

Instructions for Use

Sartobind® Rapid A

A Membrane Technology to Enable Rapid Cycling Chromatography
From Nano to Cassette



1000097815



SARTORIUS

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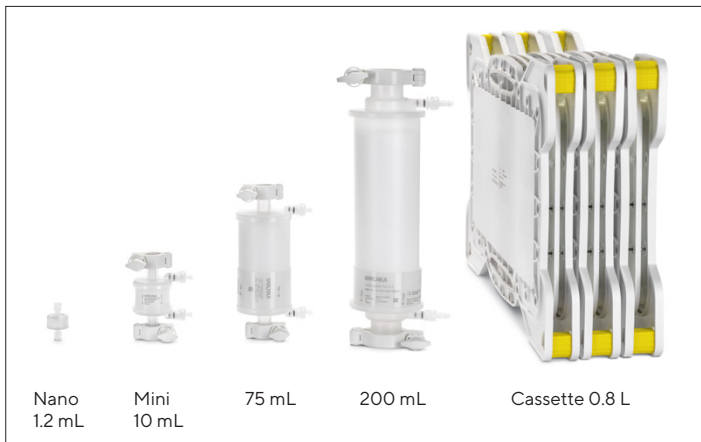
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1 About these Instructions

1.1 Validity

These instructions are part of the product; they must be read in full and retained. These instructions apply to the following versions of the product:



1.2 Related Documents

In addition to these instructions, please observe the following documents:

- Operating instructions of the device in which the product is used
- Pilot Filter Holder Instructions for Use
- Process | Double Process Filter Holder Instructions for Use
- Validation Guide of the respective product
- Regulatory Support File Sartobind® Rapid A Membrane Adsorbers

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







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





Result: Describes the result of the activities carried out.

2 Safety

-  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 and stored in 20 – 24% ethanol for shipment.
-  Eine Verwendung dieser Produkte für andere Anwendungen oder in einer anderen Weise als in diesem Handbuch beschrieben, kann zu Verletzungen, Fehlfunktion, oder einer Beschädigung des Produkts oder des Materials führen. Die Capsulen werden unsteril und in 20 – 24%-igem Ethanol lagernd versendet.
-  L'utilisation des produits dans des applications non spécifiées ou non décrites dans ce manuel peut entraîner un mauvais fonctionnement, des blessures ou des dommages sur le produit ou le matériel. Les capsules sont livrées non stériles et sont conservées dans de l'éthanol à 20 – 24 % pour l'expédition.

 El uso de los productos para otras aplicaciones no especificadas o descritas en este manual puede provocar un funcionamiento inadecuado, daños personales o daños en el producto o el material. Los cápsulas se suministran sin esterilizar y se almacenan en una solución de etanol al 20-24 % para el envío.

 在本手册未指定或未描述的应用中使用该产品,可能会导致功能不正常、人身伤害或产品或材料损坏。胶囊以非无菌形式提供,并储存在 20-24 % 的乙醇中以备运输。

 本取扱説明書に指定または説明されていない用途で製品を使用することは、機能の不具合、ケガ、または製品や材料の損傷につながります。輸送時のカプセルは、非滅菌状態で20～24 %エタノール内に保存されています。

2.1 Intended Use

The product is intended for *in vitro* use only. The product is intended exclusively for use in accordance with these instructions. Any other use is considered improper.

The membrane chromatography products also described as membrane adsorbers are intended and validated for single batch use to avoid carryover as well as tedious and costly cleaning validation procedure.

Sartobind® Nano 1.2 mL capsules have been developed for working with small sample volumes while retaining the cylindrical design of large scale membrane adsorbers. They are perfect for small scale applications, such as screening or process development. The Nano is the smallest scalable product size within the Sartobind® product portfolio. Scalability is given for breakthrough curves, elution peak profile and flow rate.

Sartobind® Mini 10 mL capsules are used for first scale up trials and preclinical production.

Sartobind® 75 mL capsules are sized for intermediate and pilot scale.

Sartobind® 200 mL capsules are perfect for production purposes in the biopharmaceutical industry.

