



Smac A-ME

Index

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

1. Product Description

Smac A-ME is an affinity chromatography medium specifically designed for monoclonal antibody (mAb) capture and purification. The characteristics of **Smac A-ME** are summarized in Table 1. Its ligand originates from a bioengineered mutant of native Protein A, enabling gentle Fc-containing molecule elution under mild pH conditions with compact elution volumes. This medium demonstrates superior process performance, high protein recovery rates and high reduction in host cell protein (HCP) levels. The alkali resistance, high loading capacity, high resolution, and rigid agarose matrix make **Smac A-ME** particularly suitable for large-scale purification of industrialized antibodies or antibodies for clinical applications.

Table 1. Characteristics of Smac A-ME

Item	Description
Matrix	rigid agarose matrix
Particle size	~60 µm
Ligand	Recombinant alkali-tolerant protein A
Binding Capacity	40-55 hlgG/ml medium
Chemical stability	all reagent in the process of antibody purification
pH stability	3-12
Cleaning-in-place	0.1 M NaOH
Recommended maximum flow rate	300 cm/h
Storage	20% ethanol, 2-8℃

2. Purification Procedure

2.1 Buffer Preparation

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.

Binding/Wash Buffer: 0.15 M NaCl, 20 mM Na₂HPO₄, pH 7.0

Elution Buffer: 0.1 M sodium acetate, pH 3.0-5.0

Neutralization Buffer: 1 M Tris-HCl, pH 8.5

2.2 Sample Preparation

To insure that proper ionic strength and pH are maintained for optimal binding, it is necessary to dilute serum samples, ascites fluid or cell culture supernatant at least 1:1 with Binding/Wash Buffer. Alternatively, the sample may be dialyzed overnight against Binding/Wash Buffer.

2.3 Packing of Column

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 magnetic 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, the medium is packed at a constant pressure of approximately 3 bar (0.3 MPa). If the recommended pressure or flow velocity can not be obtained, use the maximum flow velocity the of the pump. This should also give a well-packed bed. Do not exceed 75% of the packing flow velocity in subsequent chromatographic procedures.

5) When the bed has stabilized, mark the position of the bed surface. 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, adjust the adapter to the bed surface.

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 push the adapter a further 2-3 mm. Connect the pump. The column is now ready to use.

2.4 Sample Purification

1) Fill the pump tubing with binding buffer. Connect the column to purification system, "drop to drop" to avoid introducing air into the column.

2) Wash the column with 10 column volumes of binding buffer .

3) Apply the sample, using a syringe fitted to the connector or by pumping it onto the column.

4) Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.

5) Elute with 5 column volumes of elution buffer. Other volumes may be required if the interaction is difficult to break.

6) The elution fractions need to be neutralized immediately, and it is generally recommended to use a neutralizing solution of 1/10 of the volume of the elution fractions for neutralization.

2.5 Analysis

Identify the fractions using UV absorbance, SDS-PAGE, or western blot.

3. Cleaning-in-Place

In general, **Smac A-ME** are well suited for reuse a number of times. When precipitation and protein aggregation cause the loss of velocity and combined loads, you need to clean the medium.

Smac A-ME is a alkali tolerant medium and allows the use of 0.1M NaOH for cleaning-in-place (CIP), which improves product quality and reduces overall costs. The steps are as follows:

1) 3 column volumes binding buffer, pH 7.4;

2) 0.1 M NaOH, 15min contact time;

3) 5 column volumes binding buffer, pH 7.4

Note: The viscosity of 0.1M NaOH may increases the column pressure. Customers can reverse flushing according to need.

4. Troubleshooting

Problem	Possible Cause	Solution
Back pressure exceeds 3 bar	Column is clogged.	Cleaning in place (part 4).
		Filtering the sample solution by passing them through a 0.22 µm or 0.45 µm filter.
The curve is not stable during sample purification	Tiny air bubbles from buffer or sample.	De-gas buffers and samples. Do not allow the column to dry.
No antibody in the elute.	The concentration of antibody of interest is very low.	Purify the antibody using the specific antigen coupled to a beads.
	The antibody is unstable at low pH.	Neutralize the eluted fractions with neutralization buffer immediately after elution.
The recovery rate gradually decreases.	The sample is overloaded.	Reduce the loading volume.
	The reduced performance of the medium.	Cleaning in place (part 4).





5. Related Products

Product	Cat. No.	Size
Smac A-ME	SA123005	5 ml
	SA123025	25 ml
	SA123100	100 ml
	SA123500	500 ml
	SA12301L	1 L
	SA12310L	10 L
PreCap Smac A-ME	SA123C11	1×1 ml
	SA123C51	5×1 ml
	SA123C15	1×5 ml
	SA123C55	5×5 ml
	SA123CS	3×1 ml+1×5 ml

