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rProtein A/G Beads 4FF

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1. Product Description

rProtein A/G Beads 4FF is an affinity chromatography medium designed for the purification of monoclonal antibody and polyclonal antibody at both laboratory and process acale. The rProtein A/G is coupled to highly cross-linked 4% agarose beads by a technique which generates a stable thioether linkage between rProtein A/G and the agarose beads. The coupling technique is optimized to give a high binding capacity for IgG.This binding capacity,together with the excellent kinetic and flow properties of the highly cross-linked beads.**rProtein A/G Beads 4FF** can be used for antibody purification, immuneprecipitation and Co- immuneprecipitation.

The characteristics of **rProtein A/G Beads 4FF** are summarized in Table 1.

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

Item	Description
Matrix Spherical	Highly cross-linked 4% agarose beads
Ligand	recombinant protein A/G
Static Binding Capacity	10-15 mg Rabbit IgG/ml medium
Particle size	45-165 μm
Maximum Pressure	0.3 MPa, 3 bar
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 protein A and protein A/G as measured in a competitive ELISA test.

Species	Subclass	Protein A	Protein G	Protein A/G
Human	IgA	varible	—	++
	IgD	—	—	—
	IgE	—	—	—
	IgG1	++++	++++	++++
	IgG2	++++	++++	++++
	IgG3	—	++++	++++
	IgG4	++++	++++	++++
	IgM	varible	—	++
Avian egg yolk	IgY	—	—	—
Cow		++	++++	++++
Dog		++++	++	++++
Goat		—	++++	++++
Guinea pig	IgG1	++++	++	++++
	IgG2	++++	++	++++
Hamster		+	++	
Horse	Total IgG	++	++++	++++
Koala		—	+	
Llama		—	+	
Monkey(rhesus)		++++	++++	++++

Mouse	IgG1	+	++++	++
	IgG2a	++++	++++	++++
	IgG2b	+++	+++	+++
	IgG3	++	+++	+++
	IgM	variable	—	—
Pig		+++	+++	++++
Rabbit	Total IgG	++++	+++	++++
Rat	IgG1	—	+	++
	IgG2a	—	++++	++++
	IgG2b	—	++	++
	IgG3	+	++	++
Sheep	Total IgG	+/-	++	++

++++=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 μm filter before use.

Binding/Wash Buffer: 0.15 M NaCl, 20 mM Na_2HPO_4, pH 7.0

Acid Elution Buffer: 0.1 M glycine, pH 3.0

Neutralization Buffer: 1 M Tris-HCl, pH 8.5

Coupling Buffer: 0.2 Mtriethanolamine, pH 8.2

Cross-linking agent: DMP （dimetyl pimelimidate dihydrochloride）

Stop Buffer: 50 mM Tris, pH 7.5

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. For adherent cells in a 100 mm culture dish, the cell culture medium was removed and washed once with pre-cooled PBS, then 2ml cell lysis buffer was added to lyse the cells. For suspension cells, the cells were collected by centrifugation, and then washed with PBS once for lysis refer to adherent cells. Plant or animal tissue samples can be decomposed by liquid nitrogen grinding. Please refer to the instructions of different lysates for the specific lysis method. The final total protein concentration of the lysate is appropriate in the range of 0.5-1 ug/ul. In general, the expression of target protein is different, so the total protein concentration needs to be adjusted by pre-experiment.

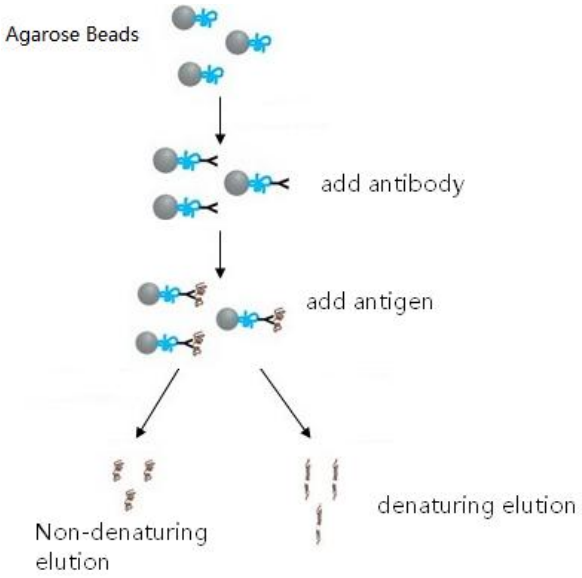
2.3 Remove Nonspecific Binding (Optional)

1) Take 200 μl to 1 ml of protein samples. Add 1 mg Normal IgG with the same species of IgG used in immunoprecipitation and 20 μl of fully re-suspended **rProtein A/G Beads 4FF**. The samples were shaken slowly for 30 minutes at 4℃.

2) Centrifuge at 2500 rpm (about 1000 g) for 1 minute, and take the supernatant for immunoprecipitation.

Note: There are several components in mammalian cells that bind to IgG, and nonspecific bands may appear in subsequent western blots. Pretreatment of lysate using Normal IgG and **rProtein A/G Beads 4FF** may reduce nonspecific adsorption.

2.4 Immuneprecipitation



2.4.1 Antibody adsorption

1) Completely resuspend **rProtein A/G Beads 4FF** and add 100 µl to 1.5-2 ml micro centrifuge tube. Centrifuge for 1 minute at 800 rpm and discard supernatant.

2) Add 0.5 ml of Binding Buffer, centrifuge for 1 minute at 800 rpm and discard supernatant. Repeat this step two times.

