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User's Guide
Protein G Magnetic Polymer Resin

I. Description

Protein G magnetic resins contain a recombinant protein G covalently coupled on non-porous magnetic polymer resins. Recombinant protein G only contains the two Fc-binding domains of protein G, that can bind to antibodies from various mammalian species, including human, mouse, rabbit, pig, dog, goat and sheep. Protein G magnetic resins can be used for isolating antibodies from serum, cell culture supernatants or ascites, and for immunoprecipitation and co-immunoprecipitation of antigens from cell lysates or tissue extracts.

Comparing to agarose-based resins, non-porous magnetic polymer resins have much less non-specific binding, and suitable for rapid isolation and elution using a magnetic stand.

II. Protein G Magnetic Polymer Resin Specifications:

Resin: Non-porous superparamagnetic polymer
Ligand: Recombinant Protein G containing only the Fc binding domains
Binding Capacity: ~ 50 µg human IgG per mg resin or 100 µL supplied resin (total rein volumes including buffer)
Resin Concentration: 10 mg/ml
Storage Buffer: 1X PBS, 0.01% Tween-20, 0.02% Sodium azide (NaN_3)
Storage Temperature: 4 ^{0}C

III. Important Notes:

1. Do not centrifuge, dry or freeze the magnetic resins, otherwise would cause resin aggregation.
2. Include 0.01 - 0.05% non-ionic detergent (e.g., Tween-20) in the binding buffer to prevent resin aggregation.
3. Low pH elution may be used for single-use applications. Optimal time for low pH elution is 10 minutes. Resins can be cleaned and reused after low pH elution.
4. Elution can also be performed in 1X SDS reducing sample buffer. Not recommended to reuse resins subjected to SDS sample buffer elution.
5. Protein A and Protein G have different binding affinity to various antibody subtypes (see Table 1 below).

Table 1. Relative Binding Strength of Protein A and Protein G

Species	Subclass	Protein A binding	Protein G binding
Human	IgA	variable	—
	IgD	—	—
	IgE	—	—
	IgG1	++++	++++
	IgG2	++++	++++
	IgG3	—	++++
	IgG4	++++	++++
	IgM	variable	—
Avian egg yolk	IgY	—	—
Cow		++	++++
Dog		++	+
Goat		—	++
Guinea pig	IgG1	++++	++

	IgG2	++++	++
Hamster		+	++
Horse		++	++++
Koala		—	+
Larma		—	+
Monkey		++++	++++
Mouse	IgG1	+	++++
	IgG2a	++++	++++
	IgG2b	+++	+++
	IgG3	++	+++
	IgM	variable	—
Pig		+++	+++
Rabbit		++++	+++
Rat	IgG1	—	+
	IgG2a	—	++++
	IgG2b	—	++
	IgG3	+	++
Sheep		—	++

++++ = strong binding; ++ = medium binding; — = weak or no binding

IV. Antibody Purification and Immunoprecipitation Protocols

Important Note: Conditions should be optimized by users for each application.

A. Additional Materials Required

- 1.5mL microcentrifuge tubes
- Binding/Wash Buffer: Tris-buffered saline containing 0.02% Tween-20, or 1X PBS containing 0.02% Tween-20
- Elution Buffer: 0.1M glycine, pH 2.0
- Neutralization Buffer: 1M Tris, pH 8.5
- Magnetic stand

B. Antibody Purification Protocol

Note: Mix resins thoroughly before each use by repeated inversion or gentle vortexing. Protease inhibitors may be added in the sample to prevent degradation of antigens and antibodies.

1. Pipette 50 µL (0.5 mg) of Protein G Magnetic Polymer Resins into a 1.5mL microcentrifuge tube. Add 500 µL of Binding/Wash Buffer to the resins and gently vortex to mix.
2. Place the tube into a magnetic stand to collect resins against the wall of the microcentrifuge tube. Remove and discard the supernatant.
3. Add 1 mL Binding/Wash Buffer to the tube. Invert the tube several times to mix. Collect resins with a magnetic stand, then remove and discard the supernatant.
4. Add 500 µL sample to pre-washed magnetic resins and invert to mix well.

Note: Sample concentration should be optimized. If the sample volume is < 500 µL, dilute to 500 µL with Binding/Wash Buffer.

