rProtein A Beads Gravity Column

Index

1. Product Description

Smart-Lifesciences **rProtein A Beads** 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 4% sepharose gel. This coupling method can improve the binding capacity for immunoglobulin. The static binding capacity of **rProtein A Beads** 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** 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.

rProtein A Beads Gravity Column are packed with 1ml and 5ml of **rProtein A Beads**. It is easy to operate.

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

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 against Binding/Wash Buffer.

2.3 Column Purification

For the use of **rProtein A Beads Gravity Column**, please refer to the following instructions. The amount of each solution is calculated according to the volume of the column (e.g., for 2 CV, the 1 ml size corresponds to 2 ml of solution, and the 5 ml size corresponds to 10 ml of solution). The entire purification process takes about 30 min (depending on the sample volume and the viscosity of the solution). Please refer to Figure 1 for the procedure.

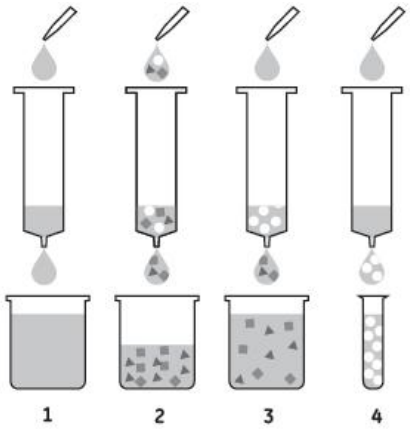

Figure 1. Schematic diagram of the protein purification process using rProtein A Beads gravity columns

Step 1: Column equilibration, Step 2: Sample loading, Step 3: Washing, Step 4: Elution

1) Fix **rProtein A Beads Gravity Column** on the iron stand, remove the lower and upper plugs in turn, and drain the buffer from the column. Add 5 column volumes Lysis Buffer onto the column to equilibrate the beads.

2) 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.
3) Wash the column with 10-15 column volumes.
4) Elute the sample with 5-10 column volumes Elution Buffer and collect the eluate.
5) Add 10µl Neutralization Buffer to each 100 µl of eluate to neutralize the pH.
6) Equilibrate the column with 3 column volumes of Lysis Buffer , 5 column volumes of distilled water. Finally store the resin with 1×PBS containing 20% ethanol at 2℃-8℃.

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 A 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 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 Gravity Column	SA012GC01	1 ml
	SA012GC05	5 ml