also capture of large molecules like blood coagulation factors, virus, virus like particles or vaccine material.

Sartobind pico is the smallest member of the Sartobind capsule family with 4 mm bed height. The small membrane volume of 0.08 ml reduces material consumption during testing and virus spiking studies to save cost during the initial development phases.

Sartobind pico 0.08 ml has a relatively large void volume compared to the Sartorius void volume optimized product line and therefore it is intended for polishing applications in flowthrough mode. If Sartobind is tested for capture application, the binding capacity has to be confirmed with void volume optimized devices, e.g. Sartobind nano 3 mL since the smaller void volume has a positive effect on binding capacity.

Fig. 1: Four types of Sartobind pico 0.08 mL (the Phenyl pico, HICP above, is not in the selection kit due to different storage requirements.)

3. Technical Data

Package contents	Each one of Sartobind Q pico Sartobind STIC PA pico Sartobind S pico Operating instruction 2 adapters Luer male to UNF 10-32 female PEEK
Dimension pico (height × diameter)	31 × 11 mm
Connectors	Luer female
Approx. weight pico	1.5 g
Housing material	Polypropylene
Base membrane	Stabilized reinforced cellulose
Nominal pore size	>3 µm
Bed height	4 mm
Bed volume	0.08 mL
Adsorption area	2.9 cm^2

Membrane types and ligands	Strong basic anion exchanger: quaternary ammonium (Q) $R\text{-}CH_2\text{–}N^+(CH_3)_3$ Salt tolerant anion exchanger STIC PA: Primary amine (PA) Strong acidic cation exchanger: sulfonic acid (S) $R\text{-}CH_2\text{–}SO_3^-$
Ligand dencity [µeq/cm²]	Q, S: 2–5 STIC PA: 18–22
Typical dynamic binding capacity* at 10 % breakthrough per area or volume of membrane	Q: 0.8 mg/cm² | 29 mg/mL S: 0.7 mg/cm² | 25 mg/mL STIC PA 1.4 mg/cm² | 50 mg/mL
Recommended flow rate**	10 – 30 membrane volumes per minute
Maximum pressure at 20°C	6 bar (0.6 MPa, 87 psi)

Short term*** pH stability	Q and STIC PA: 2–14 S: 3–14
Chemical stability	Stable against commonly used buffers in chromatography. Avoid oxidizing agents. Membranes are dried from glycerol to avoid shrinking.

* See also section 3.1 Binding capacity
** See also section 4.7 Recommended flow rates
*** Short term refers to cleaning procedure described in section 4.6 Preconditioning

3.1 Binding capacity

The following data is based on the typical dynamic binding capacity at 10 % breakthrough measured with 3 layers of 5 cm^2 membrane discs (total area 15 cm^2) stacked into a holder and run at10 mL/min.

Membrane type	Reference protein and buffer	Binding capacity pico 0.08 mL
Quaternary ammonium (Q)	1 mg/mL bovine serum albumin in 20 mM Tris/HCl pH 7.5	2.3 mg
Primary amine (PA)	As above + 150 mM NaCl	2.0 mg
Sulfonic acid (S)	1 mg/mL Lysozyme in 10 mM potassium phosphate, pH 7.0	4.0 mg

4. Operation

4.1 Buffer conditions

4.1.1 Q and S

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. It is recommended to use a buffering ion with the same charge as the membrane, i.e. buffers with positive charges (e.g. amine buffers such as Tris) shall be used with Q type exchangers. Negatively charged buffers (e.g. phosphate buffers) shall be used with S type exchangers. The buffer should have a pKa within 0.5 pH units of the working pH. Buffers and prepared samples should ideally have an ionic strength below 50 mM. Higher salt levels may restrict binding of proteins but not DNA or endotoxins. Standard PBS buffer should not be used as it contains, along with other salts, 137 mM NaCl, which will significantly reduce protein binding to the ion exchange membrane.

⚠ Important

Application of pure water may lead to a reversible swelling of the membrane and may reduce permeability.

4.1.2 STIC PA

PA membrane is an anion exchange membrane. Its unique character is that ionic strength of buffers during loading can be much higher than for conventional anion exchange Membrane Adsorbers. Otherwise refer to 4.1.1 for recommended conditions for ion exchange membranes.

⚠ Important

It is recommended to use monovalent buffers e.g. TRIS or acetate. Multivalent buffers like phosphate or citrate can reduce binding capacity for proteins but not necessarily for contaminants such as DNA or endotoxins.

4.2 Sample preparation

The sample should be adjusted to the equilibration buffer conditions and be pre-filtered through a 0.2 µm membrane filter.

⚠ Important

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

4.3 Flow direction

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

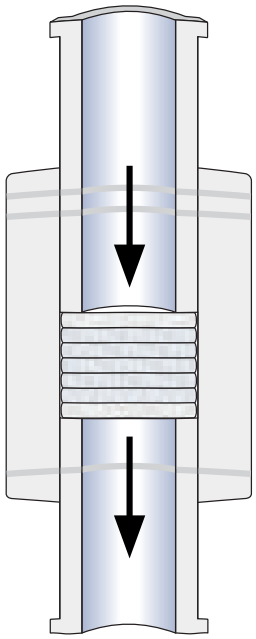

Fig. 2: Flow pattern inside Sartobind pico capsule

⚠ Important

Capsules should be visually inspected before use.
In case of damage, the device has to be replaced.

4.4 Venting

It is important to remove air from the device completely. Fill a 10–20 mL Luer syringe with equilibration buffer and connect it to the pico device. Hold capsule upright (outlet is up) and expel air as shown in Fig. 3. Then connect syringe to outlet and purge in the opposite direction to remove very small air bubbles.

