HiSelect Smac SP 40

Index

1. Product Description

Ion exchange resins are widely used in biomedical and bioengineering for separation and purification of proteins, nucleic acids and polypeptides.**Smac SP 40** 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.

HiSelect Smac SP 40 is prepacked ready to use column. It is packed with 4.7 ml **Smac SP 40**. The arrow on the column label indicates the recommended flow direction. **HiSelect Smac SP 40** can be adapted to all kinds of chromatography system, such as ÄKTA. It is easy to operate.

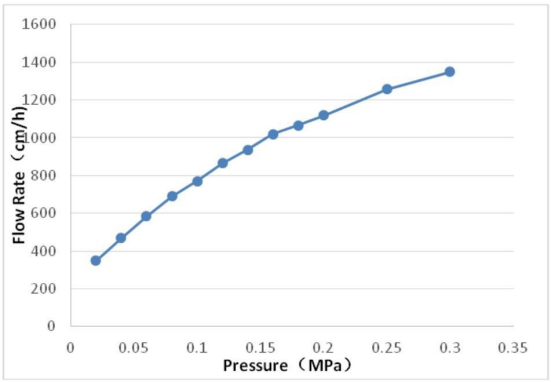

Fig.1. A typical pressure/flow rate curve

Smac SP 40 is a strong cation exchange resin. The ion exchange group is a sulphopropyll group -O-$CH_2CH_2CH_2SO_3^-$, see below.

Table 1. Characteristics of **Smac SP 40**

Item	Description
Size	4.7 ml
Column size	0.77×10 cm
Matrix	Highly cross-linked agarose
Ion exchange type	Strong cation
Total ionic capacity	0.13-0.17 mmol H^+/ml medium
Particle Size	30-60 μm
Binding capacity	> 70 mg lysozyme/mL medium
Flow rate	< 200cm/h
pH stability	4-13
Storage buffer	20% ethanol，0.2M sodium acetate
Storage	2 - 8°C
Chemical stability	1 M NaOH, 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol and 70% ethanol

2. Purification Procedure

2.1 Buffer Preparation

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

2.2 Sample Preparation

It is recommended to filter the sample solution by passing them through a 0.22 μm or 0.45 μm filter before use.

2.3 Sample Purification

HiSelect Smac SP 40 is prepacked, ready to use column for purification. The prepacked column provides fast, simple and easy separations in a convenient format.

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 stopper at the column outlet and connect the column to the chromatographic system.

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

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

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

5) Wash with Wash 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.

2.4 Analysis

Identify the fractions containing the target protein. Use UV absorbance, SDS-PAGE, or Western Blot.

3. Cleaning-in-Place

After each separation, elute any reversibly bound material either with a high ionic strength solution (e.g. 1M NaCl in buffer) or by increasing pH. Regenerate the beads 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 2M NaCl. Contact time 10-15min.

Remove the precipitation or hydrophobically bound proteins or lipoproteins

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

Remove lipids and very hydrophobic proteins

Wash with 2-4 column volumes of 0.5% non-ionic detergent, 70% ethanol or 30% isopropanol. Contact time 1-2h.

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 0.22μm or 0.45μm 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
HiSelect Smac SP 40	SI028C47	4.7 ml
HiPur Smac SP 40	SI028C20	20 ml