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Protein A/G Magnetic Beads

1 Contents

Cat. No.	Product Name	Package
HY-K0202-1 mL	Protein A/G Magnetic Beads	1 mL
HY-K0202-5 mL	Protein A/G Magnetic Beads	1 mL × 5

2 General Information

Protein A/G Magnetic Beads provide a fast and convenient method for magnetic isolation of proteins using affinity binding. MCE Protein A/G Magnetic Beads are typically used for isolating antibodies from serum, cell culture supernatant or ascites and for Immunoprecipitation and Co-Immunoprecipitation of antigens from cell or tissue extracts.

The MCE Protein A/G Magnetic Beads contain a recombinant Protein A/G that combines the IgG binding domains of both Protein A and Protein G. Protein A/G contains four Fc-binding domains from Protein A and two from Protein G, making it a more general and convenient tool for investigating and purifying immunoglobulins.

3 Characteristics

Composition	Recombinant Protein A/G monolayer covalently coupled to a blocked magnetic bead surface
Magnetization	Superparamagnetic
Mean Diameter	200 nm
Bead Concentration	10 mg/mL
Binding Capacity	0.7 mg/mL

4 General Protocol

Recommended Buffer

Binding/Wash Buffer	PBST : 1× PBS + 0.5% Tween-20, pH 7.4
Elution Buffer	0.15 M Glycine, pH 2.5-3.1

1. Preparation of Magnetic Beads

1.1 Resuspend the Magnetic Beads in the vial (tilt and rotate for 2 minutes or gently pipette for 10 times).

1.2 Transfer 25-50 µL of Protein A/G Magnetic Beads into a 1.5 mL tube (Transfer amount may be adjusted as required).

1.3 Add 400 µL of binding/wash buffer to the beads and gently pipette to mix. Place the tube into a magnetic stand to collect the beads against the side of the tube (Hereinafter referred to as magnetic separation). Remove and discard the supernatant. Repeat this step for 2 times.

2. Binding of Antibody

2.1 Dilute antibody (Ab) to the final concentration of 5-50 µg/mL with binding/wash buffer. The optimal amount of Ab may be adjusted as required.

2.2 Add 400 µL of diluted Ab to the Protein A/G Magnetic Beads. Rotate tube for 30 minutes at room temperature or 2 hours at 4°C.

2.3 Perform magnetic separation. Transfer the supernatant into a new tube for further analysis, if desired. The supernatant is the non-binding fraction.

2.4 Add 400 µL of binding/wash buffer to the beads and gently pipette to mix. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant. Repeat this step for 4 times.

Note: During the binding process, it won't affect the result if magnetic beads occasionally aggregated together.

3. Immunoprecipitation of Target Antigen

3.1 Remove the tubes from the magnetic separator and add your sample containing the antigen (Ag) (typically 5-50 µg in 400 µL lysis buffer) and gently pipette to resuspend the Protein A/G Magnetic Beads-Ab complex.

3.2 Incubate with rotation for 30 minutes at room temperature or 2 hours at 4°C to allow Ag to bind to the Protein A/G Magnetic Beads-Ab complex.

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

3.3 Perform magnetic separation. Remove and discard the supernatant.

3.4 Wash the Magbeads-Ab-Ag complex 5 times using 400 µL binding/wash buffer for each wash. Perform magnetic separation between each wash, remove supernatant and resuspend by gentle pipetting.

3.5 Resuspend the Protein A/G Magnetic Beads-Ab-Ag complex in 400 µL binding/wash buffer and transfer the bead suspension into a clean tube. This is recommended to avoid co-elution of the proteins bound to the tube wall.

4. Elution

This is a non-denaturation elution method.

4.1 Perform magnetic separation and remove the supernatant. Add 400 µL of binding/wash buffer into the tube and rotate for 5 minutes. Perform magnetic separation for 1 minute and remove the supernatant. Then add

25-50 μL elution buffer into the tube with magnetic beads-Ab-Ag complex, rotate for 5 minutes.

4.2 Perform magnetic separation, collect the supernatant.

4.3 The final solution can be used as samples for denaturing SDS-PAGE. Or the elution can be adjusted to neutral pH with neutralization buffer immediately and used for further analysis.

5 Storage

Stored at 4°C, and is stable for up to 2 years.

6 Precautions

1. The pH of Protein A/G Magnetic Beads is 6-8.
2. Do not centrifuge, dry or freeze the magnetic beads.
3. This product is for R&D use only, not for drug, house hold, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

7 Troubleshooting

Problem	Possible Cause	Solution
Low binding efficiency of antibody and magnetic beads	The binding efficiency of magnetic beads and antibody depends on the origin and subtype of the antibody	Check the affinity of antibody and the protein A/G matrix in the appendix
	The antibody subtype and protein A/G matrix shows low affinity	Elongate the incubation time of antibody and magnetic beads
		Increase the pH vaule of binding buffer (8-9) and reduce the ion strength (25-100 mM NaCl)
Magnetic beads aggregated	Magnetic beads were frozen or centrifuged	Handle the beads as directed in the instructions
	Buffer was incompatible with magnetic beads	
Multiple nonspecific bands	Nonspecific proteins bound to the magnetic beads	Add 50-350 mM of NaCl to the binding/washing and elution buffers
Protein not eluted	Elution conditions were too mild	Increase incubation time with elution buffer or use more stringent elution buffer
Low amount of protein recovered	The protein degraded	Add protease inhibitors (e.g., HY-K0010 or HY-K0011)
	Not enough magnetic beads were used	Increase the amount of magnetic beads used for capture
	Sample had an insufficient amount of target protein	Increase amount of antigen sample

Appendix: Binding Affinity of Protein A/G for Different Antibodies and Subtypes

Species	Antibody Subtype	Protein A/G
Human	Total IgG	+++++
	IgG1, IgG2	+++++
	IgG3	+++++
	IgG4	+++++
	IgM	+
	IgD	-
	IgA	+
	IgA1, IgA2	+
	IgE	+++
	Fab	+
	ScFv	+
Mouse	Total IgG	+++++
	IgM	-
	IgG1	+++
	IgG2a	+++
	IgG2b	+++
	IgG3	+++
Rat	Total IgG	+++
	IgG1	+++
	IgG2a	+++++
	IgG2b	+
	IgG2c	+++++
Cow	Total IgG	+++++
	IgG1	+++++
	IgG2	+++++
Goat	Total IgG	+++++
	IgG1	+++++
	IgG2	+++++
Sheep	Total IgG	+++++
	IgG1	+++++
	IgG2	+++++
Horse	Total IgG	+++++
	IgG(ab), IgG(c)	+
	IgG(T)	+++++
Rabbit	Total IgG	+++++
Guinea Pig	Total IgG	+++++
Hamster	Total IgG	+++
Pig	Total IgG	+++++
Donkey	Total IgG	+++++
Cat	Total IgG	+++++
Dog	Total IgG	+++++
Monkey	Total IgG	+++++
Chicken	Total IgG	-

Notes: + weak binding +++ medium binding +++++ strong binding - no biding