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Operating Instructions

Sartobind[®] pico 0.08 mL

Sartobind Q, S, STIC PA and Phenyl membrane adsorbers

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



85037-545-67

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



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



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



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.



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.



경고

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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 with Q, S or STIC PA membranes should be stored clean, dry and away from direct sunlight in the box at room temperature.

Sartobind Phenyl pico should be stored at room temperature in a clean, dry and dark place. When not in use, the end caps of the pico should be attached to the units to avoid oxygen all the times. Change of membrane color can appear after inappropriate storage (oxygen and | or light exposure). A color change however does not affect adsorptive properties of the membrane.

2. Introduction

Sartobind membranes

Traditional chromatography uses porous particles packed into columns. Target molecules in the liquid diffuse into the pores of the beads where the majority of the binding sites are located. The limiting factor is the time required for the molecules to diffuse into and out of the pores. The various steps of equilibration, loading, washing, elution and regeneration can take hours. Sartobind membranes are macroporous and can be operated at high flow rate. The base material is regenerated and stabilized cellulose. The stabilization and cross-linking brings high chemical

stability. Conventional ion exchange ligands are covalently attached to the membrane support. The chromatographic bed is formed by membrane layers and is incorporated into multi-well plates or housings. Sartobind membrane adsorbers are known for their ease of handling and can simplify the tedious procedures associated with chromatography.

They can be used in downstream processing for protein purification as single-use chromatography capsules with a re-use option. 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

Sartobind pico is the smallest member of the Sartobind capsules. It has the 4 mm bed height as does the larger devices recommended for polishing applications. The small membrane volume reduces material consumption during testing and virus spiking studies. It leads to cost savings during initial development phases.

Sartobind pico devices can be used also for screening of operating conditions such as pH, conductivity and buffer compositions in the downstream processing of therapeutic proteins, e.g. for impurity removal from proteins in flow-through mode.

Sartobind pico 0.08 ml has a relatively large void volume compared to the rest of the 4 and 8 mm capsules. These capsules have been void volume optimized, but this fact does not preclude use of the Sartobind pico capsules in process development.

(The 4 mm product line consists of: nano 1 mL, 75 mL, 200 mL, 400 mL, 600 mL and 2.5 L; the 8 mm bed height line consists of nano: 3 mL, 150 mL, 400 mL, 800 mL, 1.2 L and 5 L, see also 7. Ordering information). The smaller void volume has a positive effect on binding capacity. Therefore after optimal conditions are found with pico devices, Sartobind nano capsules should be used for estimation of binding capacity or the absolute removal of contaminants (virus, endotoxin etc.) for further scale up. This is especially important for capture applications.

Chromatography Principles

The Sartobind pico 0.08 mL devices are available in four different membrane functionalities to cover ion exchange applications and hydrophobic interaction chromatography.

Ion exchange chromatography

Sartobind Q, S and STIC PA pico devices use the basic principle of ion exchange (IEX) separation accomplished on the basis of charges carried by solvent molecules. Sartobind STIC PA is a salt tolerant anion exchange chromatography membrane and can be used at higher conductivity than Sartobind Q.

Hydrophobic interaction chromatography

Sartobind Phenyl pico use the principle of hydrophobic interaction chromatography (HIC) that separates and purifies biomolecules based on differences in their hydrophobicity. On average 50% of a protein or peptide surface is accessible for hydrophobic interaction. Buffers with high concentrations of salt promote the adsorption of proteins on the hydrophobic membrane matrix. The effect of anions and cations on protein precipitation is described in the Hofmeister series:

Anions: PO_4^{3-} , SO_4^{2-} , $\text{CH}_3\text{-COO}^-$, Cl^- , Br^- , NO_3^- , ClO_4^- , I^- , SCN^-
Cations: NH_4^+ , K^+ , Na^+ , Cs^+ , Li^+ , Mg^{2+} , Ca^{2+}

Increasing precipitation
Salting out effect
Stronger binding

Increasing chaotropic
Salting in effect
Weaker binding

Typically ammonium sulfate containing salting-out buffers are used to promote ligand-protein interaction. With increased concentration more protein is bound until the protein precipitates. Preferably, the protein binding is performed in the region where the amount of bound protein increases linearly with the salt concentration. Proteins are eluted by decreasing the salt concentration in the elution buffer. Using step or linear gradient elution proteins are eluted in the order of their hydrophobicity. In contrast to HIC, reverse phase chromatography uses ligands in much higher density. This results in higher hydrophobicity: HIC matrices allows more gentle separation conditions in which the molecular structure of the protein is preserved.



