SARTORIUS

85034-534-94

Instructions for Use

Sartobind® Protein A 2 mL

A Separation Technology Based on Macroporous Membranes

Storage conditions
Sartobind Protein A 2 mL Membrane Adsorbers are shipped in 20% ethanol | sodium phosphate buffer pH 7.0 and have to be stored refrigerated at +4 to +8°C immediately after arrival.

1. Introduction

Sartobind Protein A adsorbers represent a new generation of antibody purification devices based on membranes. They can simply be used in a liquid chromatography system, with a peristaltic pump or operated by hand with a syringe connected by Luer Lock.

Recombinant Protein A is coupled to a membrane which is fitted into a filter holder for easy and quick handling – making antibody purification nearly as easy as filtration. Combining the specificity of Protein A with the Sartobind technology, i.e. proprietary Membrane Adsorbers, guarantees for rapid handling and high purity of the desired antibody and make them ideally suited for antibody screening.

Protein A, a 42 kDa protein derived from the cell wall of specific Staphylococcus aureus strains has the ability to specifically bind to the Fc region of various immunoglobulins without affecting their three dimensional structure. Antibody purification on the basis of the Protein A affinity membrane is achieved by binding antibodies at neutral to high pH and by elution upon a shift to low pH. However, the interaction between Protein A and IgG is not equivalent for all species (see Table 1). Even within a species, Protein A interacts with some subgroups of IgG and not with others. For instance, human IgG3 and the majority of rat immunoglobulins do not bind to Protein A.

The unit can be used to quickly purify mg quantities of antibodies.

The Sartobind Protein A 2 mL is perfectly designed as a down scale unit for Sartobind large scale modules.

Table 1: Protein A affinity for different IgGs

Species	Isotype	Affinity to Protein A
Human	IgG1	++++
	IgG2	++++
	IgG3	-
	IgG4	++++
Mouse	IgG1	+
	IgG2a	++
	IgG2b	+++
	IgG3	++
Rat	IgG1	-
	IgG2a	-
	IgG2b	-
	IgG2c	+
Hamster		+
Guinea pig		++++
Rabbit		++++
Horse		++
Cow		++
Pig		+++
Sheep		+/-
Goat		-
Chicken		-

++++ = Strong affinity
+++ = Moderate affinity
++ = Weak affinity
+ = Slight affinity
+/- = Some IgGs bind weakly
- = No affinity

Sartobind Protein A 2 mL package content

Material No.	93PRAP06HB-12--A
Quantity	4
UNF 10-32 female to Luer Lock male	1
UNF 10-32 female to Luer Lock female	1
Manual	1

Accessory

Order number	Description
16534Q	Minisart® 0.2 µm Minisart® with Luer Lock outlet, non sterile, 500 items
1ZA---0004	Luer Lock adapter for UNF 10-32 to Luer Lock male, 1 item
1ZA---0005	Luer Lock adapter for UNF 10-32 to Luer Lock female, 1 item

2. Technical data

Membrane material	Stabilized reinforced cellulose nominal pore size 0.45 µm
Number of layers	20
Bed height (mm)	4
Bed volume (mL)	2
Ligand	Recombinant Protein A
Binding capacity for polyclonal human IgG*	10 – 15 mg/unit 5.0 – 7.5 mg/mL
Recommended flow rate	5 – 10 mL/min
Housing	Polypropylene
Maximum pressure	0.6 MPa | 87 psi| 6 bar
pH stability	3 – 9 (long term) 2 – 10 (short term)*
Storage	20% ethanol in 1 × PBS at +4 to +8° C

* Binding capacity at 10% breakthrough at a flow rate of 10 mL/min; sample 1 mg/mL human polyclonal antibody in PBS buffer.
** pH below 3 is sometimes required to elute all strongly bound IgG. Under these condition proteins may hydrolyze.

3. Operation

No further hardware than a 10 mL syringe with Luer Lock connector and beakers are required: A syringe can be used to push the fluids through the unit at velocity up to approximately 10 mL/min. When using syringes follow steps 3.1 to 3.7 . When using systems or peristaltic pumps, read 3.9 first.

