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HiPur A 4FF

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1. Product Description

Smart-Lifesciences **rProtein A Beads 4FF** is an affinity chromatography medium designed for the purification of monoclonal antibody, polyclonal antibody and Fc tag fusion protein.The recombinant protein A is coupled to highly cross-linked 4% sepharose gel. This coupling method can improve the binding capacity for immunoglobulin. The static binding capacity of **rProtein A Beads 4FF** is greater than 40 mg human IgG/ml settled beads. The dynamic binding capacity will vary depending on several factors such as target antibody, flow rate etc. The characteristics of **rProtein A Beads 4FF** are summarized in Table 1 .

Protein A ,a bacterial cell wall protein isolated from Staphylococcus aureus, combines mammalian IgGs mainly through Fc regions. Recombinant protein A contains five high affinity IgG binding domains with other non-essential domains removed to reduce nonspecific binding.

HiPur A 4FF is a prepacked ready to use column. It is packed with 20ml rProtein A Beads 4FF .The arrow on the column label indicates the recommended flow direction.HiPur A 4FF can be adapted to all kinds of chromatography system, such as ÄKTA. It is easy to operate.

Table 1. Characteristics of **HiPur A 4FF**

Item	Description
Size	20 ml
Column size	1.6×10 cm
Matrix Spherical	Highly cross-linked 4% agarose beads
Ligand	recombinant protein A
Static Binding Capacity	＞40mg Human 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 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 with Binding/Wash Buffer.

2.4 Sample Purification

HiPur A 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.

2.5 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 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 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
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 0.22μm 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.
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 IgG subclass does not bind to protein A.	Try other affinity chromatography media to purify the antibody, such as rProtein G Beads or rProtein A/G Beads.
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 A 4FF	SA015C20	20 ml
HiSelect A 4FF	SA015C47	4.7 ml