Fig. 1: Four types of Sartobind pico 0.08 mL are available. For the content of the package refer to chapter Ordering Information chapter 7.

3. Technical Data

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
Frontal surface area	0.19 cm^2
Void volume	0.4 mL
Connectors	Luer female
Housing material	Polypropylene
Dimension (height \times diameter)	31 \times 11 mm
Approx. weight	1.5 g

Membrane types and ligands	<p>Strong basic anion exchanger: quaternary ammonium (Q) $R-CH_2-N^+(CH_3)_3$</p> <p>Salt tolerant anion exchanger STIC PA: Primary amine (PA)</p> <p>Strong acidic cation exchanger: sulfonic acid (S) $R-CH_2-SO_3^-$</p> <p>Phenyl</p> <p>Hydrophobic interaction membrane: Phenyl</p>
Ligand density [$\mu eq/cm^2$]	<p>Q, S: 2–5</p> <p>STIC PA: 18–22</p> <p>Phenyl: 3</p>
Typical dynamic binding capacity* at 10 % breakthrough per area or volume of membrane	<p>Q: 0.8 mg/cm² 29 mg/mL</p> <p>S: 0.7 mg/cm² 25 mg/mL</p> <p>STIC PA: 1.4 mg/cm² 50 mg/mL</p> <p>Phenyl: 0.2 mg/cm² 7.3 mg/mL</p>
Recommended flow rate	<p>10 – 30 membrane volumes/min </p> <p>0.8 – 2.4 mL/min</p>

Max. pressure at 20°C	6 bar (0.6 MPa, 87 psig)
Max. pressure during venting***	0.5 bar (0.05 MPa, 7 psig)
Short term**** pH stability	Q STIC PA and Phenyl: 2–14 S: 3–14
Chemical stability	Stable in common chromatography buffers, unstable to peroxide and other oxidizing or reactive reagents

Membranes are dried from glycerol to avoid shrinking.

- * See also section 3.1. Binding capacity
- ** See also section 4.7 Recommended flow rates
- *** Venting not valid for Pico | Nano for handling by syringe
- **** 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 cm² membrane discs (total area 15 cm²) stacked into a holder and run at 10 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	4.0 mg
Sulfonic acid (S)	1 mg/mL Lysozyme in 10 mM potassium phosphate, pH 7.0	2.0 mg
Phenyl	1 mg/mL bovine blood gamma globulin in 50 mM potassium phosphate, pH 7.5, 0.9 M (NH ₄) ₂ SO ₄	0.6 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.

In ion exchange chromatography, a charged molecule is bound to oppositely charged groups attached to the insoluble matrix. This binding is reversible by application of salt ions to the buffer eluting the molecule. The pH value at which a biomolecule has

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



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

4.1.2 STIC PA

Primary amine (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 (e.g. at a level of 150 mM NaCl). Please refer also to the general recommendation for ion exchanger in the chapter 4.1.1 Q and S.

Consider the character of the amine ligand of Sartobind STC PA as a weak anion exchanger. It means that approaching higher pH the positive charge lowers. To optimize the binding capacity and load volume, multiple pH values should be tested (e.g. 96 well plates). To elute bound proteins from the membrane, higher salt concentration than for conventional ion exchanger shall be needed.



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.

