



# rProtein A Beads 4FF

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## 1. Product Description

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

Table 1. Characteristics of **rProtein A Beads 4FF**

Item	Description
Matrix Spherical	Highly cross-linked 4% agarose beads
Ligand	recombinant protein A
Static Binding Capacity	>40mg Rabbit IgG/ml medium
Particle size	45-165um
Maximum Pressure	0.3MPa, 3bar
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	variable	—
	IgD	—	—
	IgE	—	—
	IgG1	++++	++++
	IgG2	++++	++++
	IgG3	—	++++
	IgG4	++++	++++
	IgGM	variable	—
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 to filter the buffers by passing them through a 0.22 or 0.45  $\mu\text{m}$  filter before use.

**Binding/Wash Buffer:** 0.15 M NaCl, 20 mM  $\text{Na}_2\text{HPO}_4$ , 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 Packing of Column

**rProtein A Beads 4FF** is easy to pack and use, and its high flow properties make it excellent for industrial scaling-up. Here we describe the packing procedure of **rProtein A Beads 4FF** to medium pressure chromatography columns.

- 1) Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air has been trapped under the column net.
- 2) Close the column outlet leaving the net covered with packing buffer.
- 3) Resuspend the beads stored in its container by shaking (avoid stirring the sedimented medium). Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.  
If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.
- 4) Open the bottom outlet of the column and set the pump to run at the desired flow velocity. Ideally, **rProtein A Beads 4FF** is packed at a constant pressure of approximately 0.3bar (0.3MPa). If the packing equipment does not include a pressure gauge, use a packing flow velocity of approximately 400 cm/h (10 cm bed height, 25°C, low viscosity buffer). If the recommended pressure or flow velocity can not be obtained, use the maximum flow velocity the pump can deliver. This should also give a reasonable well-packed bed. Do not exceed 75% of the packing flow velocity in subsequent chromatographic procedures.
- 5) When the bed has stabilized, close the bottom outlet and stop the pump.  
If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. If using the column, carefully place the top filter on top of the bed before fitting the adapter.
- 6) With the adapter inlet disconnected, push the adapter down, approximately 2 mm into the bed, allowing the packing solution to flush the adapter inlet.
- 7) Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will be formed between the bed surface and the adapter.
- 8) Close the bottom outlet. Disconnect the column inlet and lower the adapter approximately 2 mm into the bed. Connect the pump. The column is now ready to use.





## 2.4 Sample Purification

- 1) Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, “drop to drop” to avoid introducing air into the column. Remove the snap-off end at the column outlet.
- 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. 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 4FF** 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 capacity 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

