Protein At Beads LX

Index

1. Product Description.....1
2. Purification Procedure.....1
3. Removal of leached ligand.....2
4. Cleaning-in-Place.....2
5. Troubleshooting.....3
6. Related Products.....3

1. Product Description

Protein At Beads LX is a novel, alkali-tolerant protein A-derived medium for capturing monoclonal antibodies from larger volumes of feed by packed bed chromatography. Table 1 is Characteristics of **Protein At Beads LX**. Alkali-tolerant protein A (protein At) is obtained by gene recombination technology on the base of natural protein A. The ligand has no animal origin during purification. The ligand is specially designed to enhance its stability to alkali and protease.**Protein At Beads LX** has high dynamic binding capacity (DBC) after extended retention time and is specially designed for process feed from high-expression cell cultures with increased antibody titers.

Ligand is directional immobilized to the agarose beads. The level of leakage of the ligand during elution is very low (less than 10ng ligand/mg antibody).

The base matrix of **Protein At Beads LX** is 85 μm beads of highly cross-linked agarose derivative with excellent chemical and physical stabilities, making it ideal for process scale applications.

Table 1. Characteristics of **Protein At Beads LX**

Item	Description
Matrix	Highly cross-linked agarose beads
Ligand	Protein At (Alkali-tolerant protein A)
Static Binding Capacity	＞60 mg Rabbit IgG/ml medium
Particle size	～85 μm
Chemical stability	all reagent in the process of antibody purification
Cleaning-in-place	0.1-0.5 M NaOH
Recommended flow rate	50-500 cm/h
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: 0.1 M glycine, pH 3.0-3.6

Neutralization Buffer: 1 M Tris-HCl, pH 9.0

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 packing equipment does not include a pressure gauge, use a packing flow velocity of approximately 500 cm/h (10 cm bed height, 25℃, low viscosity buffer). 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 2-3mm. 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) Add 10μl Neutralization Buffer to each 100 μl of eluate to neutralize the pH.

2.5 Analysis

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

3. Removal of leached ligand

The ligand leakage of **Protein At Beads LX** is generally low (less than 10ng ligand/mg antibody). However, in many monoclonal antibody applications it is a requirement to eliminate leached ligand from the final product. There are a number of chromatographic solutions, such as cation exchange chromatography, anion exchange chromatography, or size exclusion chromatography.

4.Cleaning-in-Place

In general, **Protein At Beads LX** 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.
Protein At Beads LX is a alkali tolerant medium and allows the use of 0.1-0.5 M NaOH for cleaning-in-place (CIP), which improves product quality and reduces overall costs. The steps are as follows:

- 3 column volumes binding buffer,pH 7.4;
- 0.1 or 0.5 M NaOH,15 min contact time;
- 5 column volumes binding buffer, pH 7.4

Note: The viscosity of 0.1–0.5 M NaOH may increases the column pressure. Customers can reverse flushing according to need.

5. 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 (i.e., PabPur Sulfolink Beads, Cat. No. SA018005).
	The antibody is unstable at low pH.	Neutralize the eluted fractions with neutralization buffer immediately afterelution.
The recovery rate gradually decreases.	The sample is overloaded.	Reduce the loading volume.
	The reduced performance of the medium.	Cleaning in place(part 4).

6. Related Products

Product	Cat. No.	Size
Protein At Beads LX	SA085005	5 ml
	SA085025	25 ml
	SA085100	100 ml
	SA085500	500 ml
	SA08501L	1 L
	SA08510L	10 L
MabCap At LX	SA085C11	1×1 ml
	SA085C51	5×1 ml
	SA085C15	1×5 ml
	SA085C55	5×5 ml
	SA085CS	3×1 ml+1×5 ml