Instructions for Use

Sartobind® 96-Well Plates

IEX (Q, S, STIC PA) and HIC (Phenyl) in 8-strips

For High Throughput Screening
Based on Macroporous Membranes, 0.8 mm Bed Height

85032-543-27

SARTORIUS

Contents

1 About these Instructions

1.1 Validity

These instructions are part of the product; they must be read in full and kept in a safe place. These instructions apply to the following versions of the product: Sartobind® 96-well plate.

1.2 Related Documents

▶ In addition to these instructions, please read the following documents:
- Operating instructions of the device in which the product is used
- Instructions for use of the respective accessories, e.g. vacuum manifold | centrifuge

1.3 Target Groups

These instructions are addressed to the following target groups.
The target groups must possess the knowledge specified below.

Target Group	Knowledge and Qualifications
Operator	The operator is familiar with the product and the associated work processes. The operator understands the hazards which may arise when working with the product, and knows how to prevent them.

1.4 Symbols Used

1.4.1 Warnings in Operation Descriptions

⚠ CAUTION

Denotes a hazard that may result in moderate or minor injury if it is **not** avoided.

NOTICE

Denotes a hazard that may result in property damage if it is **not** avoided.

1.4.2 Other Symbols Used

▶ Required action: Describes activities that must be carried out. The activities in the sequence must be carried out in succession.

▷ Result: Describes the result of the activities carried out.

2 Instructions

2.1 Intended Use

The products are screening tools for ion exchange and hydrophobic interaction chromatography based on macroporous membranes.

They can be used for screening of operating conditions such as pH, conductivity and buffer compositions in the downstream processing of therapeutic proteins, e.g. for contaminant removal from proteins in flow-through mode (negative chromatography) to bind DNA, residual proteins, host cell proteins, endotoxins and viruses.

After optimal conditions are found, the product should be used for estimation of binding capacity or the absolute removal of contaminants (virus, endotoxin etc.) or flow rate for further scale up.

The products are intended for single use to avoid carryover as well as cleaning.

The plates are supplied as non-sterile. The membrane is dried from glycerol.

The products are intended exclusively for use in accordance with these instructions for use. Any other use is considered improper.

Modifications to the Product

If the product is modified: Personnel may be put at risk. Product specific documents and product approvals may lose their validity. If you have any queries regarding modifications to the product, contact Sartorius.

2.2 Qualifications of Personnel

Personnel who do **not** possess adequate knowledge about how to use the product safely may injure themselves and other persons.

2.3 Personal Protective Equipment

Personal protective equipment protects against risks arising from the product. If the personal protective equipment is missing or is unsuitable for the work processes on the product: Personnel may be injured. The following personal protective equipment must be worn:

- Safety gloves
- Safety glasses

2.4 Leaking Liquids from the Product

If the product is damaged or incorrectly used: Liquids can leak from the product and contamination can occur.

- Do **not** exceed the maximum pressure.
- Perform a visual inspection before use.
- Ensure correct use.

3 Operating Principle

3.1 Sartobind® Membrane Adsorbers

Traditional chromatography uses porous particles packed into columns. Target molecules in the liquid diffuse into the pores of the beads to the binding sites. 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, robust 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.

3.2 Chromatography Principles

The products are available in 4 different membrane functionalities to cover every ion exchange application and a hydrophobic interaction chromatography (see chapter “7.3 Membrane Type | Ligand“, page 20).

Sartobind® IEX 96-well plates with 8-strips use the basic principle of ion exchange: separation accomplished on the basis of charges carried by solvent molecules.

Sartobind® Phenyl 96-well plates use the principle of hydrophobic interaction chromatography.

3.3 8-Strip Design Features

Sartobind® 96-well plates feature a modular design. The plates are built up from 8-well units, called "strips", allowing the number of wells to be matched to the number of samples being processed.

