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ReadyToProcess Adsorber Q, S and Phenyl Pico

Macroporous membranes with 4 mm bed height

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Read the operational instructions carefully before using ReadyToProcess™ Adsorber 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.

Avertissement

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 malfu zionamento, un danno al prodotto stesso o a persone o cose. Le capsule sono fornite non-sterilizzate. La membra-na è asciugata da glicerina. Solo per uso in vitro.

경고

본 설명서에 언급 또는 열거하지 않은 공정에서 본제품을 사용 하면 목적에 부합하지 않는 결과나, 인체 상처 및 제품 또는 필 터 재질에 손상을 초래할 수있습니다. 캡슐필터는 비무균상태 로 배송합니다.필터의 여과막(멤브레인)은 글리세롤(glycerol) 로부터 건조되었습니다.

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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.
ReadyToProcess Adsorber Pico, 0.08 mL is used for process development when only small sample quantities are available.

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1. Storage conditions

ReadyToProcess Adsorber Q and S Pico must be stored clean, dry and away from direct sunlight in the box at room temperature.

ReadyToProcess Adsorber Phenyl Pico must be stored between 2°C and 8°C in a clean, dry and dark place. When not in use, the end caps of the capsules must 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

ReadyToProcess Adsorber 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. ReadyToProcess Adsorber 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. ReadyToProcess Adsorber 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 flow-through 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.

ReadyToProcess Adsorber Pico

ReadyToProcess Adsorber Pico is the smallest member of the ReadyToProcess Adsorber 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.

ReadyToProcess Adsorber Pico capsules 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.

The ReadyToProcess Adsorber Pico capsules (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 Pico capsules in process development.

The 4 mm bed height product line consists of: Pico (0.08 mL, Nano (1 mL), 75 mL, 200 mL, 400 mL, 600 mL and 2.5 L size capsules; the 8 mm bed height line consists of: Nano (3 mL), 150 mL, 400 mL, 800 mL, 1.2 L and 5 L size capsules, see also *Chapter 7. Ordering information*. The smaller void volume has a positive effect on binding capacity. Therefore after optimal conditions are found with the Pico capsules, 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 ReadyToProcess Adsorber Pico devices are available in three different membrane functionalities to cover ion exchange applications and hydrophobic interaction chromatography.

Ion exchange chromatography

The ReadyToProcess Adsorber Q and S Pico capsules use the basic principle of ion exchange (IEX) separation accomplished on the basis of charges carried by solvent molecules.

Hydrophobic interaction chromatography

ReadyToProcess 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-}, $CH_3{\cdot}COO^-$, Cl^-, Br^-, NO_3^-, ClO_4^-, I^-, SCN^-
Cations: NH_4^+, K^+, Na^+, Cs^+, Li^+, Mg^{2+}, Ca^{2+}

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.

Fig 1. Three types of ReadyToProcess Pico capsules (0.08 mL) are available. For the content of the package refer to *Chapter 7 Ordering information*.

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
Dimension (height × diameter)	31 × 11 mm
Approximate weight	1.5 g

Membrane types and ligands	Strong basic anion exchanger: Quaternary ammonium (Q) $R\text{-}CH_2\text{–}N^+(CH_3)_3$ Strong acidic cation exchanger: Sulfonic acid (S) $R\text{-}CH_2\text{–}SO_3^-$ Phenyl Hydrophobic interaction membrane: Phenyl
Ligand density (µeq/cm^2)	Q, S: 2 to 5 Phenyl: 3
Typical dynamic binding capacity[1] at 10 % break-through per area or volume of membrane	Q: 0.8 mg/cm^2, 29 mg/mL S: 0.7 mg/cm^2, 25 mg/mL Phenyl: 0.2 mg/cm^2, 7.5 mg/mL

1 See also *3.1 Binding capacity, on page 19*.

Recommended flow rate[2]	10 to 30 membrane volumes/min, 0.8 to 2.4 mL/min
Max. pressure at 20°C	6 bar (0.6 MPa, 87 psig)
Short term[3]	Q and Phenyl: 2 to 14
pH stability	S: 3 to 14
Chemical stability	Stable in common chromatography buffers, unstable to peroxide and other oxidizing or reactive reagents

2 See also *4.8 Recommended flow rates, on page 29*.

3 Short term refers to cleaning procedure described in *4.7 Preconditioning, on page 27*.

3.1 Binding capacity

Data are based on dynamic capacity measuremements 10% 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.