3.1 Buffer recommendation

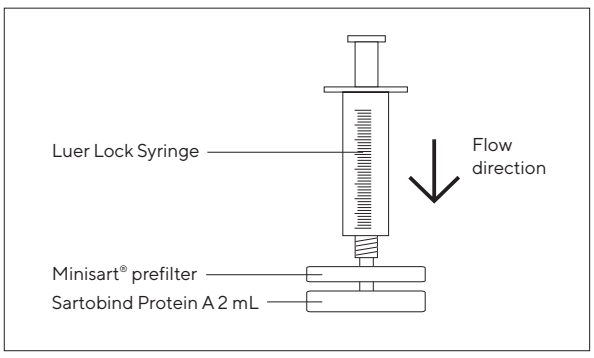
Equilibration Load Wash	PBS (phosphate buffered saline) 0.1 M sodium phosphate pH 7.0 – 8.0
Elution	0.1 M glycine/HCl pH 2.8 / 3.5 0.2 M citrate pH 2.8 or 3.5 0.1 M acetate pH 3.0
Neutralizing buffer	1.0 M Tris pH 9.0

The choice of the buffers depends on the stability of the target antibody and has to be verified previously. All buffers and protein solutions must be prefiltrated (0.2 µm).

3.2 Equilibration
The flow direction is indicated on the unit by an arrow. Remove the upper inlet cap of the unit. Connect a 10 mL syringe to the top and fill it with 10 mL of starting buffer. Remove the lower cap of the unit. Fill the upper part of the unit and remove any remaining air by moving the plunger up and down with short strokes. Make sure that the unit is completely filled with fluid. Close the outlet of the unit with the cap, remove the syringe from the unit, remove the plunger from the syringe and connect the syringe again to the unit. The unit is now ready for loading.

⚠ **The upper part has to be filled completely with fluid throughout the operation to ensure even flow and distribution of the feed stream.**

⚠ **Inline pre-filtering during sample loading is recommended (see figure) to protect the Membrane Adsorber from precipitated proteins.**



3.3 Loading
Connect the inlet of the unit via Luer Lock with a prefilter e.g. 0.2 µm membrane filter Sartorius Stedim Biotech Minisart® (order number 16534) with Luer Lock outlet. If you detect any pressure increase during loading, replace the prefilter.

Fill the sample into the syringe and remove the lower cap. When the fluid level has dropped, close the outlet with the cap. Make sure that no air enters the unit.

3.4 Washing
Wash with 10 mL of equilibration buffer.

3.5 Elution
Elute with 3 – 5 mL of elution buffer into a tube with sufficient neutralizing buffer to make sure that the pH of the sample will be close to neutral. The unit may not run dry during the operation.

3.6 Regeneration
Regenerate the Membrane Adsorber by purging 10 mL of starting buffer through the unit to remove the acidic eluate.

To remove stronger binding impurities from the membrane, load 10 – 50 mL 50 mM NaOH in 1 N NaCl on the adsorber at room temperature. Wash with 1 × PBS. Under these conditions Protein A may hydrolyze to some extent.

3.7 Storage
Keep the used unit filled with 20 % ethanol in 1 × PBS (137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, pH 7.4) at +4 °C.

3.8 Stability
The membrane is stable at pH 3 to 9 in all common chromatography buffers except oxidizing or reactive reagents.

3.9 Use of peristaltic pumps or liquid chromatography (LC) systems
For the operation of the adsorber units with workstations, use the UNF 10-32 Luer Lock adapters as enclosed Proceed as described under „Equilibration“ until the unit is filled completely with equilibration buffer, the outlet is closed and the syringe is removed. Start your HPLC or peristaltic pump at a low flow rate. When fluid emerges, stop the pump, connect the tubing via a Luer Lock adapter to the inlet of the unit. Make sure that no air is introduced. Remove the lower cap. Run the pump until fluid emerges from the outlet of the unit and stop it. Then connect outlet of the unit via Luer Lock adapter to the tubing of the LC detector.

During sample loading use an inline prefilter 0.2 µm to protect the membrane adsorber from precipitates. Replace the prefilter if you detect pressure increase during operation.

⚠ **The above described material has been manufactured in a standardized production process according to cGMP including a Quality Management System according to DIN | ISO 9001.**

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. Follow safety regulations and wear gloves, safety glasses and a lab coat during operation.

For more information about other Membrane Adsorber types and sizes please contact your local Sartorius Stedim Biotech representative or visit our homepage.

Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0
www.sartorius.com

DIR: 2677847-000-00
Status: 02 | 2021

List of Sartorius material numbers applying to EPA-FIFRA

93PRAP06HB-12-A