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

4.1.3 Phenyl

Proteins are bound to the phenyl membrane at salt concentrations typically above 400 mM. Larger proteins or monoclonal antibody aggregates tend to bind at ammonium sulphate concentrations above 200 mM. This allows for the removal in flow-through mode. Differences in protein hydrophobicity have influence on the choice of salt concentration. The strength of the interaction depends mainly on salt concentration but also on the number of exposed hydrophobic groups of the sample and on membrane ligand type and density. Sample properties, temperature, type and pH as well as additives influence the binding process as well. The character of the binding buffer will decide the success of the separation. It is therefore important to optimize the equilibration | start buffer with respect to pH, type of solvent and salt concentration.

Binding buffer examples

To bind IgG	0.8 M $(\text{NH}_4)_2\text{SO}_4$ in 50 mM potassium phosphate, pH 7.5
To bind bovine serum albumin or lysozyme	2 M $(\text{NH}_4)_2\text{SO}_4$ in 50 mM potassium phosphate, pH 7.0

Choose salt concentrations as low as possible to bind the protein. Higher salt concentrations may result in precipitation.

Commonly used salts Remarks

$(\text{NH}_4)_2\text{SO}_4$	Typical choice, often best results, not stable at >pH 8
Na_2SO_4	Solubility of proteins reduced
NaCl	3 – 4 M needed
KCl	No special remarks
$\text{CH}_3\text{COONH}_4$	No special remarks

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 (e.g. 16532-K for polyethersulfone or 16534-K for cellulose acetate membrane).



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.

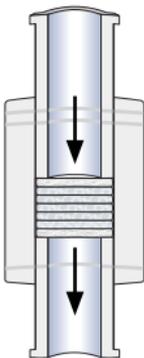


Fig. 2: Flow path inside Sartobind pico capsule



**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).

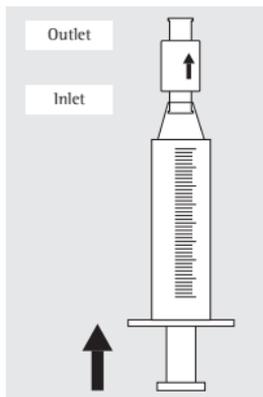


Fig. 3: Filling the Sartobind pico with a Luer syringe

4.5 Operation with syringe

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

Refer to the following chapters 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).

4.6 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 break-through measurements to binding capacity results with the larger capsules. The Sartobind device should be filled as described in chapter 4.4 (p. 23). 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".

4.7 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.

4.7.1 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.

4.7.2 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.

4.7.3 Phenyl

1. For sanitization use 30 membrane volumes (MV) of 1 N NaOH solution at a flow rate of 1 MV/min.
2. Flush with at least 50 membrane volumes water and 50 membrane volumes buffer at a flow rate of 5 MV/min. Check if pH and conductivity are stable after this step.

After the step 4.6.1, 4.6.2 or 4.6.3 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 with 1 N NaOH; see also section 4.4).

4.8 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 typically decreases the flow rate.

4.9 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 will instead pass through the Sartobind Q or STIC PA anion exchanger. 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 negatively charged. With the pH of the buffer above the pI, the protein product flows through without binding.

For Sartobind Phenyl the loading conditions should be chosen to selectively retain contaminants with higher hydrophobicity and allow the target molecule with less hydrophobicity (the monomeric antibody for example) to pass through the capsule.

4.10 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® 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.

4.11 Washing

When using capsules in bind & elute mode, wash with equilibration buffer after sample loading until pH value and conductivity are stable.

4.12 Elution

To elute target protein, virus or virus like particle (VLP) from ion exchanger (Q, S or STIC PA), use the salt concentration previously determined to be appropriate for elution of the bound molecules. Take into account that STIC 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. To elute the target protein from Sartobind Phenyl, use buffers with salt typically below 100 mM.

4.13 Draining

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

4.14 Regeneration and storage

The pico devices are validated and certified as single use devices. However, if re-use and storage is necessary, the protocols recommended below can be followed.

Q, S and STIC

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 at +2 to +8 °C. The binding capacity of STIC PA typically cannot be restored completely.

Phenyl

After use, regenerate with e.g. 50% ethylene glycol, 70% ethanol or 30% isopropanol in pure water, wash extensively with pure water and 20% ethanol and store airtight in 20% ethanol at +2 to +8°C in a dark place. Do not store in high salt solution.