Sartobind® 0.8 L cassettes are used in the Pilot Scale Filter Holder of up to 10.4 L membrane volume for the biopharmaceutical production. They must be used in an appropriate cassette holder and one Manifold Set (see “8.2 Accessories”, page 41).

When using different filter holders from other manufacturers, please contact your Sartorius office for technical advice.

2.2 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.

2.3 Qualifications of Personnel

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

2.4 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:

- Protective work clothing
- Safety gloves
- Safety glasses

2.5 Leaking Liquids from the Product

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

- ▶ Do **not** exceed the maximum pressure (see chapter “7 Technical Data”, page 36).
- ▶ Perform a visual inspection before use.
- ▶ Ensure correct installation.

3 Device Overview

3.1 Operating Principle

The Sartobind® Rapid A capsules and cassettes are ready-to-use products and can be used out of the box. They can be operated with a peristaltic pump or liquid chromatography system. The Nano can as well be operated with a syringe. For the Nano and the Mini there are adapters enclosed in the shipment.

The 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.

Affinity chromatography with protein A as ligand is used to specifically capture monoclonal antibodies or other Fc-region containing molecules. Therefore, 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 that exhibits very low unspecific binding. To release the target molecule from the protein A ligand a pH shift is performed. This allows elution of the target molecule with a high yield and purity of > 95%.

Sartobind® Rapid A is intended to be used in Rapid Cycling mode. High permeability and an optimized bed height allow that a single bind and elute cycle will be done within minutes. As a result, high productivity is achieved and large volumes can be captured with a minimum of chromatographic material while the life time of this material will be utilized in a short time (within one batch) to avoid long storage times and the associated bioburden risks.

↓ Flow direction

← Vent valve



Fig. 1: Flow direction and position of vent valves of 4 mm capsules and cassettes

The flow pattern inside the capsule is from the outside of the cylinder through the membrane bed to the inside, and then to the outlet.

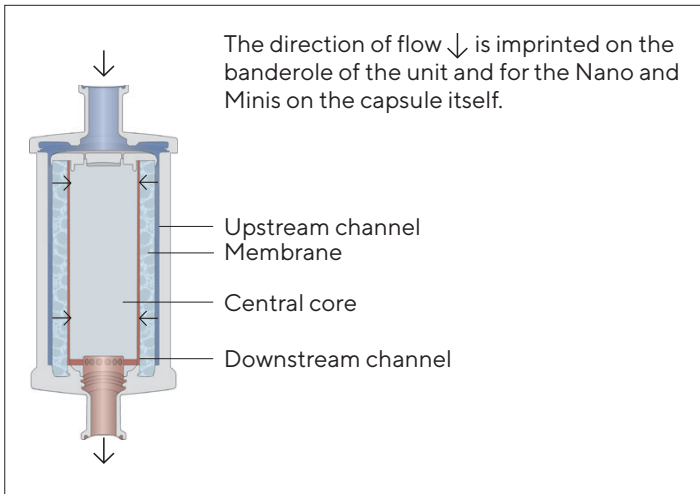


Fig. 2: Cross section of Sartobind® capsules.

The capsules contain a central core and the cassettes a spacer element to minimize void volume.

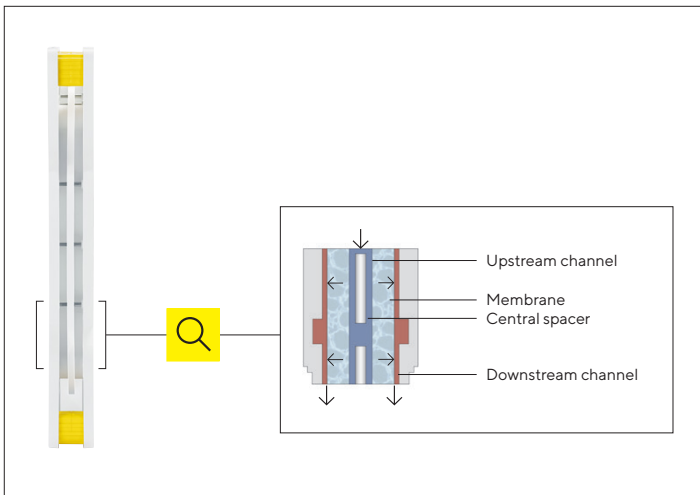


Fig. 3: Side view cassette;
highlighted section see Fig. 4

Fig. 4: Construction and flow path inside
the 4 mm cassettes

4 Installation

NOTICE

Malfunctions due to damaged products. A damaged product can cause malfunctions.

