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HiPur A/G 4FF

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1. Product Description

rProtein A/G Beads 4FF is an affinity chromatography medium designed for the purification of monoclonal antibody and polyclonal antibody at both laboratory and process acale. The rProtein A/G is coupled to highly cross-linked 4% agarose beads by a technique which generates a stable thioether linkage between rProtein A/G and the agarose beads. The coupling technique is optimized to give a high binding capacity for IgG.This binding capacity,together with the excellent kinetic and flow properties of the highly cross-linked beads.**rProtein A/G Beads 4FF** can be used for antibody purification, immuneprecipitation and Co- immuneprecipitation.

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

The characteristics of **rProtein A/G Beads 4FF** are summarized in Table 1.

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

Item	Description
Size	20 ml
Column size	1.6×10 cm
Matrix Spherical	Highly cross-linked 4% agarose beads
Ligand	recombinant protein A/G
Static Binding Capacity	10-15 mg 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℃

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

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

Llama		—	+	
Monkey(rhesus)		++++	++++	++++
Mouse	IgG1	+	++++	++
	IgG2a	++++	++++	++++
	IgG2b	+++	+++	+++
	IgG3	++	+++	+++
	IgM	variable	—	—
Pig		+++	+++	++++
Rabbit	Total IgG	++++	+++	++++
Rat	IgG1	—	+	++
	IgG2a	—	++++	++++
	IgG2b	—	++	++
	IgG3	+	++	++
Sheep	Total IgG	+/-	++	++

++++=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/G 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. Related Products

Product	Cat. No.	Size
HiPur A/G 4FF	SA032C20	20 ml
HiSelect A/G 4FF	SA032C47	4.7 ml