3) Add the sample containing target IgG to the tube and gently invert the tube to mix. Incubate the tube at room temperature with mixing (on a shaker or rotator) for 30minutes.

4) Centrifuge for 1 minute at 800 rpm and discard supernatant. Repeat this step two times. If necessary, keep the supernatant for analysis.

5) Add 500 µl Binding/Wash Buffer to the tube and mix well, Centrifuge for 1 minute at 800rpm and discard supernatant. Repeat this step two times.

2.4.2 Cross - linking IgG to the Beads (Optional)

If you need to elute antibodies and target antigen together, please ignore this step.

1) Add 1 ml 0.2 M triethanolamine, pH 8.2 to the **rProtein A/G Beads 4FF** with immobilised IgG. Centrifuge for 1 minute at 800rpm and discard supernatant.

2) Resuspend the beads in 1 ml of 20 mM dimetyl pimelimidate dihydrochloride (DMP) in 0.2 M triethanolamine, pH 8.2 (5.4 mg DMP/ml buffer). This cross - linking solution must be prepared freshly.

3) Incubate the tube at room temperature with mixing (on a shaker or rotator) for 30minutes. Centrifuge for 1 minute at 800 rpm and discard supernatant.

4) Resuspend the beads in 1 ml of 50 mM Tris, pH 7.5 to stop the reaction and incubate for 15 minutes at room temperature with rotational mixing.

5) Centrifuge for 1 minute at 800 rpm and discard supernatant.

6) Wash the cross - linked beads (Beads-Ab complex) three times with 500ul Wash Buffer.

2.4.3 Binding Antigen to the Beads-Ab complex

1) Add sample containing the antigen (Ag) (typically 100–1000 µl) and gently mix to resuspend the Beads-Ab complex.

2) Incubate with rotation for 30min at room temperature to allow Ag to bind to the Beads-Ab complex.

3) Centrifuge for 1 minute at 800 rpm and discard supernatant.Wash the beads three times with 1 ml PBS, pH7.4.

Note: Depending on the affinity of the antibody, it may be necessary to increase incubation times for optimal binding.

2.4.4 Elution of Target Protein

A. Denaturing elution

1) Add 50 µl 1XSDS-PAGE Sample Buffer to the tube and mix well.

2) Heat the tube at 100℃ for five minutes.

3) Centrifuge for 1 minute at 800 rpm and transfer the supernatant containing desired sample into a new tube.

4) Analyze the sample by SDS - PAGE followed by Western blot analysis.

B. Non-denaturing elution

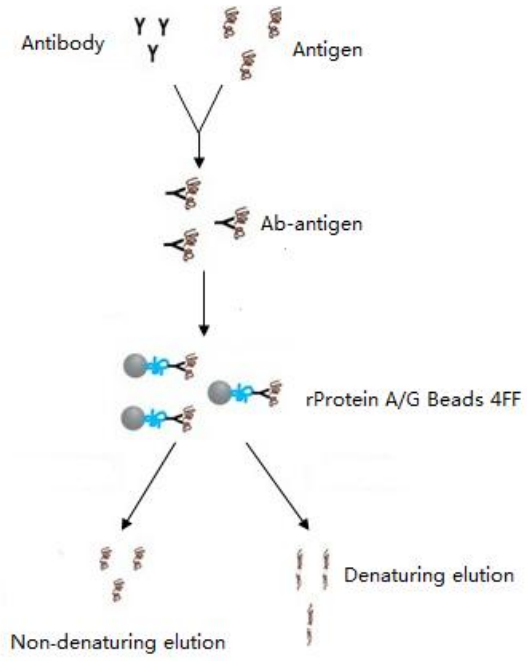
1) Add 150 µl Elution Buffer to the tube and mix well. Incubate for five minutes at room temperature with occasional mixing.

2) Centrifuge for 1 minute at 800 rpm and transfer the supernatant into a new tube.

3) Repeat Step 1 and 2 twice.

4) Add 10µl Neutralization Buffer to each 100 µl of eluate to neutralize the pH.

2.5 Co- Immunoprecipitation



1) Mix antibody with the target protein, incubation for 30-60 min at room temperature or at 2-8 degree overnight .

Note: Depending on the affinity of the antibody, it may be necessary to increase incubation times for optimal binding.

2) Completely resuspend rProtein A/G Beads 4FF and add 100µl to 1.5-2 ml microcentrifuge tube. Centrifuge for 1 minute at 800 rpm and discard supernatant.

Add 0.5ml of Binding Buffer, centrifuge for 1 minute at 800 rpm and discard supernatant. Repeat this step two times.

3) Mix the antibody-antigen with the beads. Incubate at room temperature with mixing (on a shaker or rotator) for 30minutes. Centrifuge for 1 minute at 800 rpm and collect supernatant for detection.

4) Add 500 µl Binding/Wash Buffer to the tube and mix well, Centrifuge for 1 minute at 800 rpm and discard supernatant. Repeat this step two times.

5) Elute the sample as 2.4.4.

3. Related Products

Product	Cat. No.	Size
rProtein A/G Beads 4FF	SA032005	5 ml
	SA032025	25 ml
	SA032100	100 ml
	SA032500	500 ml
	SA03201L	1 L
	SA03210L	10 L
AbCap A/G 4FF	SA032C11	1 X 1 ml
	SA032C51	5 X 1 ml
	SA032C15	1 X 5 ml
	SA032C55	5 X 5 ml
	SA032CS	3X1 ml+1X5 ml