Product Specification

Product Name: rProtein A MagPoly Beads

Product Code: MAGP034 / MAGP035 / MAGP036 / MAGP037 / MAGP038

PRODUCT DESCRIPTION:

rProtein A MagPoly Beads are magnetic affinity chromatography beads designed for the streamlined, one-step isolation of various immunoglobulin classes, subclasses, and fragments. These beads feature recombinant Protein A ligands covalently coupled to the magnetic beads, enabling efficient capture from cell culture media and other biological fluids.

Characteristics of rProtein A MagPoly Beads:

Item	Description
Matrix Spherical:	Polymer Magnetic Beads
Ligand:	Recombinant Protein A
Binding Capacity:	> 50μg 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 Temperature:	2℃ - 8℃

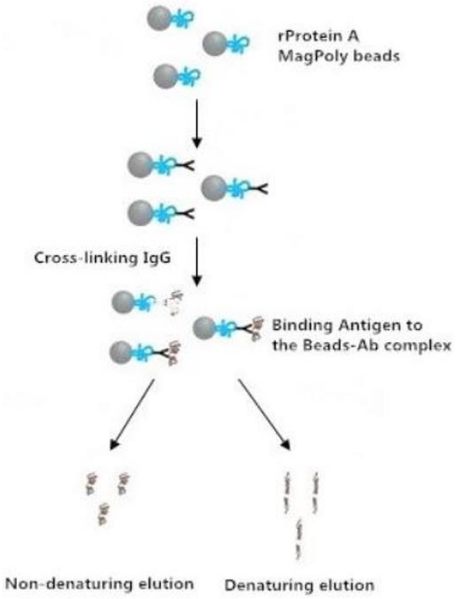
Relative Binding Strengths of Antibodies from Various Species to Protein A, Protein G And Protein A/G as Measured in a Competitive ELISA Test.

Species	Subclass	Protein A	Protein G	Protein A/G
Human	IgA	Variable	—	++
	IgD	—	—	—
	IgE	—	—	—
	IgG1	++++	++++	++++
	IgG2	++++	++++	++++
	IgG3	—	++++	++++
	IgG4	++++	++++	++++
	IgM	Variable	—	++
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

PURIFICATION PROCEDURE

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



1. Buffer Preparation

Water and chemicals used for the buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.22 or 0.45µm filter before use.

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

Elution Buffer: 0.1M glycine, pH 2-3

Neutralization Buffer: 1M Tris, pH 8.5

Coupling Buffer: 0.2M Triethanolamine, pH 8.2

Cross-linking agent: DMP (dimethyl pimelimidate dihydrochloride)

Stop Buffer: 50 mM Tris, pH 7.5

2. Preparation of the Magnetic Beads

1) Resuspend the beads by shaking or vortexing the vial.

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

3) Place the tube on a Magnetic Rack to sequester the beads. Remove and discard the supernatant.

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

3. Antibody Adsorption

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

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

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

4) Use the Magnetic Rack to collect the beads at the tube wall and discard the supernatant. If necessary, retain the supernatant for analysis.

5) Resuspend the beads in 500µl of Binding/Wash Buffer, then use the Magnetic Rack to sequester the beads and discard the supernatant. Repeat this washing step three more times.

4. Cross-linking IgG to the Beads (Optional)

If the antibodies and target antigen needs to be eluted together, please ignore this step.

1) Resuspend the **rProtein A MagPoly Beads** containing the immobilized IgG in 1 ml of 0.2M triethanolamine, pH 8.2, then use the Magnetic Rack to collect the beads and discard the supernatant. Repeat this washing step once more.

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

3) Incubate the beads at room temperature for 30 minutes in a rotating mixer. Then, use the Magnetic 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 continuous mixing in a rotating mixer.

5) Use the Magnetic Rack to collect the beads at the tube wall and discard the supernatant.

6) Resuspend the cross-linked beads (Beads-Ab complex) in 1 ml of PBS, pH7.4, then use the Magnetic Rack to collect the beads and discard the supernatant. Repeat this washing step two more times.

5. Binding Antigen to the Beads-Ab complex

1) Place the tube (from step 6 in "4. Cross-linking IgG to the Beads") on the Magnetic Rack to sequester the beads and discard the supernatant.

2) Add 100–1000µL of your antigen (Ag) sample to the Beads-Ab complex, then gently pipette to resuspend.

3) Incubate with rotational mixing for 10 minutes at room temperature to allow the Ag to bind to the Beads-Ab complex.

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

6. Elution of Target Protein

6.1 Denaturing Elution

1) Place the tube from Section 5 on the Magnetic Rack to collect the beads and discard the supernatant.

2) Resuspend the beads in 50-100µl of 1X SDS Sample Buffer and mix well.

3) Heat the tube at 100°C for 5 minutes.

4) Use the Magnetic Rack to sequester the beads then transfer the supernatant (sample eluate) into a fresh microcentrifuge tube.

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

6.2 Non-Denaturing Elution

1) Place the tube from Section 5 on the Magnetic Rack to collect the beads and discard the supernatant.

2) Resuspend the beads in 150µl of Elution Buffer and mix well. Then, incubate the tube for five minutes at room temperature with occasional mixing.

3) Use the Magnetic Rack to collect the beads then transfer the supernatant (sample eluate) into a fresh microcentrifuge tube.

4) Repeat Steps 2 and 3 twice.

5) Add 5µl of Neutralization Buffer to each 50µl of eluate to neutralize the pH.

RELATED PRODUCTS

Product Name	Product Code	Size
rProtein A MagPoly Beads	MAGP034	1 ml
	MAGP035	5 ml
	MAGP036	10 ml
	MAGP037	50 ml
	MAGP038	100 ml

2 International Business Park Road Strategy #01-04, Singapore(609930)
Tel: (+65) 6954 2519, (+65) 6491 5938
Email: sg@biobasic-asia.com Web: www.biobasic-asia.com