5. Rotate the tube to mix the sample and resins under room temperature for 1 hour, or under 4 ^{0}C for 4 hours or overnight.
6. Collect resins with a magnetic stand, then remove and discard the supernatant.

Note: you may keep the supernatant at Step 6 for characterization such as by SDS-PAGE.

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7. Add 500 µL of Binding/Wash Buffer to the tube, rotate the tube for 5 min, collect resins with a magnetic stand, and then remove and discard the supernatant. Repeat the wash step twice.
8. Add 100 µL of Elution Buffer to the tube, mix well and incubate 10 minutes at room temperature with occasional mixing.
9. Collect resins with a magnetic stand, remove and save the supernatant that contains the eluted antibody.
10. Neutralize eluted antibody immediately by adding 15 µL Neutralization Buffer for each 100 µL of eluate.

C. Immunoprecipitation Protocol

Note: Mix resins thoroughly before each use by repeated inversion or gentle vortexing. Protease inhibitors may be added in the sample to prevent degradation of antigens and antibodies.

1. Add 2 - 5 µg antibody into cell lysates or tissue extracts. Adjust the reaction volume to 500 µL with the Binding/Wash Buffer. Incubate at 4 ^{0}C for 4 hours or overnight.
2. Pipette 30 µL (0.4 mg) of Protein G Magnetic Polymer Resins into a 1.5mL microcentrifuge tube. Add 500 µL Binding/Wash Buffer to resins and gently vortex to mix.
4. Place the tube into a magnetic stand to collect resins against the wall of the microcentrifuge tube. Remove and discard the supernatant.
5. Add 1 mL Binding/Wash Buffer to the tube. Invert the tube several times to mix. Collect beads with a magnetic stand. Remove and discard the supernatant.
6. Add the antigen sample/antibody mixtures prepared in Step 1 to the pre-washed magnetic resins. Incubate at room temperature for 1 hour with mixing, or at 4 ^{0}C for 4 hours or overnight.
7. Collect resins with a magnetic stand, remove the supernatant and save for characterizations.
8. Add 500 µL of Binding/Wash Buffer to the tube and gently mix for 5 min. Collect resins using a magnetic stand, remove and discard the supernatants. Repeat the wash step twice.

Note: You may add 0.3 - 0.5M NaCl in the Binding/Wash Buffer in the wash steps to reduce non-specific binding. You may also save the supernatants in the wash steps for analysis.

9. Add 100µL of Elution Buffer to the tube. Mix the tube at room temperature for 10 minutes. Collect resins using a magnetic stand. Collect and save the supernatant containing the target antigen. Add 15µL Neutralization Buffer immediately for each 100 µL of eluate to neutralize the eluate.
10. Alternatively, add 100 µL 1X SDS reducing sample buffer to the tube and boil samples for 5 minutes. Collect resins using a magnetic stand and save the supernatant containing the target antigen.

Note: If you perform Western blot using rabbit antibodies (primary or secondary), incubate at room temperature for 10 minutes with mixing to elute, do not heat the sample.

D. Troubleshooting

Problem	Cause	Solution
Low levels of antigen/antibody were recovered	Low antigen/antibody levels in the sample	Increase sample volume or concentrate the sample
	Antibody and antigen were degraded	Add protease inhibitors in the sample
	Not enough magnetic resins	Use more resins in the reaction
	Antibody did not elute	Increase elution incubation time, and/or use distilled water to rinse the resins once before you add low pH elution buffer to elute. For immunoprecipitation, you can use 1X SDS reducing sample buffer to elute.

Non-specific proteins appear in eluate	Non-specific protein bounds on resins	Add NaCl to 0.3 - 0.5M and/or increase Tween-20 concentration to 0.05% in washing buffer
Resin aggregation	Centrifuging, drying or freezing resins	Avoid centrifuging, drying or freezing resins
	No non-ionic detergents in samples and in binding/wash buffer	add 0.02% - 0.05% Tween-20 in sample buffer, and binding/wash buffer
Bands at ~50 kDa appeared on the Western blot	Elution was performed under boiling when a rabbit primary or secondary antibody was used for Westernblotting	Perform elution under room temperature