The 8-strips make multi-well plate technology far more economical when fewer than 96 samples need to be processed simultaneously, or when different membrane types should be tested at the same time. When working with less than 96 samples using traditional plates, either the partially used plates are disposed of, increasing the cost per preparation, or partially used plates must be treated for storage use and reuse, which raises problems of storage conditions and cross contamination of samples. The modular design of Sartobind® 96-well plates eliminates these problems. Used strips can be economically disposed of, while unused strips can be stored for future use.

3.4 Operation Modes

The plates can be operated with a vacuum manifold or a centrifuge with a swing-out multi-well plate rotor equipped to hold standard footprint deep-well plates, as well as manually or with an automatic liquid handling system.

A silicone gasket seals the plate setup of 12 individual 8-strip units for vacuum processing. Two specific vacuum manifolds are available: Vac8 and Vac96. For large sample quantities, the full plate set up can be processed quickly with Vac96. Using Vac8, individual 8-strips can be run for medium throughput applications.

3.5 Scale Up

The products are ideal tools for developing methodologies to screen target proteins against different loading | washing | eluting conditions or contaminant removal conditions in flow-through mode as well as membrane types.

After the screening of condition with the 96-well plates, it is necessary to follow with scale down devices of fully validated large-scale membrane chromato graphy capsules. For example, Sartobind® Pico 0.08 mL, Sartobind® Nano 1 mL

or Sartobind® Nano 3 mL can be used. The products are ideal tools to screen target proteins against different loading | washing | eluting conditions or contaminant removal conditions.

4 Getting Started

4.1 Recommended Buffer Conditions

The products are compatible with all commonly used aqueous buffer systems. There is **no** need to degas any buffers before use.

4.1.1 Q and S Membrane

In the majority of applications, an equilibration buffer concentration of 10 mM provides sufficient buffering capacity and prevents the protein of interest from precipitation.

- Keep the ionic strength as low as possible to avoid reduction of binding capacity.
- Use buffering ion with the same charge as the membrane. This means:
  - buffers with positive charge (e.g. amine buffers such as Tris) with Q type exchangers
  - buffers with negative charge (e.g. phosphate buffers) with S type exchangers. Use buffer that has a pKa value within 0.5 pH units of the working pH value.

Buffers and prepared samples ideally have an ionic strength below 50 mM. Higher salt levels may ristrict binding of proteins but not DNA or endotoxins.

- Do **not** use Standard PBS buffer. It contains, along with other salts, 137 mM NaCl, which will significantly reduce protein binding to the ion exchange membrane.

NOTICE

Swelling of the membrane!

Pure water can cause reversible swelling of the membrane and reduce its permeability.

▶ Do **not** use pure water.

4.1.2 PA Membrane

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 chapter chapter "4.1.1 Q and S Membrane", page 11 for recommended conditions for ion exchange membranes.

NOTICE

Reduced binding capacity for proteins!

Multivalent buffers like phosphate or citrate can reduce binding capacity for proteins but not necessarily for contaminants such as DNA or endotoxins. The buffering ion should carry the same charge as the ionexchange ligand. Use monovalent buffers, e.g. TRIS or acetate.

4.1.3 Phenyl Membrane

Proteins are bound to the phenyl membrane at salt concentrations typically above 500 mM. Larger proteins tend to bind better than smaller ones. Differences in protein hydrophobicity have influence on the choice of salt concentration. The strength of the interaction depends mainly on salt concentrations but also on the sufficient 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.

The effect of pH on binding is much less than in ion exchange chromatography. Higher temperature typically promotes stronger binding of the sample solute as known from entropy driven reactions. Thus temperature control is important to achieve reproducible results.

