rProtein G Magarose Beads

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1. Product Description

Magarose Beads have superparamagnetism and rapid magnetic responsiveness. Peptides, proteins, antibodies, oligomeric nucleotide biological ligands can be coupled to the surface of microspheres. **Magarose Beads** is an important carrier of the medical and molecular biology research tools.The particle size of **Magarose Beads** is about 30 to 100 μm and it is more suitable for biological examination and purification experiment.

rProtein G Magarose 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 G ligand is coupled to Magnetic beads. Protein G, a bacterial cell wall protein isolated from group G Streptococci, binds to mammalian IgGs mainly through Fc regions. Native protein G has 3 IgG binding domains and also sites for albumin and cell-surface binding. The recombinant protein G have been eliminated the nonspecific binding site. Although protein G has very similar tertiary structures to protein A, their amino acid compositions differ significantly, resulting in different binding characteristics. Protein G can be used for purification of mammalian monoclonal and polyclonal IgGs that do not bind well to protein A. Protein G has greater affinity than protein A for most mammalian IgGs, especially for certain subclasses including human IgG3, mouse IgG1 and rat IgG2a. Unlike protein A, protein G does not bind to human IgM, IgD and IgA.

Table 1. Characteristics of **rProtein G Magarose Beads**

Item	Description
Matrix spherical	Magnetic agarose
Ligand	Recombinant protein G
Binding capacity	> 30 mg Goat IgG/ml medium
Particle size	30-100 μm
Beads concentration	20% (V/V) slurry
Storage solution	1X PBS containing 20% ethanol
Storage	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

This protocol offers a general guideline for immunoprecipitation. Optimization may be required for each antibody and target antigen. The protocol uses 100 µl of **rProtein G Magarose Beads**, but this may be scaled up or down as required.

2.1 Buffers 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: PBS, pH 7.4 (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4)

Acid Elution Buffer: 0.1 M glycine, pH 3.0

Neutralization Buffer: 1 M Tris-HCl, pH 8.5

2.2 Preparation of the Magnetic Beads

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

2) Transfer 500 µl **rProtein G Magarose Beads** (20% v/v) into a clean tube.

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

4) Add 1 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 Protein Purification

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

2) Add the sample 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. It also can be done at 4℃ over night.

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

5) Add 1ml 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 Elution of Target Protein

Add 300-500µl Elution Buffer, mix well and incubation for 5-10 min (on a shaker or rotator). Use the magnetic separation rack to collect the beads and transfer the supernatant into a new tube. Repeat this step one more time.

Add 50 µl Neutralization Buffer to each 500 µl of eluate to neutralize the pH.

3. Related Products

Product	Cat. No.	Size
rProtein A Magarose Beads	SM003001	1 ml
	SM003005	5 ml
	SM003025	25 ml
	SM003100	100 ml
	SM00301L	1 L
rProtein G Magarose Beads	SM004001	1 ml
	SM004005	5 ml
	SM004025	25 ml
	SM004100	100 ml
	SM00401L	1 L