- ▶ Visually inspect the product before use.
 - ▶ Do **not** keep or place the products with the connector directly on the floor.
 - ▶ In case of damage: Replace the product.
-

Procedure for Capsules

- ▶ Protect inlet and outlet connectors when unpacking.
- ▶ Install the product in an upright position according to the process flow.
- ▷ The inlet is up.
- ▶ Install the product in the process flow as indicated on the capsule.

It is recommended to protect the membrane of the product with a prefilter (0.2 μm).

Procedure for Cassettes (see fig. 5)

- ▶ Unpack the manifold set containing 1 inlet (2) and 1 outlet (5).
- ▶ Place the "INLET" plate at one end of the holder.
- ▷ "THIS SIDE UP" is readable on top.

- ▶ Place the "OUTLET" plate at the other end of the holder.
- ▷ "THIS SIDE UP" is readable on top.

CAUTION

Leaking liquids due to incorrect installation!

- ▶ Place the cassettes (4, see Fig. 5) in the lowest possible position in the holder.
 - ▶ The cassettes (4) used for chromatographic separation must originate from the same lot.
-
- ▶ Put the desired number of Sartobind® cassettes (4) between the manifolds (see Fig. 5).
 - ▷ “THIS SIDE UP” is readable on top.
 - ▶ Adjust the clamping force for cassettes in Pilot and Process holders to a minimum of 25 kN (optimal range: 25–30 kN).
 - ▷ In the Pilot holder up to 13 cassettes and a manifold set can be installed.
 - ▷ Close all DRAIN and VENT valves (1) of the manifold plates manually using the pinch clamp.

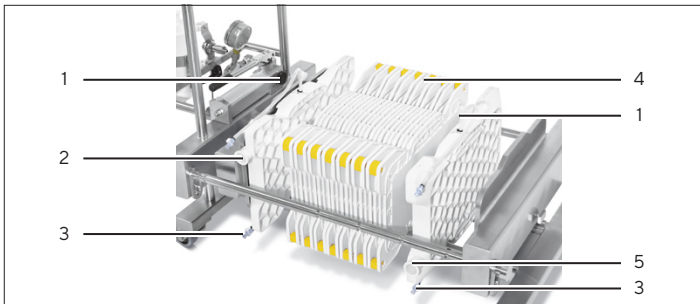


Fig. 5: Insert cassette(s) between the manifold inlet and outlet plates on the Pilot Filter Holder.

Pos.	Name
1	Vent valve
2	Inlet manifold
3	Drain valve
4	Cassettes
5	Outlet manifold

- ▶ Connect the inlet and outlet plates with 1½ inch tri-clamp to the process solution.
- ▷ Maximum pressure for the set-up of 1 to 13 cassette(s) is 2 bar (0.2 MPa, 29 psig).
- ▶ Make sure that the pump peak pressure caused by pulsation stays below this limit.

5 Operation

5.1 Venting

All capsules except Nano have vent valves (see Fig. 1).

Procedure

- ▶ Remove any air from the unit before use.
- ▶ Connect the valves with flexible tubing (inner diameter 6 mm) to waste.
- ▶ **NOTICE** Insufficient closing of the valve due to excessive pressure! During venting of capsules do **not** exceed 0.05 MPa (0.5 bar | 7.3 psi).
- ▶ Open the vent valve screw $\frac{1}{3}$ turn to left until all air is replaced by buffer fluid.
- ▶ For venting the cassettes, tubes with quick connectors are attached to the inlet and outlet manifolds and closed with a pinch clamp (see chapter "6 Troubleshooting", page 32).

