Protein A/G Magnetic Beads for IP

Description

Protein A/G Magnetic Beads for IP use a biological nanosurface technology (S-TEC). Protein A/G is orientated as a coat on the surface of super paramagnetic microspheres with high coating density up to 9.3 × 10^{13} molecules/cm^2. Compared to other similar immune magnetic beads, Selleck Protein A/G magnetic beads display more antibody binding sites, therefore during IP, less magnetic beads are needed. Non-specific binding is low, enabling Selleck Protein A/G magnetic beads to be used in IP conveniently and efficiently. With a large, specific surface area, these beads can greatly shorten the equilibrium antibody and antigen adsorption time, enabling complete antibody antigen adsorption process within 10 min, and complete total purification and precipitation in just 30 min. This product can be used on a wide variety of samples, such as cell lysates, supernatants collected from cell secretion, serum, ascites, and other immune antigens.

Components

Contents	Cat#: B23201	Cat#: B23202
Protein A/G Magnetic Beads for IP	1 mL	5 mL

1. The particle size of magnetic beads is 100 nm.
2. The concentration of magnetic beads is 10 mg/mL, and the binding capacity of human IgG is 0.4-0.5 mg/mL.
3. Supplied in 25 mM Tris-HCl (pH 7.2), 50% StabiGuard, containing 0.01% (w/v) sodium azide, and shipped with blue ice.
4. Protein A/G Magnetic Beads for IP is stable for long term under pH 6-8.

Storage

Protein A/G Magnetic Beads for IP should be stored in 50% glycerol at 4°C for maximum stability. The unopened product is stable for 2 years when stored as indicated. After use, the magnetic beads should be cleaned and stored in 25 mM Tris-HCl (pH 7.2), 50% StabiGuard, containing 0.01% (w/v) sodium azide. Do not freeze in the absence of glycerol.

Notice

1. This product requires use of a magnetic separator.
2. Protein A/G Magnetic Beads should be suspended uniformly before use.
3. Protein A/G Magnetic Beads should be kept in storage solution and prevent dry.
4. Do not freeze or centrifuge protein A/G magnetic beads.
5. In order to ensure the best results, please select an antibody with strong specificity.
6. For the IP experiments, different antibodies and antigens will display different binding affinities. Some operator optimization may be necessary.
7. This product is only intended to be used as directed. All other uses are prohibited.

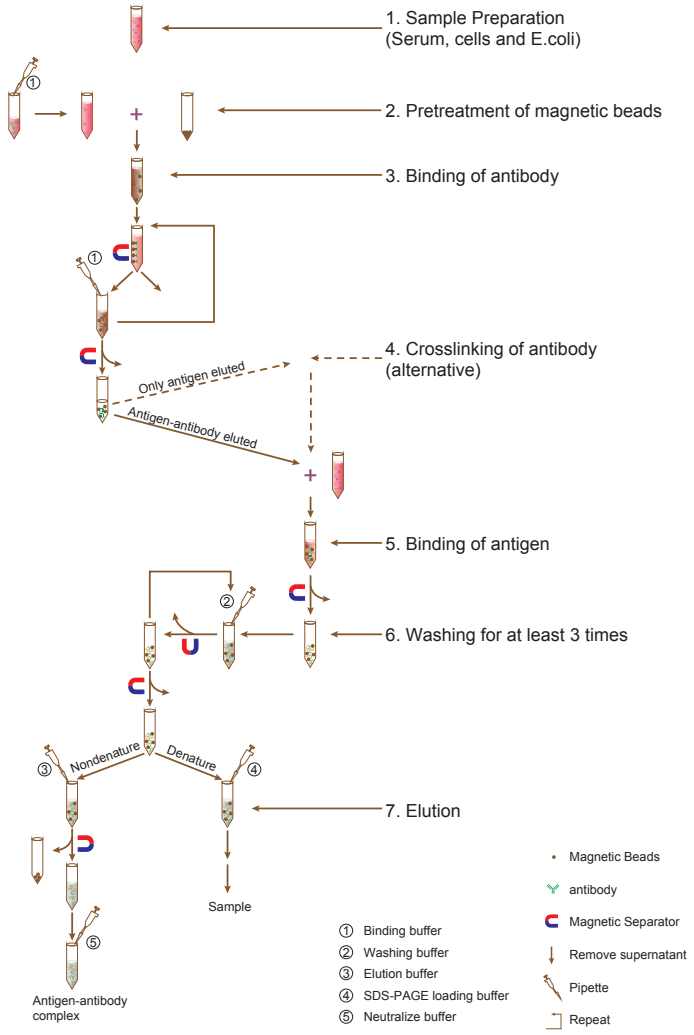

Figure 1. General Protocol for Immunoprecipitation

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Protocol

Reference Protocol for Immunoprecipitation (IP)

Optimization may be required for each antibody and target antigen. Protein A/G Magnetic Beads for IP are ideally suited for IP reactions.

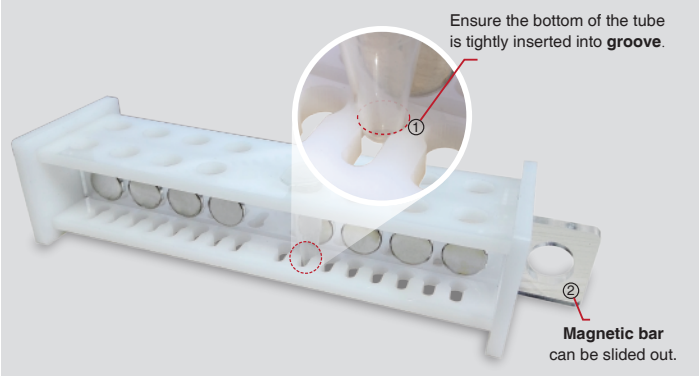

Figure 2. Magnetic separator

1 Preparation of Protein A/G Magnetic Beads Matrix

1.1 Resuspend the Magnetic Beads in the vial (vortex >30 sec or tilt and rotate for 2 min).

1.2 Transfer 50 µL (the amount may be scaled up or down as required) Protein A/G Magnetic Beads to a 1.5 mL tube. Perform magnetic separation (ensure the bottom of the tube is tightly inserted into the ①Groove, incubate for 5-10 sec until all beads have migrated to the side of the tube near the magnetic bar) and discard the supernatant.