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.

Then connect a filled syringe to pico outlet, connect an inline prefilter to pico inlet and vent in the opposite side. The prefilter should be stable against 1 N NaOH. If not, it shall be connected after preconditioning (see 4.6). Use of inline prefilter 0.2 µm is strongly recommended.

Now the pico device can be connected to a liquid chromatography (LC) system or a peristaltic pump (for whole procedure with syringe without LC system, refer to 4.8 Operation with syringe).

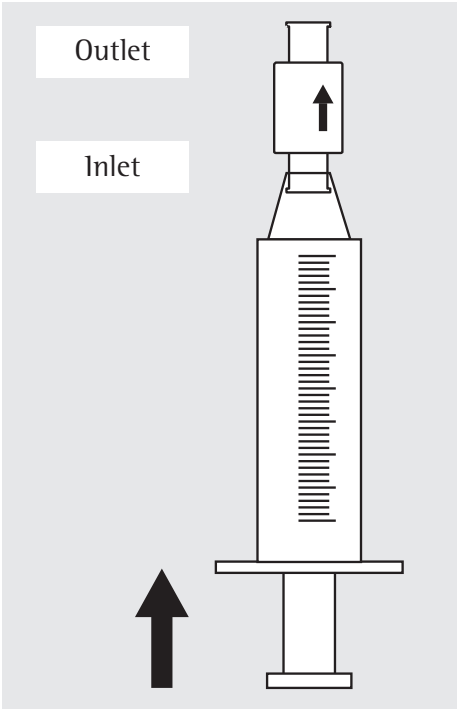

Fig. 3: Filling the Sartobind pico with a Luer syringe

4.5 Installation in LC system or peristaltic pump

To prepare the LC system for use with the Sartobind pico device, measure the systems flow rate per minute – e.g. with a graduated cylinder or through weighing with a laboratory balance at the chosen flow rate. This prevents deviations of pico breakthrough measurements to binding capacity results with the larger capsules. The Sartobind device should be filled as described in chapter 4.4 (p. 16). Start the LC system or peristaltic pump at a

low flow rate. When fluid emerges, connect the tubing to the inlet of the Sartobind pico. 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. Additionally, it may be necessary to simplify the flow path as much as possible, by removing unnecessary valves, mixers, etc., in order to achieve the desired flow rates within the pressure limitations of the pico. For additional information please refer to Sartorius Application Note 85037-545-50 “Using the Sartobind Pico”.

4.6 Preconditioning

Prior to sample loading, a sanitization and flushing procedure should be performed.

A sufficient flushing with equilibration buffer is required to stabilize the pH value. Due to the void volume of the LC 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.

4.6.1 Q and S

1. For sanitization use 30 membrane volumes (MV) of 1 N NaOH solution at a flow rate of 1 MV/min. This sanitization step should take at least 30 minutes. If a higher flow rate is applied, the volume of the NaOH solution should be increased accordingly.
2. First flushing with 50 MV of 1 N NaCl at 5 MV/min
3. Second flushing with 50 MV equilibration buffer (e.g. 20 mM Tris/HCl, pH 7.5) at 5 MV/min

If it is difficult to set the flow rate above, use 10 MV/min.

4.6.2 STIC PA

1. For sanitization use 30 membrane volumes (MV) of 1 N NaOH solution at a flow rate of 1 MV/min. This sanitization step should take at least 30 minutes. If a higher flow rate is applied, the volume of the NaOH solution should be increased accordingly.
2. Flush with 100 MV of equilibration buffer (e.g. 20 mM Tris/HCl, 150 mM NaCl, pH 7.5) at 5 MV/min.

If it is difficult to set the flow rate above, use 10 MV/min.

After the step 4.6.1 or 4.6.2 connect a filled sterile prefilter to pico inlet. Use of inline prefilter 0.2 µm is strongly recommended (The prefilter is added at this step in the event that the chosen filter is not compatible with1 N NaOH; see also section 4.4).

4.7 Recommended flow rates

Membrane adsorbers can be run at much higher flow rate than 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 since buffers and samples have different compositions and viscosities. Membranes can be operated also at lower flow rates without any loss of performance. Please consider that lowering the flow rate will not improve binding capacity and cold room temperature decreases the flow rate.

4.8 Operation with syringe

Sartobind pico can be operated manually with a syringe. However, it requires some effort to push some solutions through the pico device.

Refer to previous section 4.5 and replace the procedure with a syringe instead of a LC system or a peristaltic pump (e.g. For manual preconditioning, use the same volume of sanitization solutions and buffers and push through the pico with a syringe slowly).

5. Troubleshooting

Problem	Possible cause	Action
Break through data of Sartobind pico do not fit to larger capsules	LC pump provides different flow rates 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. 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. 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 visible 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 4.4 Venting.
Capsule is installed 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 such 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.

For optimization of the procedure see also Sartorius Application Note 85037-545-50 “Using the Sartobind pico”.

6. Ordering information

Sartobind pico

Order number	Description	Quantity
92MU0142DD-11	Sartobind Selection Kit pico	1 each of Q, S and STIC PA
92IEXQ42DD-11--D	Sartobind Q pico 0.08 mL	10
92IEXS42DD-11--D	Sartobind S pico 0.08 mL	10
92STPA42DD-11--D	Sartobind STIC PA pico 0.08 mL	10
92HICP42DD-11--D	Sartobind Phenyl pico 0.08 mL	10

Each pico package contains 2 adapters Luer male to UNF 10 – 32 female, PEEK

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