85030-519-45

Sartobind® Epoxy 75

A Microporous Coupling Membrane for Affinity Chromatography
Operating Instructions

Storage conditions
Sartobind epoxy-activated membrane units can be stored at room temperature.

Introduction
The Sartobind Epoxy unit is a powerful tool for protein immobilization to create an affinity Membrane Adsorber. Any molecule containing amino-, hydroxyl- or thiol-groups may be immobilized by covalent coupling to the epoxy-activated membrane. The membrane is fitted into a filter holder with Luer Lock connectors for easy handling to quickly couple biomolecules like proteins or peptides covalently.
A lengthy blocking step of the epoxy membrane is unnecessary, thus shortening the whole coupling procedure to a minimum. Another advantage of this membrane technology is the sample handling in a Luer Lock syringe filter holder. With Sartobind Epoxy membranes all steps can be carried out manually with a syringe making protein coupling and the following purification procedure as easy as filtration.

Handling
Sartobind Epoxy 75 units can be used to couple small amounts of a given protein (2 - 11 mg) with high coupling efficiency. Total setup time is less than 30 minutes for the preparation steps plus 15 to 180 minutes incubation time for the coupling reaction. Two coupling reactions can be done in parallel making the Sartobind Epoxy 75 unit an ideal tool for affinity chromatography of proteins. The resulting covalent bond by an ether, amine or thioether linkage between a given protein and the membrane forms an affinity purification matrix with desired specificity. The highest binding capacity is achieved with a coupling time of 3 hours and circulating the coupling solution with a peristaltic pump. For example, this can be used for the purification of antibodies with the respective antigen coupled to the column or vice versa.

The affinity unit may be used at flow rates of 5 to 10 ml/min depending on the binding kinetics. The Sartobind Epoxy 75 is perfectly designed as a down scale unit for Sartobind large scale modules having as well a bed height of 4 mm (15 layers).

Sartobind Epoxy 75

Cat. No.	93EPOX06DB-12--V
Down Scale units	2 x 75 cm^2 units
Instruction manual	1

Technical Data

Membrane area	75 cm^2 (2.1 ml membrane volume)
Number of layers | bed height	15 | 4 mm
Binding capacity per cm^2	30 – 150 µg protein*
Binding capacity per unit	2 – 11 mg protein*
Recommended volume for binding	5 ml
Concentration of ligand (protein)	1 – 10 mg/ml
Circulation flow rate (coupling)	1 ml/min
Recommended coupling time	15 – 180 min
Recommended working flow rate of loaded affinity adsorber during chromatography	5 – 10 ml/min**
Maximum pressure	0.6 MPa | 87 psi | 6 bar

* The values are generated with proteins ranging from 12.5 to 600 kD under standard coupling conditions using gravity flow.

** Maximum flow rate depends strongly on binding kinetics, therefore starting with a lower flow rate is recommended.

Materials

Housing	Polysulfone
Matrix	Stabilized reinforced cellulose, nominal pore size 0.45 µm
Ligand | ligand density	Epoxy | 1.5 µeq/cm^2

Operation
Only a 10 ml syringe with Luer Lock connector and beakers are required. You may use also peristaltic or HPLC pump to circulate the protein solution.

Important note:
The protein sample should not contain nucleophilic agents (e.g. Tris, glycine buffers or glycerol). These compounds should be removed prior to coupling as they will compete with the protein of choice for coupling on the epoxy groups of the membrane. Since high pH must be used to couple hydroxyl and amino ligands, epoxy-activated supports are not suitable for some base-sensitive ligands (some proteins). Since thiol groups are better nucleophiles than amine and hydroxyl groups, epoxy-activated supports can be used to couple thiol-containing proteins at lower pH.

Preparation of coupling buffer
To 50 ml of potassium phosphate stock A stock B is added until the desired pH is reached. Filter the buffer e.g. with a glass holder (order number 16307) for membranes 0.45 µm (111-06-050N). If coupling yield is low, use other buffers up to pH 12 at 0.5 to 1 M concentration. Our recommendation is to couple at pH 8.

Recommended stock buffers for coupling buffer preparation (pH 8-9)
Stock A: 1 M K_2HPO_4
Dissole K_2HPO_4 174.18 g in approx. 800 ml demineralized water by heating up the solution and continuous stirring. Fill up to one liter after buffer has reached room temperature.
Stock B: 1 M KH_2PO_4
Dissolve 136.09 g KH_2PO_4 in approx. 800 ml demineralized water and fill up to 1 liter

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Preparation of sample
- Dissolve the lyophilized protein in coupling buffer or dialyze the dissolved protein against the coupling buffer. Alternatively, dissolve 0.5 ml of your protein solution with 4.5 ml of coupling buffer.
- Prefilter the sample through a 0.45 µm membrane filter e.g. Sartorius Minisart 16555 or 17597.
- The concentration of the target substance should be between 1 and 10 mg/ml. A volume of 5 ml of coupling solution is optimal.

Preparation of the Sartobind epoxy 75 unit
Remove the upper cap of the unit. Connect a 10 ml syringe to the top which has been filled before with 5 ml coupling buffer. Remove the lower cap. Fill the unit. Make sure that no air has entered the unit by gently moving the plunger up and down. Close the outlet of the unit with the cap.

Coupling
- Connect a 10 ml syringe without plunger on top and fill with 5 ml of the protein dissolved in the coupling buffer.
- Remove the lower cap and let the fluid pass through the unit by gravity flow. This may last 1 to 2 hours. Alternatively you may connect the unit to a pump and let the solution circulate for three hours at approximately 1 ml/min.
- When all of the fluid has entered the unit, close the outlet with the cap.
- Wash with 10 ml PBS (phosphate buffered saline) or any other suitable buffer. Now the Sartobind affinity adsorber unit is ready for use.

Recommendations Sample loading of the Sartobind affinity 75 unit
- Connect a 10 ml Luer Lock syringe without plunger to the top and fill in the sample. Take care that no air enters the unit
- Remove the lower cap.
- Let the fluid pass through the unit by gravity.

Washing and elution
Wash with the 10 ml of a suitable equilibration buffer and discard the effluent. Elute the target with a minimum amount of a suitable elution buffer, e.g. 3-5 ml. Let the fluid pass through the unit by gravity until the fluid level has reached the constriction of the syringe. Push out the fluid with the plunger to remove any fluid. Immediately regenerate to avoid the drying out of the unit. That could irreversibly damage the protein ligand.

Regeneration
Regenerate the Membrane Adsorber by passing 10 ml of equilibration buffer. Repeat this step twice. Ensure that the pH of the eluate has reached the value of the equilibration buffer.

Storage
Keep the used unit filled with equilibration buffer in the presence of an antimicrobial agent such as sodium azide at a concentration of 0.02%.

Use of a peristaltic pump or chromatography systems
For the operation of the adsorber unit with chromatography systems, specific connectors can be ordered.

Accessory

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

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

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