1.3 Slide out the ②magnetic bar and add 150 µL binding buffer into the tube. Wash by gentle pipetting. Then incert ②magnetic bar and perform magnetic separation and discard the supernatant. Repeat this step for 2 times.

2 Binding of Antibody

2.1 Add your antibody (Ab) (typically 5-10 µg) to 50 µL binding buffer, in order to dilute Ab to the appropriate concentration. The optimal amount of Ab needed depends on the individual Ab used.

2.2 Add Ab mixture to the Protein A/G Magnetic Beads from above step. Rotate tube for 1 h at room temperature or 4 h at 4°C, then resuspend thoroughly by pipetting up and down.

2.3 Perform magnetic separation and discard the supernatant.

2.4 Slide out the ②magnetic bar and add 150 µL binding buffer into the tube. Wash by gentle pipetting.

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

3 Immunoprecipitation of Target Antigen

3.1 Incert ②magnetic bar, perform magnetic separation and discard the supernatant.

3.2 Remove the tubes from the magnetic separator and add your sample containing the antigen (Ag) (typically 100-150 µL) and gently pipette to resuspend the Protein A/G Magnetic Beads-Ab complex.

3.3 Incubate with rotation for 10 min at room temperature 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.

3.4 Perform magnetic separation. Transfer the supernatant to a clean tube for further analysis, if desired. The supernatant is the non-binding fraction.

3.5 Wash the Magbeads-Ab-Ag complex 3 times using 300 µL binding buffer for each wash. Perform magnetic separation between each wash, remove supernatant and resuspend by gentle pipetting .

3.6 Resuspend the Protein A/G Magnetic Beads-Ab-Ag complex in 150 µL binding buffer and transfer the bead suspension to 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. Slide out the ②magnetic bar, add 300 µL wash buffer into the tube and rotate for 5 min. Incert ②magnetic bar, perform magnetic separation for 1 min and remove the supernatant. Then add 10-50 µL elution buffer into the tube with magnetics beads-Ab-Ag complex, rotate for 5 min.

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 subjected for further analysis.

Recommended buffer examples

Buffer	Content
Binding buffer	50 mM Tris, 150 mM NaCl, 0.1%-0.5% detergent (TritonX-100, Tween 20 or NP40), pH 7.5
Wash buffer	50 mM Tris, 150 mM NaCl, 0.1%-0.5% detergent, pH 7.5
Elution buffer	0.1 M-0.2 M Glycine, 0.1%-0.5% detergent, pH 2.5-3.1 (or 0.1 M citric acid, 0.1%-0.5% detergent, pH 2.5-3.1)
Neutralize buffer	1 M Tris, pH 8.0

Trouble Shooting

Problem	Suggestion
How to improve the binding efficiency of antibody and magnetic beads	The binding efficiency of magnetic beads and antibody depends on the origin and subtype of the antibody, please check the affinity of your antibody and the Protein A/G matrix in the attached table1. If your antibody subtype and protein A/G matrix shows low affinity, please elongate the incubation time of antibody and magnetic beads, improve the pH value of binding buffer (8-9) and reduce the ion strength (25-100 mM NaCl) to improve the binding efficiency.
How to improve the binding specificity of magnetic beads in the IP reaction	It is suggested to incubate the antibody and your sample at first to obtain the antibody-antigen complex, and then the complex is captured by the protein A/G magnetic beads. This method could improve the binding efficiency of antibody and antigen, while reduce the contact time of magnetic beads and sample, and enhance the product specificity of IP experiment.
How to avoid the aggregation of magnetic beads in storage or in usage	The magnetic beads should be stored in 4°C. Reagent pollution and dryness could cause irreversible aggregation. During the elution step using low pH buffer, it is normal that magnetic beads aggregates, and which will not influence the magnetic beads' function. After low pH elution, the magnetic beads could be washed by pH neutral buffer, resuspended in Tris buffer (pH 7.5) containing 0.1% nonionic detergent, and treated by ultra-sonic for 2 min, and then the magnetic beads will return to uniform status. All these operation will not influence the antibody binding efficiency.
How to solve the problem that magnetic beads sticking to the inner surface of the pipette tips	It is suggested to use supplies of low absorption in the handling of magnetic beads. And it is suggested to add 0.1%-0.5% nonionic detergent (like TritonX-100, Tween-20 or NP-40) to the buffer system to reduce the sticking situation.
Why is the magnetic beads clogged during the experiment	If the magnetic beads are located within the magnetic field for a very long period, it is possible that they are clogged and hard to be evenly distributed. It is suggested that they are under ultra-sonic treatment in water pot for two min, after which clogged magnetic beads will disperse evenly again. But it should be noticed that ultra-sonic treatment will damage the antibody bond, so this treatment should not be used during the experiment, after binding or before elution.

Attached table1: Relative Binding Strengths of Antibodies to Protein A/G

Species	Antibody class	sProtein 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	+++
	IgG 1	+++
	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 binding