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
Sulfonic acid (S)	1 mg/mL lysozyme in 10 mM potassium phosphate, pH 7.0	4.0 mg
Phenyl	1 mg/mL bovine blood gamma globulin in 50 mM potassium phosphate, pH 7.5, 0.9 M $(NH_4)_2SO_4$	0.6 mg

4. Operation

4.1 Buffer conditions for Q and S membranes

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 pK_a 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 (ReadyToProcess Adsorber S). Above its isoelectric point (at least 1 pH unit) it will bind to an anion exchanger (ReadyToProcess Adsorber Q).

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

4.2 Buffer conditions for Phenyl membranes

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 or start buffer with respect to pH, type of solvent and salt concentration.

Binding buffer examples

Example	Buffer
To bind IgG	0.8 M $(NH_4)_2SO_4$ in 50 mM potassium phosphate, pH 7.5
To bind bovine serum albumin or lysozyme	2 M $(NH_4)_2SO_4$ 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
$(NH_4)_2SO_4$	Typical choice, often best results, not stable at >pH 8
Na_2SO_4	Solubility of proteins reduced
NaCl	3 to 4 M needed
KCl	No special remarks
CH_3COONH_4	No special remarks

4.3 Flow direction

In ReadyToProcess Adsorber Pico the flow is from top inlet through 4 mm membrane bed to the outlet.

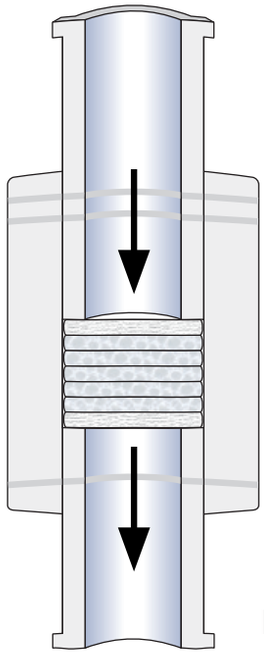

Fig 2. Flow path inside the ReadyToProcess Adsorber Pico capsule

Note: *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 unit completely.

Step	Action
1.	Fill a 10 to 20 mL Luer syringe with equilibration buffer and connect to the capsule.
2.	Hold the capsule upright (outlet is up) and expel air as shown in *Fig 3, on page 25*. Then connect the syringe to outlet and purge in the opposite direction to remove very small air bubbles.
3.	If you still detect any air in the filled unit, close it at the outlet, hold the syringe up and move the plunger slightly up and down that air bubbles can ascend into the syringe.
4.	Connect a filled syringe to the outlet of the capsule, connect an inline prefilter to the Pico capsule inlet and vent in the opposite side. The prefilter should be stable against 1 M NaOH. If not, it shall be connected after preconditioning (see *4.7 Preconditioning, on page 27*). Use of inline prefilter 0.2 µm is strongly recommended.

Step	Action

5. Connect the capsule to a liquid chromatography (LC) system or a peristaltic pump (for whole procedure with syringe without LC system, refer to *4.5 Operation with syringe, on page 25*).

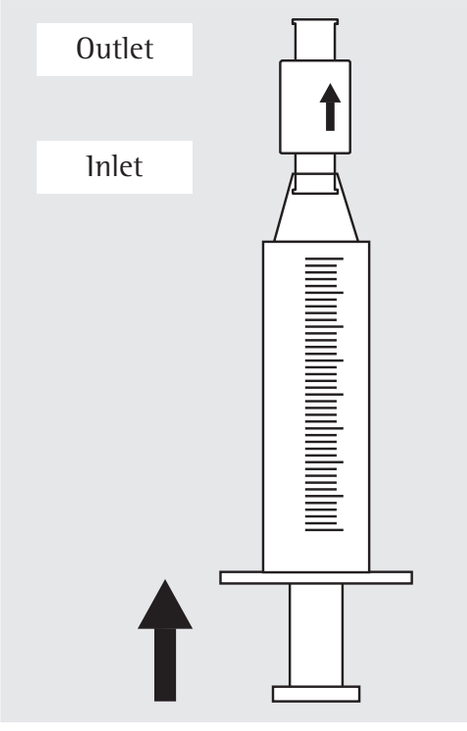

Fig 3. Filling the ReadyToProcess Adsorber Pico capsule with a Luer syringe

4.5 Operation with syringe

The ReadyToProcess Adsorber Pico capsule can be operated manually with a syringe. However, it requires some effort to push more viscous solutions through the capsule. 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 reconditioning, use the same volume of sanitization solutions and buffers and push through the Pico capsule with a syringe slowly).