4.15 Chemical stability

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

4.16 Scaling up

Sartobind pico 0.08 ml devices are ideal tools for developing methodologies to screen target proteins against different loading | washing | eluting conditions or contaminant removal conditions in flow-through mode. After the screening conditions with Sartobind pico, it is necessary to follow with scale down devices of fully validated large-scale membrane chromatography capsules. For example, Sartobind® nano 1 ml or Sartobind® nano 3 ml can be used, keeping in mind that these capsules are void volume optimized and will not require proportionately larger volumes of buffer as predicted by trials with the Sartobind pico.

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. Consider that the pico device fits only when the void volume of the larger device is equivalent.
Reuse is needed	For economic or practical reasons	Q, S and Phenyl: 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, but reuse validation has to be performed by the user. 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.

Problem	Possible cause	Action
Reuse is needed	For economic or practical reasons	STIC PA: The ligand strongly binds the contaminants and cleaning with 1 N NaOH does typically 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 revalidation.
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.

Problem	Possible cause	Action
Capsule is installed upside down	The arrow indication on the device was overlooked	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).
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.

Problem	Possible cause	Action
High back pressure during sample loading	Material has not been filtered	Prefilter with 0.2 μm or 0.45 μm filter before processing through the unit (preferentially inline).
	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	Replace unit (for all types). Perform a regeneration cycle (for Q, S, Phenyl).

Problem	Possible cause	Action
	Pure water leads to swelling of membrane (Q, S and STIC PA)	Add sodium chloride or use ionic buffers
Target molecule is not bound	Conditions for binding are insufficient	<p>For Q and S, decrease salt concentration, control other process parameters such as pH and keep temperature constant (ph change).</p> <p>Sartobind STIC membrane is salt tolerant. Process conditions, e.g. prefiltration, pH, conductivity, multivalent buffers etc. have to be checked and optimized.</p> <p>For Phenyl increase salt concentration, control other process parameters as type of salt, pH and temperature.</p>

Problem	Possible cause	Action
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.

Problem	Possible cause	Action
I use STIC PA to remove DNA endotoxins using a buffer containing PBS	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 applies for endotoxins. You may even work at pH conditions which normally would not be accessible as target protein could be bound.
The Phenyl membrane has changed color	Wrong storage	No action. A slight change of the membrane color is due to oxygen and light exposure, and does not affect the adsorptive properties of the membrane or performance of the device.

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

6. Quality assurance

Sartobind membranes have been tested for dynamic protein binding capacity, thickness, evenness and flow rate. Capsules and membranes are manufactured in a controlled environment.

The product meets all Sartorius Stedim Biotech standards for traceability, production and specifications as given here or exceeded them as certified in the quality assurance certificate enclosed.

7. Ordering information

7.1 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 two PEEK adapters Luer male to UNF 10–32 female. Also included are one set of operation instructions and quality assurance certificate.

7.2 Sartobind nano

Order number	Description	Quantity
96IEXQ42DN-11	Sartobind Q nano 1 mL, 4 mm	1
96IEXQ42DD-11--A	Sartobind Q nano 1 mL, 4 mm	4
96IEXS42DN-11	Sartobind S nano 1 mL, 4 mm	1
96IEXS42DN-11--A	Sartobind S nano 1 mL, 4 mm	4
96STPA42DN-11--A	Sartobind STIC PA nano 1 mL, 4 mm	4
96IEXQ42EUC11--A	Sartobind Q nano 3 mL, 8 mm	4
96IEXS42EUC11--A	Sartobind S nano 3 mL, 8 mm	4
96HICP42EUC11--A	Sartobind Phenyl nano 3 mL, 8 mm	4

Each nano package contains two PEEK adapters Luer male to UNF 10 – 32 female. Also included are one set of operations instructions and one quality assurance certificate.

7.3 Accessory

Order number	Description	Quantity
1ZA---0004	Adapter Luer male to UNF-10 – 32 female, PEEK	1

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