5.1.1 Alternative: for venting the Nano | Degas of the Product Using a Benchtop Chromatography System

Procedure

- ▶ Flush the chromatography system with buffer and make sure that all air bubbles in the flow path are removed.
- ▶ Connect the product to the pre-flushed chromatography system. Disconnect the tubing at the inlet of the product again and flush the product with equilibration buffer in upflow direction at 1 mL/min flow rate.
- ▶ Rinse the product until no bubbles are visible anymore (this will take approx. 2 min).
- ▶ Connect the tubing to the inlet again and continue to flush the product in downflow direction.
- ▶ Another way to remove persistent air bubbles: connect a buffer filled 10 – 20 mL syringe to the inlet of the Nano and immerse the whole Nano in a beaker; remove air by pushing and pulling the plunger up and down. Then connect with the chromatography system at a flowrate of 1 mL/min.

5.2 Cleaning and Equilibration

Procedure

- ▶ Optional: Sanitization for 30 minutes with 30 MV of 0.5 M NaOH solution at the flow rate of minimum 1 MV/min.
- ▶ Flush with equilibration buffer at 5 MV/min and stop equilibration when buffer pH is reached after 30 MV or more.
- ▷ Product is sanitized, equilibrated, and ready for use.

5.3 Recommended Buffer Conditions

Monoclonal antibodies are bound to the Sartobind® Rapid A membrane at physiological buffer conditions and 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.

Equilibration wash buffer	All commonly used buffers for equilibration and wash steps are suitable
Elution buffers and conditions	pH 2.5–4 e.g. 50–100 mM citrate, acetate, glycine.
Sanitization buffer	0.5 M NaOH
Regeneration	0.1–0.2 M NaOH

5.4 Recommended Flow Rates and Buffer Volumes






Membrane adsorbers can be run at much higher flow rate per volume than resin columns. As a rule of thumb, flow rates of 1–10 MV/min are recommended (see table). This recommendation is only a guideline as buffers and samples have different compositions and viscosities. Please test your respective flow rates with a small scale product to ensure that the flow rate fits with your pump capacities and the product pressure limits.

Lower flow rates than the recommended ones can also be used but will typically not improve binding capacity or overall performance. Cold room temperature increases buffer viscosity and possibly back pressure. Flow rates < 0.25 MV/min are not recommended.

The equilibration buffer volume can vary depending on the type and concentration of buffer. For E | L and biocompatibility studies 30 MV equilibration was used | needed.

For the cassette, flow rate (L/min) and equilibration volumes have to be multiplied with the number of cassettes in use.

Chromatography step	Membrane volume [MV]	Flow rate [MV/min]
Sanitization (optional once as bioburden reduction step)	30	1
Equilibration	until pH of buffer is reached (~10 MV)	5–10
Load		3–5
Wash	10	5–10
Elution	10	3–5
Wash (optional)	until pH of buffer is reached (~10 MV)	5–10
Regeneration (0.1–0.2 M NaOH)	10	5
Re-equilibration	until pH of buffer is reached (~20 MV)	5–10

					
Membrane volume (MV)	1.2 mL	10 mL	75 mL	200 mL	0.8 L
Bed height (mm)	4	4	4	4	4
Rec. flow rate (L/min)	0.02	0.2	1.5	2	16**
Rec. equilibration volume* (L)	0.01	0.1	0.75	2	8**

* Refer to “5.2 Cleaning and Equilibration”, page 26

** Multiply with number of used cassettes

5.5 Sample Preparation

To achieve high number of cycles and avoid fouling of the membrane it is recommended to pre-filter the sample inline. For inline pre-filtration, we recommend using a Sartopore® 2 XLG prefilter with a pore size of 0.2 µm.

NOTICE

Capacity loss | increased back pressure due to unfiltered feed.

- ▶ It is recommended to use inline filtering during operation.
 - ▶ When the pressure increases: Replace the prefilter.
-

5.6 Operation of the Sartobind® Rapid A Devices with Liquid Chromatography (LC) Systems

Procedure

- ▶ After venting of the device (see chapter 5.1.1 page 26), connect the outlet of the device 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 are typically run at much higher flow rates than columns, there is no risk of bubble formation in the UV cell when removing the flow restrictor.

