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rProtein G Beads

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1. Product Description

rProtein G Beads is an affinity chromatography medium designed for purification of monoclonal antibody, polyclonal antibody and Fc tag fusion protein. The recombinant protein G ligand is coupled to 4% agarose beads. The Characteristics of **rProtein G Beads** 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 sites 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 greater affinity than protein A for most mammalian IgGs, especially for certain subclasses including human IgG3, mouse IgG1 and rat IgG2a.

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

Item	Description
Matrix Spherical	4% agarose
Ligand	recombinant protein G
Static Binding Capacity	＞30mg Human IgG/ml medium
Particle size	45-165um
Maximum Pressure	0.1MPa, 1bar
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.

2.3 Column Purification

1) Mix the slurry by gently inverting the bottle several times to completely suspend the **rProtein G Beads.** Close the column outlet leaving the net covered with packing buffer. Transfer the slurry to the column.

2) Allow the resin to settle down and the buffer to drain from the column. Add 5 column volumes Binding Buffer onto the column to equilibrate the beads.

3) Apply the sample to the column and drain the flow-through. Collect the flow-through for measuring the binding efficiency to the beads, i.e. by SDS-PAGE.

4) Wash the column with 10-15 column volumes Wash Buffer or until the absorbance of the effluent at 280 nm is stable.

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 1XPBS containing 20% ethanol. Finally store the resin with 1XPBS containing 20% ethanol at 4℃.

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** 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 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