Binding buffer examples	Condition
To bind IgG	0.8 M (NH4)2SO4 in 50mM potassium phosphate, pH 7.5
To bind bovine serum albumin or lysozyme	2 M (NH4)2SO4 in 50 mM potassium phosphate, pH 7.0

Higher salt concentrations may result in precipitation. For this reason:

- Select the salt concentration as low as possible.
- Carry out a pre-test to check the 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 – 4 M needed
KCl	No special remarks
CH_3COONH_4	No special remarks

4.2 Operation Modes

4.2.1 Capture of Target Proteins in Bind and Elute Mode

In ion exchange chromatography a charged molecule is bound to oppositely charged groups attached to the insoluble matrix. This binding is reversible by using a higher salt elution buffer to elute the molecule. The pH value at which a biomolecule has no net charge is the isoelectric point (pI). In buffers below the pI (at least 1 pH unit), a protein, for example, carries a positive net charge and will bind to a cation exchanger (Sartobind® S). In buffers above its pI (at least 1 pH unit), it will bind to an anion exchanger (Sartobind® Q or PA). During elution of your target protein maintain a constant pH of the buffer while the saltconcentration is increased.

Refer to chapter “4.1.3 Phenyl Membrane“, page 12.

4.2.2 Contaminant Removal in Flow-Through Mode

For contaminant removal from products such as monoclonal antibodies, pH conditions in the range of pH 6 – 8 are used in order to bind highly negatively-charged DNA, endotoxins, contaminating proteins, some host cell proteins and viruses with anion exchanger (Sartobind® STIC PA or Q). The product of interest, the monoclonal antibody with pI 8 – 9.5 for example, will not bind and pass through.

To remove contaminating proteins and aggregates with Sartobind® S in flow-through mode, process impurities have to be charged positively to bind while the target protein stays negative. At 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 mole-culewith less hydrophobicity to pass through the capsule.

4.3 Additional Equipments

- Multi-channel pipette or set of pipettes for dispensing small volumes of liquid (10 – 200 µL; 200 – 1,000 µL), or robotic liquid handling system
- 0.2 µm syringe filters for sample clarification
- Collection plates

4.4 Sample Preparation

Procedure

▶ Filter the buffers with 0.2 µm filters before use.
▷ The quality of water | chemicals should be of high purity.
▶ Pre-filter the samples through 0.2 µm syringe filters (e.g. Minisart® polyethersulfone 16532-K) before mixing with individual buffers.
▷ This prevents blocking of the membrane pores and increases binding capacity.
▶ Alternatively: Centrifuge your samples at 5,000 x g for 5 min to sediment any cellular debris or large visible particles.
▷ This option may result in longer sample loading times.

4.5 Vacuum Manifold: Checking the Operating Conditions

Equipment

– Vac96 or Vac8 vacuum manifold
– Vacuum pump or vacuum source capable of applying vacuum up to 350 mbar (35 kPa, 5 psi)
– Vac96 or Vac8 liquid trap or other suitable liquid trap to protect vacuum source from carry-over of liquid (optional), or to collect large wash volumes

Procedure

▶ Ensure that the operation conditions have been met: Up to 350 mbar (35 kPa, 5 psi) until the wells are empty plus an additional 8 – 10 seconds.
▶ Monitor the liquid as the vacuum draws it through the membrane in each well.
▷ It will take 8 – 10 seconds longer for all the liquid to fully pass through the membrane after the well has emptied.

4.6 Centrifuge: Checking the Operating Conditions

Equipment | Requirements

- Centrifuge with swing-out rotor accepting stacks of 4 standard or 2 deep well 96-well plates per carrier, and capable of spinning at up to 1000 x g (recommendation: use lower acceleration, e.g.~ 500 x g depending on centrifuge equipment).
- The silicone gasket on the bottom is **not** necessary.

Procedure

▶ Ensure that the operation conditions have been met until wells are completely empty.
▷ Centrifugation at higher speed is **not** recommended. Centrifugation at a lower speed will necessitate longer spin times.
▶ If the wells are not fully emptied after centrifugation, repeat it again.

5 Operation

⚠ CAUTION

Leaking liquids!

If the product is damaged or incorrectly used: Liquids can leak from the product.

▶ Perform a visual inspection before use.
▶ Do **not** exceed the product's operating conditions.

