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rProtein A-ME Beads 4FF

Index

1. Product Description

rProtein A-ME Beads 4FF is an affinity chromatography medium designed for the purification of monoclonal antibody, polyclonal antibody and Fc tag fusion protein.The characteristics of **rProtein A-ME Beads 4FF** are summarized in Table 1. Conventional protein A Affinity media need low pH elution (pH3.0, or even lower) after binding with antibodies.This elution condition is easy to cause antibody aggregation or denaturation.**rProtein A-ME** is obtained by gene recombination technology on the base of natural protein A.The antibody can elute at A relatively high pH (pH5.0) to ensure the antibody activity.

Table 1. Characteristics of **rProtein A-ME Beads 4FF**

Item	Description
Matrix Spherical	Highly cross-linked 4% agarose beads
Ligand	recombinant protein A
Static Binding Capacity	＞15mg human IgG/ml medium
Particle size	45-165um
pH	3-10
Maximum Pressure	0.3MPa, 3bar
Storage	1×PBS containing 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 Na_2HPO_4, pH 7.0

Elution Buffer: 25 mM NaH_2PO_4，0.5 M NaCl，pH 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, ascite fluid or cell culture supernatant at least 1:1 with binding/wash buffer. Alternatively, the sample may be dialyzed overnight with 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 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 reservoi8r, 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, **rProtein A-ME Beads 4FF** is packed at a constant pressure of approximately 3bar (0.3MPa). 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) When the bed has stabilized, 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, approximately 2 mm into the bed, allowing the packing solution to flush the adapter inlet.

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 binding buffer. 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 10 column volumes of binding buffer.

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

4) Wash the column 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.

2.5 Analysis

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

3. Maintenance

3.1 Regeneration

Regenerate the column by washing the resin with 3 column volumes of Elution Buffer followed by equilibration with at least 3 column volumes of Binding/Wash buffer. Columns can be regenerated up to 10 times without significant loss of binding capacity.

3.2 Cleaning-in-place

In general, **rProtein A Beads** 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.

Remove the precipitation or denatured protein

Wash the column with 2 column volumes 6M guanidine hydrochloride solution. Finally wash the column with 5 column volumes 1XPBS (pH7.4).

Remove the hydrophobically bound protein

Wash the column with 3-4 column volumes 70% ethanol or 2 column volumes 0.1% non-ionic detergent. Finally wash the column with 5 column volumes 1XPBS (pH7.4).

4. Troubleshooting

Problem	Possible Cause	Solution
Back pressure is too high	The filter is blocked.	Clean or replace the filter.
	Column is clogged.	Cleaning in place(part 4).
		Filter the sample solution by passing them through a 0.22μm or 0.45μm filter.

The curve is not stable during sample purification	There are air bubbles in buffer or sample.	De-gas buffers and samples. Do not allow the column to dry.
No antibody in the elute.	The antibody can not be eluted.	Reduce the pH of the elution buffer.
	The antibody is unstable at low pH.	Neutralize the eluted fractions with neutralization buffer immediately after elution.
	The IgG subclass does not bind to protein A.	Try to use the other affinity chromatography media, such as rProtein G Beads, rProtein A/G Beads or PabPur Sulfolink Beads(the specific antigen can be immobilized to the beads).
The recovery rate gradually decreases.	The sample is overloaded.	Reduce the loading volume.
	The reduced performance of the medium.	Cleaning in place(part 3).

5. Related Products

Product	Cat. No.	Size
rProtein A-ME Beads 4FF	SA073005	5 ml
	SA073025	25 ml
	SA073100	100 ml
	SA073500	500 ml
	SA07301L	1 L