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Operating Instructions

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

Read operational instructions carefully before using Sartobind® 96-well plates consisting of twelve 8-strips for each plate.

IMPORTANT

Use of the product 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 plates are supplied as non-sterile. The membrane is dried from glycerol.

Intended Use

They have been developed for working with small sample volumes and for screening of operating conditions such as pH, conductivity and buffer compositions. The products are intended for single use to avoid carryover as well as cleaning.

Table of Contents

1. Storage Conditions

Sartobind® 96-well plates 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 96-well plates should be stored at room temperature in a clean, dry and dark place. 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® 96-well plates 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, Sartobind® capsules should be used for estimation of binding capacity or the absolute removal of contaminants (virus, endotoxin etc.) or flow rate for further scale up.

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.

Chromatography Principles
The Sartobind® 96-well plates are available in four different membrane functionalities to cover every ion exchange application and a hydrophobic interaction chromatography (see 3. Technical data).
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.

8-Strip Design Features
Sartobind® 96-well plates feature a modular design. The plates are built up from 8-well units, "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 safely stored for future use.

Operation
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 set-up 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.

Scale Up

Sartobind® 96-well plates 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 chromatography capsules. For example, Sartobind® pico 0.08 ml, Sartobind® nano 1 ml or Sartobind® nano 3 ml can be used.

Package Contents	**Qty 2 Units (10 units) Pack**
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)
Operating instructions	1 (1)
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^2/well
Maximal loading volume	500 µl/well per step

Membrane Types and Ligands	
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

Ligand Density [µeq/cm^2]	
Q, S	2 – 5
STIC PA	18 – 22
Phenyl	3

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

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

Stability

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

3.1 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 and Buffer	Binding Capacity [mg/ml] | [mg/well]
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

4.1 Buffer Conditions and Sample Preparation

4.1.1 General Operating Notes

Sartobind® devices are compatible with all commonly used aqueous buffer systems. There is no need to degas any buffers before use with Sartobind® devices. Buffers should be filtered with 0.2 µm filters before use and the quality of water and chemicals should be of high purity.
Samples should be pre-filtered through 0.2 µm syringe filters before mixing with individual buffers, this prevents blocking of the membrane pores and increases binding capacity.
Alternatively, you can centrifuge your samples at 5,000 × g for 5 minutes to sediment any cellular debris or large visible particles, though this option may result in longer sample loading times.

4.1.2 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. 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.

4.1.3 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 4.1.2 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. The buffering ion should carry the same charge as the ion exchange ligand.

4.1.4 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

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. A pre-test to check the precipitation is recommended.

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

4.1.5 Operation Mode

4.1.5.1 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, the buffer should maintain a constant pH while the salt concentration is increased.
Refer to 4.1.4 for Sartobind® Phenyl.

4.1.5.2 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 molecule with less hydrophobicity to pass through the capsule.

4.2 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

4.2.1 Operation with Vacuum Manifold

- 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

4.2.2 Operation with Centrifuge

- Centrifuge with swing-out rotor accepting stacks of 4 standard or 2 deep-well 96-well plates per carrier, and capable of spinning at 1,000 × g
- The silicone gasket on the bottom is not necessary.

4.3 Operation Conditions

By vacuum: up to 350 mbar (35 kPa, 5 psi) until the wells are empty plus an additional 8 – 10 seconds

NOTICE

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.

By centrifuge: 1000 × g for 1 minute

NOTICE

Centrifugation at higher speed is not recommended. Centrifugation at a lower speed will necessitate longer spin times, but should not alter the purification characteristics. If the wells are not fully emptied after centrifugation, repeat it again.

4.4 Procedure

1. **Vacuum:** Position Sartobind® 96-well plate on top of your vacuum manifold according to the instructions. 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 Sartobind® 96-well plate 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.

2. Equilibrate each of the wells to be used by filling with 2 ml (4 × 500 µl) of loading buffer (see 4.1 for buffer recommendations). Make sure to stabilize pH and conductivity via the equilibration buffer before loading the sample. Apply vacuum or centrifuge and discard the flow-through.

3. Load up to 500 µl (per step) of prepared sample (see 4.1 for sample preparation) 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 Step 3 if you want to load more sample solution per well.

4. Wash the remaining unbound fraction from the membrane with 1–2 × 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.

5. Elute the bound protein fraction with 1–2 × 500 µl aliquots of elution buffer per well (see 4.1 for buffer recommendations) by vacuum or centrifugation.

Problem	Possible Cause	Action
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	Gravitiy, 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 should be kept constant for all tests
Binding capacity or LRV of viruses, phages or endotoxins is lower than with larger devices	The 96-well plate ist not scaleable to single devices (e.g. void volume, difficult control on flow rate ect.)	– Test with scaleable single devices – Check pH and conductivity to assure the same condition used for single capsules

Sartobind® 96-well plate

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

Vacuum Manifolds and Accessories

Article No.	Description	Qty
VW96VAC01	Vac96 vacuum manifold	1
VW96VAA02	Liquid trap and reservoir for Vac96 vacuum manifold	1
VW96VAA04	96 deep well collection plate 2 ml (square wells)	25
VW96VAC05	Replacement seal for Vac96 vacuum manifold	1
VW08VAA01	Vac8 vacuum manifold	1
VW08VAA02	Liquid trap and reservoir for Vac8 vacuum manifold	1
VW08VAA03	8 well collection strips 1.2 ml (round wells)	125
VW08VAA04	Replacement seal for Vac8 vacuum manifold	1
16612	Vacuum pump, 98%, 220 V, 50 Hz	1
16615	Vacuum pump, 98%, 110 V, 60 Hz	1
16534----------K	Minisart®* syringe filter, cellulose acetate, 0.2 µm, 28 mm, sterile, individually packed	50

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

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