AbCap G 4FF

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1. Product Description

rProtein G Beads 4FF is an affinity chromatography medium designed for the purification of monoclonal antibody,polyclonal antibody and Fc tag fusion protein. The recombinant protein G ligand is coupled to highly cross-linked 4% agarose beads. The characteristics of **rProtein G Beads 4FF** are summarized in Table 1 .

Protein G, a bacterial cell wall protein isolated from group G Streptococci, binds to mammalian IgGs mainly through Fc regions. Native protein G has 3 IgG binding domains and also site for albumin and cell-surface binding. The recombinant protein G have been eliminated the nonspecific binding site. Although protein G has very similar tertiary structures to protein A, their amino acid compositions differ significantly, resulting in different binding characteristics. Protein G can be used for purification of mammalian monoclonal and polyclonal IgGs that do not bind well to protein A. Protein G has better affinity than protein A for most mammalian IgGs, especially for certain subclasses including human IgG3, mouse IgG1 and rat IgG2a.

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

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

Item	Description
Matrix Spherical	Highly cross-linked 4% agarose beads
Ligand	recombinant protein G
Static Binding Capacity	＞30mg Goat IgG/ml medium
Particle size	45-165um
Maximum Pressure	0.3MPa, 3bar
pH	3-10
Storage Solution	1×PBS containing 20% ethanol
Storage Temperature	2℃-8℃

Table 2. Relative binding strengths of antibodies from various species to protein G and protein A as measured in a competitive ELISA test.

Species	Subclass	Protein A binding	Protein G binding
Human	IgA	varible	—
	IgD	—	—
	IgE	—	—
	IgG1	++++	++++
	IgG2	++++	++++
	IgG3	—	++++
	IgG4	++++	++++
	IgGM	varible	—
Avian egg yolk	IgY	—	—
Cow		++	++++
Dog		++	+
Goat		—	++
Guinea pig	IgG1	++++	++
	IgG2	++++	++
Hamster		+	++
Horse		++	++++
Koala		—	+

Llama		—	+
Monkey(rhesus)		++++	++++
Mouse	IgG1	+	++++
	IgG2a	++++	++++
	IgG2b	+++	+++
	IgG3	++	+++
	IgM	variable	—
Pig		+++	+++
Rabbit	no distinction	++++	+++
Rat	IgG1	—	+
	IgG2a	—	++++
	IgG2b	—	++
	IgG3	+	++
Sheep		+/-	++

++++=strong binding; ++= medium binding; —=weak binding or no binding

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 with binding/wash buffer. It is recommended to filter the sample solution by passing them through a 0.22μm or 0.45μm 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) 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.

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. 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 G 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 6M guanidine hydrochloride solution. Finally wash 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
The flow rate of the column is very low.	The sieve plate is blocked.	Clean or replace the filter.
	Column is clogged.	Cleaning in place(part 3).
		Filtering the sample solution by passing them through a 0.22μm or 0.45μm 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 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 G.	Try other affinity chromatography media to purify the antibody, such as rProtein A 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 G Beads	SA016005	5 ml
	SA016025	25 ml
	SA016100	100 ml
	SA016500	500 ml
	SA01601L	1 L
	SA01610L	10 L
AbPur rProtein G Kit	SA016K03	3T
rProtein G Beads 4FF	SA020005	5 ml
	SA020025	25 ml
	SA020100	100 ml
	SA020500	500 ml
	SA02001L	1 L
	SA02010L	10 L
AbCap G 4FF	SA020C11	1 X 1 ml
	SA020C51	5 X 1 ml
	SA020C15	1 X 5 ml
	SA020C55	5 X 5 ml
	SA020CS	3X1 ml+1X5 ml