4.6 Installation in LC system or peristaltic pump

To prepare the LC system for use with the ReadyToProcess Adsorber Pico capsule, 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 the Pico capsule break-through measurements to binding capacity results with the larger capsules.

Step	Action
1.	The capsule should be filled as described in *4.4 Venting, on page 24*.
2.	Start your LC system or peristaltic pump at a low flow rate.
3.	When fluid emerges, connect the tubing to the inlet of the Pico capsule. Make sure that no air is introduced. Remove the cap from the outlet.
4.	Run the pump until fluid emerges from the outlet of the unit, then stop the pump.

Step	Action
5.	Connect the outlet of the unit via Luer Lok 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 capsule.

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 capsule, NaOH residue could lead to a pH shift. In that case more flushing volume after a sanitization is needed.

For Q and S membranes

Step	Action
1.	For sanitization use 30 membrane volumes (MV) of 1 M 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 1 M NaCl at 5 MV/min
3.	Second flushing with 50 MV equilibration buffer (e.g., 20mM Tris/HCl, pH 7.5) at 5 MV/min

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

For Phenyl membranes

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

After preconditioning, connect a filled sterile prefilter to the inlet of the Pico capsule. 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 M NaOH; see also *4.4 Venting, on page 24*).

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 MV 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. 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 to 9.5 for example, will not bind and will instead pass through the ReadyToProcess Adsorber Q anion exchanger. The influence of the flow rate is very low.

To remove contaminating proteins and aggregates with ReadyToProcess Adsorber S in flow-through mode, process impurities have to be charged positively to bind to the S membrane while the target protein stays negatively charged. With the pH of the buffer above the pI, the protein product flows through without binding. For ReadyToProcess Adsorber 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 must be adjusted to the starting or equilibration buffer and be prefiltered through a 0.2 µm membrane, e.g., ULTA pure HC capsules.

Note: *Unfiltered feed will block the Membrane 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 and 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 exchangers (Q or S), use the salt concentration previously determined to be appropriate for elution of the bound molecules. To elute the target protein from ReadyToProcess Adsorber 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 capsules are validated and certified as single use devices. However, if re-use and storage is necessary, the protocols recommended below can be followed.

Q and S

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

Caution

Specific regulations may apply when using 70% ethanol and 30% isopropanol since it can require the use of explo-sion-proof areas and equipment

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°C 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

ReadyToProcess Adsorber Pico (0.08 mL) capsules are excellent tools for developing methodologies to screen target proteins against different loading, washing and eluting conditions or contaminant removal conditions in flow-through mode. After the screening conditions with the ReadyToProcess Adsorber Pico capsules, it is necessary to follow with scale down devices of fully validated large-scale membrane chromatography capsules. For example, the
ReadyToProcess Adsorber Nano (1 mL or 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 ReadyToProcess Adsorber Pico capsule.

5. Troubleshooting

Problem	Possible cause	Action
Break through data of the Pico capsule do not fit to larger capsules	LC pump provides different flow rates than indicated or given void volume of the LC system is incorrect.	Check 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 capsule is suited only when the void volume of the larger device is equivalent.

Problem	Possible cause	Action
Reuse is needed	For economic or practical reasons	The major application of Ready-ToProcess Adsorber capsules is the single use and they are manufactured with plastic housing and validated like this. Also they are validated and certified only for one use. Technically they can be reused. The 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
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 *4.4 Venting, on 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. 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 pro-cedure		The capsules have been qualified and validated according the given procedure. If a deviation is necessary, the validation 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. If you again observe 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
High back pressure during sample loading	The adsorber is clogged	Perform a regeneration cycle.
	Pure water leads to swelling of membrane (Q, and S))	Add sodium chloride or use ionic buffers
Target molecule is not bound	Conditions for binding are insufficient	For Q ,and S membranes decrease salt concentration, control other processes parameters such as pH and keep temperature constant (pH change) For Phenyl membranes increase salt concentration, control other process parameters as type of salt, pH and temperature.

Problem	Possible cause	Action
Binding capacity is not sufficient	Process optimization 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. We do not recommend to run two adsorbers in parallel.
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.

6. Quality assurance

ReadyToProcess Adsorber 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 Cytiva standards for traceability, production and specifications as given here or exceeded them as certified in the quality assurance certificate enclosed.

7. Ordering information

Product code	Description	Qty
17372101	ReadyToProcess Adsorber Q Pico (0.08 mL)	10
17372131	ReadyToProcess Adsorber S Pico (0.08 mL)	10
17372161	ReadyToProcess Adsorber Q Phenyl (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.

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