

Sartobind® Protein A 75 Membrane Adsorbers

Operating Instructions

A Separation Technology Based on Microporous Membranes

Storage conditions

Sartobind Protein A 75 Membrane Adsorbers have to be stored refrigerated at 4 °C immediately after arrival. This product has a shelf life of approx. 6 months.

Introduction

Sartobind Protein A adsorbers represent a new generation of antibody purification devices based on membranes. They can simply be used in an HPLC, FPLC or by hand with a syringe connected by Luer Lock. 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. 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 laboratory scale unit equipped with Luer Lock connectors can be used to quickly purify amounts of antibody up several mg. The 15 layer device Sartobind Protein A 75 is perfectly designed as a down scale unit for Sartobind large scale modules which contain 15, 30 or 60 layers.

Sartobind Protein A 75

Cat. No.	93PR-A06DB-12--V
Number of units	2
Instruction manual	1

Specifications

Membrane area	75 cm ² (2.1 ml membrane volume)
Number of layers bed height	15 4 mm
Binding capacity per cm ²	80 µg polyclonal IgG from human serum
Binding capacity per unit	6 mg polyclonal IgG from human serum

Materials

Housing	Polysulfone
Matrix	Stabilized reinforced cellulose, nominal pore size 0.45 µm
Ligand	Recombinant Protein A

Usage

Recommended flow rates	5 ml/min up to 10 ml/min
Max pressure	0.7 MPa 102 psi 7 bar
pH stability	3 - 9
Storage temperature	+4 °C
Storage buffer	PBS, 0.1 M sodium phosphate pH 7.0 - 8.0 and 0.02% azide

Table 1: Protein A affinity to different IgGs

Species	Protein A affinity
Human	++++
Mouse	++
Rat	+/-
Hamster	+
Guinea Pig	++++
Rabbit	++++
Horse	++
Cow	++
Pig	+++
Sheep	+/-
Goat	-
Chicken	-

Species Isotype	Protein A affinity
Human IgG1	++++
Human IgG2	++++
Human IgG3	-
Human IgG4	++++
Mouse IgG1	+
Mouse IgG2a	++
Mouse IgG2b	+++
Mouse IgG3	++
Rat IgG1	-
Rat IgG2a	-
Rat IgG2b	-
Rat IgG2c	+

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

Operation instructions for Sartobind Protein A 75 unit by gravity flow:

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.



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Equilibration

Hold the unit in a vertical position. Remove the upper cap. Connect a 10 ml syringe to the top and fill with 10 ml of starting buffer. Remove the lower cap. 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 to the unit. The unit is now ready for loading.

Important Note:

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

Loading

Prefilter the sample through a 0.45 µm membrane filter e.g. Sartorius Minisart 16555 or 17597. 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.

Washing

Wash with 10 ml of equilibration buffer.

Eluting

Elute with 2-3 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.

Regeneration

Regenerate the Membrane Adsorber by passing 10 ml of starting buffer to remove the acidic eluate.

Storage

Keep the used unit filled with starting buffer in the presence of an antimicrobial agent such as sodium azide at a concentration of 0.02% and refrigerate at +4 °C.

Stability

The membrane is stable at pH 3 to 9.

Suggested buffers: Equilibration, binding, wash: PBS (phosphate buffered saline) 0.1 M sodium phosphate pH 7.0 - 8.0 Elution: 0.1 M glycine/HCl pH 2.8 or 0.2 M citrate pH 2.8 or 0.1 M phosphate pH 3.0 Neutralizing buffer: 1.0 M TRIS pH 9.0

The choice of the buffers depend on the stability of the target antibody and has to be verified previously.

Use of peristaltic pumps or workstations such as HPLC systems

For the operation of the adsorber units with workstations, specific Luer Lock adapters are needed. They can be ordered as an accessory. 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 very 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 HPLC detector.

Accessory

Order number	17002---140
Description	Luer Lock adapters for inlet and outlet to M6 female
Quantity	2

For more information about scale-up or other membrane types please contact you nearest Sartorius office or visit our web-page: www.sartorius.com.

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