Procedure

Vacuum

- ▶ Position the product on top of your vacuum manifold (see respective instructions for use).
- ▶ If you use a part of 8-strips on a 96-well plate vacuum manifold (e.g. Vac96), the strips that are **not** required have to be sealed with tape.
- ▷ The Vac8 vacuum manifold is available for operation of an individual 8-strip.

Centrifuge

- ▶ Position the product on top of a deep-well collection plate.
- ▶ The strips that are **not** required can be removed from the holding frame by pushing upwards from the bottom, taking care **not** to damage the drip nozzles on the underside of the strips.
- ▶ Equilibrate each of the wells to be used by filling with 2 mL (4 x 500 µL) of loading buffer (see chapter "4.1 Recommended Buffer Conditions", page 11).
  - ▶ Make sure to stabilize pH and conductivity via the equilibration buffer before loading the sample.
  - ▶ Apply vacuum or centrifuge and discard the flow-through.
- ▶ Load up to 500 µl (per step) of prepared sample (see chapter "4.4 Sample Preparation", page 15) per well and apply vacuum or centrifuge.
  - ▶ If you want to analyse the different fractions, replace the collection plate with a new one, or discard the fraction if not required.
  - ▶ Repeat the step if you want to load more sample solution per well. Consider potential effect by overloading (exceeding binding capacity of the screening device).
- ▶ Wash the remaining unbound fraction from the membrane with 1–2 x 500 µL volumes of fresh loading buffer by vacuum or centrifugation.
  - ▶ If you want to analyse the different fractions, replace the collection plate with a new one, or discard the wash fraction if not required.
- ▶ Elute the bound protein fraction with 1–2 x 500 µL aliquots of elution buffer per well (see chapter "4.1 Recommended Buffer Conditions", page 11) by vacuum or centrifugation.

6 Troubleshooting

Fault	Cause	Solution
Clogging of wells at loading	Aggregation or precipitation of proteins	Pre-filter sample with 0.2 µm before loading.
Sample solution does not (or not sufficiently) run through the membrane	Vacuum does not build up correctly	Check pump for any leakage and right positioning of the sealing.
Dropping from the bottom at loading	Gravity, especially at long loading duration	— Loading with a multi-pipette. — Build up light backpressure by connecting compressed air onto the vent-port.
Recovery is too low	Dead space, elution volume too low	— Check wash fraction, increase elution volume.
High variation among wells with the same amount of loading (identical sample solution)	Pipetting failure	Check parameter of liquid handling system or pipette, minimize multi-application per step (to avoid accumulation of failure).
Large deviation at test repeating with a new pipette	Vacuum inconsistency	Vacuum or centrifuge parameters should be kept constant for all tests.
Binding capacity is lower than with larger devices	The 96-well plate is not scaleable to single devices (e.g. void volume, difficult control on flow rate etc.)	— Test with scaleable single devices. — Check pH and conductivity to assure the same condition used for single capsules.

7 Technical Data

7.1 Package Contents

	2 Units	10 Units
8 strips	24	120
Holding frames for 12 strips	2	10
96-well silicone gasket	2	10
2 mL 96-well deep-well plates	4	0
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7.2 Technical Information

Base membrane	Stabilized reinforced cellulose
Nominal membrane thickness	275 µm
Nominal pore size	>3 µm
Bed height	0.8 mm
Bed volume	19 µL/well
Adsorption area	0.7 cm^3/well
Maximal loading volume	500 µL | well per step

7.3 Membrane Type | Ligand

Strong basic anion exchanger	Quaternary ammonium (Q) $R\text{-}CH_2\text{-}N+\text{-}(CH_3)_3$
Salt tolerant anion exchanger	Primary amine (PA)
Strong acidic cation exchanger	Sulfonic acid (S) $R\text{-}CH_2\text{-}SO_3$
Hydrophobic interaction membrane	Phenyl

7.4 Ligand Density [µeq/cm²]

Q, S	2 – 5
STIC PA	18 – 22
Phenyl	3

7.5 Materials

8-strip units	Polypropylene
Holding frame	Polystyrene
Deep-well collection plate	Deep-well collection plate

7.6 Binding Capacity

The following data is based on the typical dynamic binding capacity at 10% breakthrough measured with MA 15 units (bed height 0.8 mm, bed volume 0.41 mL) at 10 mL/min.

