Smac MMC

Index

1. Product Description.....1
2. Purification Procedure.....1
3. Cleaning-in-Place.....2
4. Troubleshooting.....3
5. Related Products.....3

1. Product Description

Smac MMC is a multimodal weak cation exchange resin with ionic interaction, hydrogen bond, hydrophobic interaction for fast and efficient protein purification.**Smac MMC** based on a highly rigid agarose base matrix that offers outstanding pressure/flow properties, optimized pore structure,and very high chemical stability to support CIP procedures.

Table 1. Characteristics of **Smac MMC**

Item	Description
Matrix Spherical	rigid agarose matrix
Ion exchange type	Multimodal weak cation
Ionic capacity	0.07-0.10 mmol H^+/ml medium
Particle size	75 μm
Flow rate	300-600 cm/h
pH	2-12
Chemical stability	All commonly used aqueous buffers, 1 M sodium hydroxide, 1 M acetic acid
Storage Solution	20% ethanol
Storage Temperature	4-30℃

2. Purification Procedure

2.1 Buffer Preparation

The Binding Buffer and Elution Buffer used are selected according to different sample conditions. The basic principle is low salt sample, high salt elution.

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.22 or 0.45 μm filter before use.

2.2 Sample Preparation

To insure that proper ionic strength and pH are maintained for optimal binding, the sample may be dialyzed overnight against Binding Buffer.

2.3 Packing Columns

Smac MMC is easy to pack and use, and its high flow properties make it excellent for industrial scaling-up. Here we describe the packing procedure of **Smac MMC** to medium pressure chromatography columns.

1) Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air has been trapped under the column net.

2) Close the column outlet leaving the net covered with packing buffer.

3) Resuspend the beads stored in its container by shaking (avoid stirring the sedimented medium). Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.

If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

4) Open the bottom outlet of the column and set the pump to run at the desired flow velocity. Ideally, **Smac MMC** is packed at a constant pressure of approximately 3 bar (0.3 MPa).

If the packing equipment does not include a pressure gauge, use a packing flow velocity of approximately 400 cm/h (10 cm bed height, 25°C, low viscosity buffer). If the recommended pressure or flow velocity can not be obtained, use the maximum flow velocity the pump can deliver. This should also give a reasonable well-packed bed. Do not exceed 75% of the packing flow velocity in subsequent chromatographic procedures.

5) Maintain packing flow velocity for at least 3 bed volumes. When the bed has stabilized, mark the bed height on the column and close the bottom outlet and stop the pump.

If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. If using the column, carefully place the top filter on top of the bed before fitting the adapter.

6) With the adapter inlet disconnected, push the adapter down into the column until it reaches the mark, allowing the packing solution to flush the adapter inlet. Lock the adapter in position.

7) Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will be formed between the bed surface and the adapter.

8) Close the bottom outlet. Disconnect the column inlet and lower the adapter approximately 2 mm into the bed. Connect the pump. The column is now ready to use.

2.4 Sample Purification

1) Fill the syringe or pump tubing with distilled water. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, “drop to drop” to avoid introducing air into the column. Remove the snap-off end at the column outlet.

2) Wash the column with 3-5 column volumes of distilled water.

3) Equilibrate the column with at least 5 column volumes Binding Buffer.

4) Apply the pre-treated sample, using a Loop fitted to the connector or by pumping it onto the column.

5) Wash with Binding Buffer until the absorbance reaches the baseline or no material appears in the effluent (Generally at least 10-15 column volumes).

6) Elute with elution buffer using a stepwise or linear gradient. For one-step elution, 5 column volumes are usually enough. Other volumes may be required if the interaction is difficult to break. Linear gradient elution can be used to separate proteins of different binding strengths with a small gradient, such as 20 column volumes or more.

3. Cleaning-in-Place

After each separation, elute any reversibly bound material either with a high ionic strength solution (e.g. 1 M NaCl in buffer) or by increasing pH. Regenerate the media by washing with at least 5 bed volumes of buffer, or until the column effluent shows stable conductivity and pH values.

Cleaning-in-place (CIP) is a cleaning procedure that removes contaminants such as lipids, precipitates, or denatured proteins that may remain in the packed column after regeneration. Regular CIP also prevents the build-up of these contaminants in the media bed and helps to maintain the capacity, flow properties and general performance of the media.

A specific CIP protocol should be designed for each process according to the type of contaminants present. CIP cycle is generally recommended every 1-5 separation cycles.

- **Remove the ionically bound proteins**

Wash with 3-4 column volumes of 2 M NaCl. Contact time 10-15 min.

- **Remove the precipitation or hydrophobically bound proteins or lipoproteins**

Wash with at least 2 column volumes of 1 M NaOH. Contact time1-2 h.

- **Remove lipids and very hydrophobic proteins**

Wash with 3-4 column volumes of 1% non-ionic detergent, 70% ethanol or 30% isopropanol. Contact time 1-2 h.

4. Troubleshooting

Problem	Probable Cause	Solution
Back pressure is too high	Column is clogged	Cleaning in place (part 3).
	Sample solution contains precipitate	Filtering the sample solution by passing them through a 0.22 μm or 0.45 μm filter.
Eluate is not pure	The medium repeat too much times.	Cleaning in place (part 3).
	Wash is not enough.	Increase the volume of Wash Buffer.

5. Related Products

Product	Cat. No.	Size
Smac MMC	SI033025	25 ml
	SI033100	100 ml
	SI033500	500 ml
	SI03301L	1 L
	SI03310L	10 L