AbCap 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.

AbCap L 4FF is a prepacked ready to use ,column for purification of monoclonal and polyclonal antibodies. **AbCap L 4FF** 1ml and 5ml columns are packed with 1ml and 5ml of rprotein L beads 4FF. **AbCap L 4FF** can be adapted to all kinds of chromatography system, such as ÄKTA. It is easy to operate.

Table 1. Characteristics of **rProtein L Beads 4FF**

Item	Description
Matrix Spherical	Highly cross-linked 4% agarose beads
Ligand	recombinant protein L
Static Binding Capacity	＞15mg Mouse 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 filtering 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 filtering the sample solution by passing them through a 0.22 μm or 0.45 μm filter before use.

2.3 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) 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 the sample with 5-10 column volumes Elution Buffer. Collect the eluate containing the target immunoglobulin and immediately neutralize to pH 7.4 with Neutralization Buffer (1/10 volume of total eluate).

6) Equilibrate the column with 3 column volumes of Binding Buffer, 5 column volumes of distilled water and 5 column volumes of 1×PBS containing 20% ethanol. Finally store the resin with 1×PBS containing 20% ethanol at 4℃.

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
rProtein L Beads	SA045005	5 ml
	SA045025	25 ml
	SA045100	100 ml
	SA045500	500 ml
	SA04501L	1 L
	SA04510L	10 L
rProtein L Beads 4FF	SA033005	5 ml
	SA033025	25 ml
	SA033100	100 ml
	SA033500	500 ml
	SA03301L	1 L
	SA03310L	10 L
AbCap L 4FF	SA033C11	1×1 ml
	SA033C51	5×1 ml
	SA033C15	1×5 ml
	SA033C55	5×5 ml
	SA033CS	3×1 ml+1×5 ml