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AbPur rProtein A Kit

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1. Product Description

Smart-Lifesciences rProtein A Beads is an affinity chromatography medium designed for easy, one-step purification of classes, subclasses and fragments of immunoglobulins from biological fluids and from cell culture media. Table 1 lists the characteristics of rProtein A Beads. Protein A, a bacterial cell wall protein isolated from Staphylococcus aureus, binds to mammalian IgGs mainly through Fc regions. Protein A does not bind to dog IgG, nor does it bind to human IgM, IgD or IgA, see table 2. Recombinant protein A contains five high affinity IgG binding domains with other non-essential domains removed to reduce nonspecific binding. Since only the Fc region is involved in binding, the Fab region is available for binding antigens.

AbPur rProtein A Kit provides one pre-loaded 3ml rProtein A 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 A Beads**

Item	Description
Matrix Spherical	4% agarose
Ligand	recombinant protein A
Static Binding Capacity	＞40mg 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 A Kit**

Name	Cat.No	Size	Amount
AbPur rProtein A Column	SA012K-1	3ml	1
Binding/Wash Buffer	SA012K-2	100ml	2
Elution Buffer	SA012K-3	100ml	1

2. Purification Procedure

2.1 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 filtering 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.

2.2 Column Purification

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

Column Purification

1). Fix AbPur rProtein A 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℃.

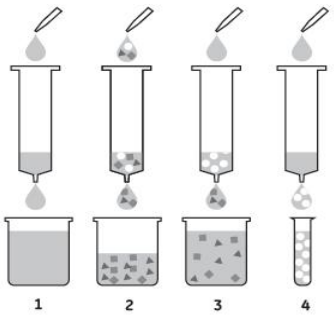

Fig.1. Purification process of AbPur rProtein A Column

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

2.3 Analysis

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

3. Clean-In-Place

In general, **AbPur rProtein A 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).

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
rProtein A Beads	SA012005	5 ml
	SA012025	25 ml
	SA012100	100 ml
	SA012500	500 ml
	SA01201L	1 L
	SA01210L	10 L
AbPur rProtein A Kit	SA012K03	3T
rProtein A Beads 4FF	SA015005	5 ml
	SA015025	25 ml
	SA015100	100 ml
	SA015500	500 ml
	SA01501L	1 L
	SA01510L	10 L
AbCap A 4FF	SA015C11	1 X 1 ml
	SA015C51	5 X 1 ml
	SA015C15	1 X 5 ml
	SA015C55	5 X 5 ml
	SA015CS	3X1 ml+1X5 ml