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AbPur rProtein G Kit

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1. Product Description

rProtein G Beads 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 cross-linked 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 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.

AbPur rProtein G Kit provides one pre-loaded 3ml rProtein G Beads gravity column, Binding/Wash Buffer and Elution Buffer.The Kit is simple in operation and high in purification efficiency.The kit components are shown in Table 3.

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

Table 3. The component of **AbPur rProtein G Kit**

Name	Cat.No	Size	Amount
AbPur rProtein G Column	SA016GC03	3ml	1
Ab Binding/Wash Buffer	SLB017100	100ml	2
Ab Elution Buffer	SLB018100	100ml	1

2. Purification Procedure

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 to filter the samples by passing them through a 0.22 or 0.45 μm filter before use. It helps to reduce impurities, improve the efficiency of protein purification and prevent clogging of the column.

2.3 Column Purification

AbPur rProtein G kit contains 3ml AbPur rProtein G Column, Binding/Wash Buffer and Elution Buffer. The purification process takes about 30min. It mainly depends on the sample volume and solution viscosity.

1). Fix AbPur rProtein G Column and remove the bottom plug and top plug. Allow storage buffer to drain from resin by gravity flow.

2). Equilibrate column with 5ml Binding Buffer. Allow buffer to drain from the column. Repeat this step two times.

3). Add the prepared sample to the resin. Collect the flow-through. If desired, re-apply the flow-through once to maximize binding.

4). Wash resin with 5ml Wash Buffer and collect the flow-through. Repeat this step five times or repeat this step using a new collection tube until the absorbance of the flow-through fraction at 280nm approaches baseline.

5). Elute proteins from the resin with 15-30ml Elution Buffer. Collect the fraction every 5ml in a separate tube.

6). Equilibrate the column with 5ml Binding Buffer and 5ml deionized water alternately. Repeat 2 times. Then equilibrate the column with 5ml 20% ethanol. Finally store the beads with 1XPBS containing 20% ethanol at 2-8℃.

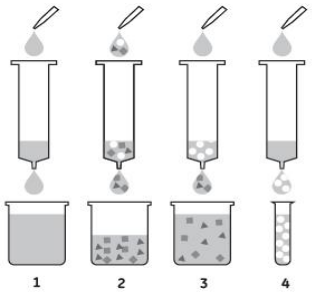

Fig.1. Purification process of AbPur rProtein A Column

Step1：Equilibrate；Step2：Binding；Step3：Wash；Step4：Elution

2.4 Analysis

Identify the fractions using UV absorbance, SDS-PAGE, or western blot.

3. Clean-In-Place

In general, **AbPur rProtein G Column** is 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).

3. 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