HiSelect L 4FF

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1. Product Description

rProtein L Beads 4FF is an affinity chromatography medium designed for purification of classes, subclasses and fragments of immunoglobulins from biological fluids and from cell culture media. The recombinant protein L ligand is immobilized to highly cross-linked 4% agarose beads. The Characteristics of **rProtein L Beads 4FF** are summarized in Table 1.

Protein L is recombinantly expressed in E. coli and retains the property of binding to the antibody κ-chain without affecting the antigenic binding site of the antibody. Protein L binds well to the kappa light chain of human and mouse, and may bind specifically to certain kappa isoforms of other species. Protein L binds weakly to rabbit immunoglobulins and does not bind bovine, goat or sheep source immunoglobulins.

HiSelect L 4FF is a prepacked ready to use column. It is packed with 4.7ml rProtein L Beads 4FF .The arrow on the column label indicates the recommended flow direction.HiSelect L 4FFcan be adapted to all kinds of chromatography system, such as ÄKTA. It is easy to operate.

Table 1. Characteristics of HiSelect L 4FF

Item	Description
Size	4.7 ml
Column size	0.77×10 cm
Matrix Spherical	Highly cross-linked 4% agarose beads
Ligand	recombinant protein L
Static Binding Capacity	＞15mg Human IgG/ml medium
Particle size	45-165 μm
Maximum Pressure	0.3 MPa, 3 bar
pH	3-10
Storage Solution	1×PBS containing 20% ethanol
Storage Temperature	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

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 against Binding/Wash Buffer.

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 L 4FF is a prepacked, ready to use column for purification. The prepacked column provides fast, simple and easy separations in a convenient format.

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.

2.4 Analysis

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

3. Cleaning-in-Place

In general, **rProtein L Beads 4FF** 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 6 M guanidine hydrochloride solution. Finally wash the column with 5 column volumes 1×PBS (pH 7.4).

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 1×PBS (pH 7.4).

4. Troubleshooting

Problem	Possible Cause	Solution
The flow rate of the column is very low.	The sieve plate is blocked.	Clean or replace the sieve plate.
	Column is clogged.	Cleaning in place(part 3).
		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.
A considerable amount of sample has been loaded, but no specific antibody of interest is detected.	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 sensitive to low-pH elution buffer	Neutralize the eluted fractions with Neutralization Buffer immediately after elution.
The recovery rate gradually decreases.	The sample is overloaded.	Reduce the loading volume.
	The column is too dirty and the binding capicity is reduced.	Cleaning in place (part 3).

5. Related Products

Product	Cat. No	Size
HiPur L 4FF	SA033C20	20 ml
HiSelect L 4FF	SA033C47	4.7 ml