Membrane Type	Reference Protein | Buffer	Binding Capacity [mg/mL]
Quaternary ammonium (Q)	1 mg/mL bovine serum albumin in 20 mM Tris/HCl, pH 7.5	29 | 0.55
Primary amine (PA)	As above +150 mM NaCl	50 | 0.95
Sulfonic acid (S)	1 mg/mL lysozyme in 10 mM potassium phosphate, pH 7.0	25 | 0.48
Phenyl	1 mg/mL bovine blood gamma globulin in 50 mM potassium phosphate, pH 7.5, 0.9 M $(NH_4)2SO_4$	14.5 | 0.28

7.7 Dimensions

L x W x H	128 x 85.5 x 25 mm (+7 mm drip nozzle)
Total height of 8-strip plus collection plate (mm)	74 mm

7.8 Storage Conditions

Material	Conditions
Q, S or STIC PA membranes	Clean, dry and away from direct sunlight at room temperature in the box.
Phenyl	Clean, dry and dark place at room temperature. Change of membrane color can appear after inappropriate storage (oxygen and | or light exposure). A color change does not affect adsorptive properties of the membrane.

7.9 Chemical Stability

Short term pH stability	Q, PA, Phenyl: 2 – 14 S: 3 – 14
Chemical stability	Stable in common chromatography buffers, unstable to peroxide and other oxidizing or reactive reagents

8 Ordering Information

8.1 Sartobind® 96-well plate

Description	Article No.	Qty plates (8-strips)
Sartobind® Q 96-well plate	99IEXQ42GC-----V	2 (24)
Sartobind® Q 96-well plate	99IEXQ42GC-----D	10 (120)
Sartobind® STIC PA 96-well plate	99STPA42GC-----V	2 (24)
Sartobind® STIC PA 96-well plate	99STPA42GC-----D	10 (120)
Sartobind® S 96-well plate	99IEXS42GC-----V	2 (24)
Sartobind® S 96-well plate	99IEXS42GC-----D	10 (120)
Sartobind® Phenyl 96-well plate	99HICP42GC-----V	2 (24)
Sartobind® Phenyl 96-well plate	99HICP42GC-----D	10 (120)

8.2 Vacuum Manifolds and Accessories

Description	Article No.	Qty
Vac96 vacuum manifold	VW96VAC01	1
Liquid trap and reservoir for Vac96 vacuum manifold	VW96VAA02	25
96 deep well collection plate 2 mL (square wells)	VW96VAA04	1
Replacement seal for Vac96 vacuum manifold	VW96VAC05	1
Vac8 vacuum manifold	VW08VAA01	1

Description	Article No.	Qty
Liquid trap and reservoir for Vac8 vacuum manifold	VW08VAA02	1
8 well collection strips 1.2 mL (round wells)	VW08VAA03	125
Replacement seal for Vac8 vacuum manifold	VW08VAA04	1
Vacuum pump, 98%, 220 V, 50 Hz	16612	1
Vacuum pump, 98%, 110 V, 60 Hz	16615	1
Minisart®* syringe filter, cellulose acetate, 0.2 µm, 28 mm, sterile, individually packed	16534----------K	50

* See our website or catalog for further Minisart® filters.

9 Trademark Information

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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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92IEXS42DD-11--D
92STPA42DD-11--D
99HICP42GC-----D
99HICP42GC-----V
99IEXQ42GC-----D
99IEXQ42GC-----V
99IEXS42GC-----D
99IEXS42GC-----V
99STPA42GC-----D
99STPA42GC-----V