Instructions for Use

Sartobind® Rapid A 96-Well Plates

For High Throughput Screening

1000116983

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® Rapid A 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 Safety Instructions

2.1 Intended Use

The products are screening tools for protein A affinity chromatography based on the newly developed Sartobind® Rapid A membrane.

The product has been developed for working with small sample volumes and for screening of optimal operating conditions such as pH, conductivity and buffer compositions in the downstream processing to specifically capture monoclonal antibodies or other Fc-region containing molecules.

After optimal conditions are found, the products should be used for estimation of binding capacity 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 and filled with 20 – 24% ethanol for shipment.

The product is intended exclusively for use in accordance with these instructions. 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.

The newly developed Sartobind® Rapid A membrane is a convecdiff membrane which combines high binding capacity from diffusion dependent chromatographic matrices and high flow rates from purely convective matrices. Large convective pores of about 4–6 µm provide high permeability for scalable bed heights and low fouling propensity for robust processing. The protein A ligands are covalently attached to this membrane.

The chromatographic bed is formed by the Rapid A membrane and is incorporated into multi-well plates or housings.

3.2 Chromatography Principles

The product uses the basic principle of affinity chromatography. Affinity chromatography with protein A as ligand is used to specifically capture monoclonal antibodies or other Fc-region containing molecules. The target molecule interacts strongly with protein A and is tightly bound to the matrix while other components of the load material will run through the chromatographic bed which exhibits very low unspecific binding.

To release the target molecule from the protein A ligand a pH shift is performed to low pH (pH 2.5 – 4.0). This allows elution of the target molecule with a high yield and purity of typically > 95%.

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.

3.4 Operation Mode

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. A specific vacuum manifold Vac96 is available.

3.5 Scale Up

The products are ideal tools to screen target proteins against different loading | washing | eluting conditions or contaminant removal conditions.

After screening of conditions with the 96-well plates, it is necessary to confirm results with small scale but fully validated and scalable bioprocessing devices. For this e.g., Sartobind® Rapid A Nano can be used.

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.

Monoclonal antibodies are bound to the Sartobind® Rapid A membrane at physiological buffer conditions and are released at low pH (pH 2.5 – 4). The membrane is stable to buffer conditions commonly used for the mAb capture step. All chromatography buffers should be 0.2 µm prefiltered.

Buffer	Condition
Equilibration	Wash	All commonly used buffers for equilibration	wash steps are suitable.
Elution	pH 2.5 – 4 e.g. 50 – 100 mM citrate, acetate, glycine, HAc
Sanitization	0.1 – 0.5 M NaOH
Optional: Regeneration	0.2 M NaOH

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

4.3 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.

4.4 Vacuum Manifold: Checking the Operating Conditions

Equipment

– Vac96 vacuum manifold
– Vacuum pump or vacuum source capable of applying vacuum up to 350 mbar (35 kPa, 5 psi)
– Vac96 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.

4.5 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.

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.
- Ensure that drip nozzles are not touch ground of the collection plate.
- 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 10).
  - 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 sample (multiple steps might be necessary depending on concentration of target molecule).
  - 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 10) by vacuum or centrifugation.

6 Troubleshooting

Problem	Possible Cause
Clogging of wells at loading	Aggregation or precipitation of proteins
Sample solution does not (or not sufficiently) run through the membrane	Vacuum does not build up correctly
Dropping from the bottom at loading	Gravity, especially at long loading duration
Recovery is too low	Dead space, elution volume too low
High variation among wells with the same amount of loading (identical sample solution)	Pipetting failure
Large deviation at test repeating with a new pipette	Vacuum inconsistency
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.)

	Action
	Pre-filter sample with 0.2 µm before loading.
	Check pump for any leakage and right positioning of the sealing.
	— Loading with a multi-pipette. — Build up light backpressure by connecting compressed air onto the vent-port.
	— Check wash fraction, increase elution volume. — Consider binding capacity of the screening device and elution conditions (pH).
	Check parameter of liquid handling system or pipette, minimize multi-application per step (to avoid accumulation of failure).
	Vacuum or centrifuge parameters should be kept constant for all tests.
	— 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

Nominal pore size	4 – 6 µm
Bed height	0.7 mm ± 0.1 mm
Bed volume	17 µL ± 4 µL
Maximal loading volume	500 µL | well per step

7.3 Membrane Type | Ligand

Membrane	Rapid A
Ligand	Protein A

7.4 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.

Reference Protein | Buffer	**Binding Capacity [mg/mL]**
Polyclonal antibody | 1 x PBS pH 7.4, 16 mS/cm	≥ 30 mg/mL

7.5 Materials

96-well plate	Polypropylene
Holding frame	Polystyrene
Deep-well collection plate	Polypropylene

7.6 Dimensions

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

7.7 Storage Conditions

Storage for Shipment in ethanol	20 – 24%
Storage in transport box	+2 – +8°C
Store in a clean, dry and dark place until use	

7.8 Chemical Stability

Short term pH stability (cleaning in place | regeneration procedures during operation)	2 – 14
Chemical stability	Stable in common chromatography buffers, unstable to peroxide and other oxidizing or reactive reagents

8 Ordering Information

Description	Article No.	Qty plates (8-strips)
Sartobind® Rapid A 96-well plate	99R-PA19GC-----V	2 (24)
Sartobind® Rapid A 96-well plate	99R-PA19GC-----D	10 (120)

9 Trademark Information

Sartobind® and Minisart® are trademarks of Sartorius Stedim Biotech GmbH.

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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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