5.7 Draining of Device After Usage

Drain the capsule or cassette by application of air or nitrogen pressure (<1 bar | 14.5 psi) to the inlet of the product.

Recommended is a pressure regulator that reduces the pressure to the <1 bar (14.5 psi) for a capsule and <0.5 bar (7.3 psi) for 1 to 13 cassettes draining pressure.

5.8 Scale up

Keep the sample concentration constant in lab and production scale. Adjustments might be required due to additional volumes from tubing and the system.

First, optimal binding conditions for the target molecule are developed at Nano scale. After optimization, the capture step can be scaled up to a larger capsule.

6 Troubleshooting

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 chapter “5.1 Venting”, page 24.
Air bubbles in Nano product	Incomplete air removal	For the Nano capsule fill a 10–20 mL Luer syringe with equilibration buffer and connect to the capsule. Hold capsule upright (outlet is up) and expel air. 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. Another method is to connect a second empty syringe to the top of the Nano and expel air and buffer into that syringe, disconnect the upper syringe to push out air and reconnect to the Nano, turn it and purge the solvent back and forth. As well, venting via Äkta is possible.

Problem	Possible cause	Action
Air bubbles in Nano product	Incomplete air removal	Very small air bubbles observed directly below the inlet of the Nano do not disturb performance. The capsule function will not be influenced as long as the small air bubbles remain outside of the membrane bed.
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. Thus 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 procedure		The capsules have been qualified and validated according to 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	Material has not been filtered	Prefilter with 0.2 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 membrane fouling	Replace unit. You may backflush within given flow and pressure limits, perform regeneration cycles (optionally repeat regeneration cycle). Work at room temperature, avoid lower temperatures
	Viscosity swelling effects	Add sodium chloride or use ionic buffers
Target molecule is not bound	Conditions for binding are insufficient	Increase pH if possible, consider other process parameters as type of buffer and conductivity.

Problem	Possible cause	Action
Binding capacity is not sufficient	Process conditions not optimized	Use larger adsorber product, 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.

7 Technical Data

7.1 Technical Information

Membrane volume (MV)	1.2 mL	10 mL
Ligand	Protein A	Protein A
Bed height	4 mm	4 mm
Design	cylindrical	cylindrical
Dynamic binding capacity at 10% breakthrough*	≥30 mg/mL matrix	≥30 mg/mL matrix
Maximum pressure [bar (MPa, psi)] at 20 °C	4 (0.4, 58)	4 (0.4, 58)
Approximate weight	10 g	65 g
Connectors	female luer	female luer ¾" sanitary clamp

* Reference protein and buffer: polyclonal antibody, 1xPBS pH 7.4, cond. 16mS/cm

75 mL	200 mL	800 mL
Protein A	Protein A	Protein A
4 mm	4 mm	4 mm
cylindrical	cylindrical	flat sheet
≥30 mg/mL matrix	≥30 mg/mL matrix	≥30 mg/mL matrix
4 (0.4, 58)	4 (0.4, 58)	2 (0.2, 29)
400 g	750 g	4.9 kg
¾" sanitary clamp	1½" sanitary clamp	1½" sanitary clamp

Short term pH stability 2 – 14 refers to cleaning in place and regeneration procedures during operation.

Long term storage pH stability 3 – 13 refers to overnight storage and longer.

7.2 Materials

Membrane materials

Matrix	Sartobind® Rapid A membrane
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Nominal pore size	>4 µm
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Ligand	Protein A
--------	-----------

Capsule materials

Outer cage, inner core, end caps, capsule housing	Polypropylene
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O-ring in vent valve (except Nano)	EPDM (ethylene propylene diene monomer)
------------------------------------	--

Cassette materials

Outer cage, seal	ABS, silicone
------------------	---------------

7.3 Storage Conditions

Shipment and storage in ethanol	20 – 24%
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Storage temperature	+2 – +8°C
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Until use keep product in the sealed aluminium bag.

When not in use the end caps | closing plates should be attached to the units to avoid evaporation of the storage solution.

