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rProtein A MagPoly Beads

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1. Product Description

rProtein A MagPoly Beads is an affinity chromatography Magnetic beads designed for easy, one-step binding of classes, subclasses and fragments of immunoglobulins from biological fluids and from cell culture media. The recombinant protein A ligand is coupled to Magnetic beads.

Table 1. Characteristics of **rProtein A MagPoly Beads**

Item	Description
Matrix spherical	Polymer magnetic beads
Ligand	Recombinant protein A
Binding capacity	> 50 μg hIgG/mg magnetic beads
Particle size (μm)	~ 1
Beads concentration	10 mg/ml
Storage solution	20 mM Tris PH 7.4, 0.01%Tw20（v/v），0.05%kv300（v/v）
Storage	2℃ - 8℃

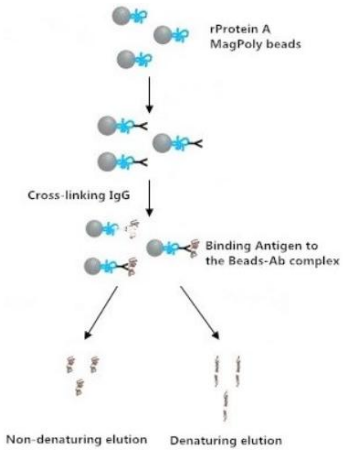
Table 2. Relative binding strengths of antibodies from various species to protein A, protein G and protein A/G as measured in a competitive ELISA test.

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

This protocol offers a general guideline for immunoprecipitation. Optimization may be required for each antibody and target antigen. The protocol uses 1 mg of **rProtein A MagPoly Beads**, but this may be scaled up or down as required.



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: 20 mM Na_2HPO_4, 0.15 M NaCl, pH 7.0

Elution Buffer: 0.1 M glycine, pH 2 - 3

Neutralization Buffer: 1 M Tris, 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 Preparation of the Magnetic Beads

1) Completely resuspend the beads by shaking or vortexing the vial.

2) Transfer 100 μl **rProtein A MagPoly Beads**(10 mg/ml) into a clean tube.

3) Place the tube on a magnetic separation rack to collect the beads. Remove and discard the supernatant.

4) Add 0.5 ml Binding/Wash Buffer to the tube and invert the tube several times to mix. Use the magnetic separation rack to collect the beads and discard the supernatant. Repeat this step twice.

2.3 Antibody adsorption

1) Resuspend the beads in 100 μl Binding/Wash Buffer.

2) Add the sample containing target IgG to the tube and gently invert the tube to mix.

3) Incubate the tube at room temperature with mixing (on a shaker or rotator) for 30 minutes.

4) Use the magnetic separation rack to collect the beads and discard the supernatant. If necessary, keep the supernatant for analysis.

5) Add 500 μl Binding/Wash Buffer to the tube and mix well, use the magnetic separation rack to collect the beads and discard the supernatant. Repeat the wash step three more times.

2.4 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 MagPoly Beads** with immobilised IgG. Wash twice using the magnetic separation rack with 0.2 M triethanolamine, pH 8.2 as the washing buffer.

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 beads with rotational mixing for 30 minutes at room temperature. Use the magnetic separation rack to collect the beads and discard the 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) Use the magnetic separation rack to collect the beads and discard the supernatant.
6) Wash the cross - linked beads (Beads-Ab complex) three times with 1 ml PBS, pH7.4.

2.5 Binding Antigen to the Beads-Ab complex

1) Place the tube (from step 6 in "2.4 Cross - linking IgG to the Beads ") on the magnetic separation rack and remove the supernatant.
2) Add your sample containing the antigen (Ag) (typically 100–1000 µL) and gently pipette to resuspend the Beads-Ab complex.
3) Incubate with rotation for 10min at room temperature to allow Ag to bind to the Beads-Ab complex.
Note: Depending on the affinity of the antibody, it may be necessary to increase incubation times for optimal binding.

2.6 Elution of Target Protein

A. Denaturing elution

1) Place the tube from section 2.5 on the magnetic separation rack to collect the beads and discard the supernatant.
2) Add 50-100 µl 1XSDS Sample Buffer to the tube and mix well.
3) Heat the tube at 100°C for five minutes.
4) Use the magnetic separation rack to collect the beads and transfer the supernatant containing desired sample into a new tube.
5. Analyze the sample by SDS - PAGE followed by Western blot analysis.

B. Non-denaturing elution

1) Place the tube from section 2.5 on the magnetic separation rack to collect the beads and discard the supernatant.
2) Add 150 µl Elution Buffer to the tube and mix well. Incubate for five minutes at room temperature with occasional mixing.
3) Use the magnetic separation rack to collect the beads and transfer the supernatant into a new tube.
4) Repeat Step 2 and 3 twice.
5) Add 5 µl Neutralization Buffer to each 50 µl of eluate to neutralize the pH.

3. Related Products

Product Name	Cat. No.	Size
rProtein A MagPoly Beads	SM037001	1 ml
	SM037005	5 ml
	SM037010	10 ml
	SM037050	50 ml
	SM037100	100 ml
	SM037500	500 ml
	SM03701L	1 L