7.4 Chemical Stability

Stable for all commonly used buffers in chromatography
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No oxidizing agents

8 Ordering Information

8.1 Products

Order number	Description	Quantity
96R-PA19D-T11--V	Sartobind® Rapid A Nano 1.2 mL capsule, manual, certificate	2
96R-PA19D-T11--A	Sartobind® Rapid A Nano 1.2 mL capsule, manual, certificate	4
96MU01-----11	Sartobind® mAb pack (Rapid A S Q STIC Phenyl) one Nano of each type, manual, certificate	1
96R-PA19D4R11	Sartobind® Rapid A Mini 10 mL capsule, Luer, 2 adapters, manual, certificate	1
96R-PA19D4RFF	Sartobind® Rapid A Mini 10 mL capsule, ¾" sanitary clamp, manual, certificate	1
96R-PA19D9MFF	Sartobind® Rapid A, 75 mL capsule, ¾" sanitary clamp, manual, certificate	1
96R-PA19D1GSS	Sartobind® Rapid A 200 mL capsule, 1½" sanitary clamp, manual, certificate	1

Order number	Description	Quantity
98R-PA19D-L	Sartobind® Rapid A 0.8 L cassette, manual, certificate	1

8.2 Accessories



Order number	Description	Quantity
1ZA---0004	Adapter Luer male to UNF 10–32 female, PEEK	1
1ZAOGV0003	Adapter UNF 10–32 female to sanitary ¾", 25 mm, polyoxymethylene	2
5ZGI--0001	Holder for 1 x 200 to 1,200 mL (10–30") capsule, stainless steel, 3 legs	1
5ZALB-0002	Distribution adapter for 3 x 200 (10–30") to 1200 mL capsules, 1 x 2", 3 x 1½", sanitary, stainless steel	1
7ZAL-V0013	Reducing adapter 1½" (50.5 mm) to ¾" (25 mm), sanitary	1
7ZAL-V0010	Reducing adapter 2" (64 mm) to 1½" (50.5 mm), sanitary	1

Order number	Description	Quantity
29Z-S00001	Manifold set for Sartoclear® Sartobind®, 1½" sanitary clamp	2
2ZGL--0005	Pilot filter holder for Sartoclear® Sartobind®	1
2ZGL--0006	Process filter holder for Sartoclear® Sartobind®	1
2ZGL--0007	Double process filter holder for Sartoclear® Sartobind®	1
2ZGL--0008	Drip pan for Pilot Filter Holder	1
2ZGL--0015	Drip pan for Process and double Process Filter Holder	1

8.3 Recommended Prefilter (Example)

Order number	Description	Quantity
5441307G4--SS--B	Sartopore® 2 XLG pore size 0.8 0.2 µm, filter area 210 cm ²	5

9 Dimensions and Connections

Membrane volume 4 mm	 1.2 mL	 10 mL
Size	Nano	Mini
Dimensions in mm	37 x 31 (H x Ø)	Luer: 70 x 54.5 (H x Ø) Sanitary: 100 x 54.5 (H x Ø)
Connectors	Luer female	<ul style="list-style-type: none"> - Luer female - Sanitary 3/4", 25 mm outer, 14 mm inner Ø
Gaskets	n.a.	3/4", inner Ø 16 mm

n.a.=not available



75 mL

5"

Sanitary: 190 x 77 (H x Ø)

Sanitary ¾",
25 mm outer,
14 mm inner Ø

¾",
inner Ø 16 mm



200 mL

10"

350 x 100 (H x Ø)

Sanitary 1½",
50.5 mm outer,
36 mm inner Ø

1½",
inner Ø 35.8 mm



0.8 L

Cassette

634 x 387 x 49 (W x L x Ø)

Via manifold:
Sanitary 1½",
50.5 mm outer,
36 mm inner Ø

For manifold:
1½", inner Ø 35.8 mm

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LM | DIR: 2776703-000-00

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 the other gender as well.

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Last updated:
09 | 2022

List of Sartorius Material Numbers Applying to EPA-FIFRA

96R-PA19D-T11--V

96R-PA19D-T11--A

96MU01-----11

96R-PA19D4R11

96R-PA19D4RFF

96R-PA19D9MFF

96R-PA19D1GSS

98R-PA19D-L
