GeneBio Systems, Inc.™

GB-MagicTM Protein A (or A/G) Immunoprecipitation-User Manual Product Introduction

GB-Magic™ Protein A/ G Matrix beads for immunoprecipitation are superparamagnetic microspheres coupled with Protein A/G at a high density. Compared to similar products currently available from other suppliers, these beads have more antibody binding capacity, allowing the researcher to use fewer beads, and low background binding with other proteins, making immunoprecipitation experiments more efficient. Each millilitre of immunoprecipitation beads can bind more than 300μg of human IgG, and a single reaction only needs 25μL of beads. The super-large specific surface area provided by the beads can significantly reduce the equilibrium time required for adsorption of antibody and antigen. The antibody adsorption process can be completed within 15 minutes and the antigen precipitation process is completed within 30 minutes. Short procedure times can help avoid target protein hydrolysis, ensuring the activity of the target protein and integrity of the protein complex. This product can be used with a wide variety of samples, including with cell lysates, cell secretion supernatant, blood plasma, ascites, tissue culture supernatant and other samples of antigen immunoprecipitation reaction. The end user can refer to the data in Table 1 for binding capacity between different species and subtypes of antibodies and Protein A beads or Protein A /G beads.

Product Information

This operating manual is for all Magic Beads™ Protein A/G for Immunoprecipitation Kits.

PLEASE NOTE CAT. # 22202B IS FOR THE PRODUCT WITH BEADS ONLY, NO BUFFER IS PROVIDED. FOR KITS CONTAINING BUFFER, SEE ORDERING INFORMATION BELOW.

Product name	GB-Magic Beads™ Protein A (or A/G) Immunoprecipitation Kit
GB-Magic Beads™ Protein A/G for IP ①	1 mL
IP Binding Buffer ②	30 mL
phosphate Buffer PBS (10×, dilute1×before use) ③	20 mL
IP Washing Buffer ④	20 mL
IP Elution Buffer ⑤	0.5 mL
IP Neutralization Buffer ⑥	0.2 mL
Magnetic Separator Stand 2/15 ⑦	optinal
Storage condition	2~8℃ for long-term preservation
Shelf life	2 years

Note: the capacity binding of Human IgG Antibody Capacity are: Protein A： 0.4~0.5 mg/mL; Protein A/G：0.5~0.6 mg/mL

Procedure

1.Antigen sample preparation: methods for processing four types of samples are provided in this operating manual. We recommend that you choose the appropriate method to process different antigen samples in order to lyse cells and release antigens to lysates.

Note: some of the procedure requires weighing an empty microcentrifuge tube, prior to starting the procedure.

Serum sample processing: If the target protein is highly abundant, it is recommended to dilute the serum sample to the final concentration 10~100 μg/mL of the target protein with binding buffer（②. Place on ice (or store at -20 ° C).

Suspended cell sample processing: collect cells by centrifugation (4 ° C, 500 x g, 10 min), discard the supernatant and weigh the pellet, and wash twice with the ratio of 50 μL 1×PBS （③） per every milligram of cells; add the binding buffer （②）with the ratio of 5~10 μL per every milligram of cells, then add protease inhibitor (such as PMSF with a final concentration of 1 ㎜), mix and place on ice for 10 min; centrifuge to collect the supernatant (4 ° C, 14000 g, 10 min), place on ice (or store at -20 ° C).

Adherent cells processing: Remove the medium and wash twice with 1×PBS(③) at a ratio of 150 μL per 1.0×10^5 cells; scrape off the cells with a scraper and collect into a 1.5 mL microcentrifuge tube. Add the binding buffer（②） at a ratio of 20~30 μL per 1.0×10^5 cells, and immediately add a protease inhibitor (such as a final concentration of 1 mM PMSF). After mixing, place on ice for 10 min; collect the supernatant by centrifugation (4 ° C, 14000 g, 10 min) and place on ice (or store at -20 ° C for a long time).

E. coli sample processing : Collect Escherichia coli (4 ° C, 12000 g, 2 min) by centrifugation , discard supernatant and weigh pellet, wash twice with 1 × PBS （③） at a ratio of 10 mL per gram (wet weight) of bacteria. Add the binding buffer （②） at a ratio of 5~10 mL per gram (wet weight) of the cells, and add a protease inhibitor (such as PMSF at a final concentration of 1 mM), resuspend the cells, lyse the cells, and centrifuge(4° C, 17000 g, 10 min) to collect the supernatant.

2.Magnetic Beads Pretreatment: Vortex the immunoprecipitation magnetic beads （①） for 1 min to be fully resuspended. Place 25~50 μL of the magnetic beads suspension in a 1.5 mL microcentrifuge tube. Wash with 200 μL of binding buffer（②, magnetic separation [Place the microcentrifuge tube on the magnetic rack/stand（⑦, so that the beads are adsorbed on the tube wall until the solution is clarified (Note: the description of the magnetic separation step is omitted below], and discard the supernatant.
Remove the microcentrifuge tube from the magnetic arck and repeat the wash once. Finally, add 200 μL Binding Buffer （②） and resuspend the beads for later use.

3.Preparation of antibody working solution: Dilute antibody sample with binding buffer （②） to prepare antibody working solution with final concentration of 5~50 μg/mL. Spare on ice.

Antibody adsorption: magnetically separate the magnetic bead suspension pretreated in step 2, discard the supernatant; add 200 μL of antibody working solution, resuspend it, place it on a vertical rocking mixer at room temperature or gently flip the microcentrifuge tube by hand, after 15 min, then perform magnetic separation, collect the supernatant and store on the ice for subsequent detection.
Washing: Add 200 μL of Binding Buffer（②） to the microcentrifuge tube and gently pipette to spread the magnetic beads-antibody complex evenly. Then magnetic separation, discard the supernatant and remove the microcentrifuge tube from the magnetic rack. Repeat the above washing operation once.

4.Antibody cross-linking reaction (optional): If the operator needs to elute the antibody and the target antigen complex, please ignore this step and proceed to step 5.
This step is suitable for experiments where the operator needs to elute the target antigen separately. It is recommended to use BS3 (Thermo Scientific, Cat. #21580) as the cross-linking reagent, please refer to its operating instructions for the related experiments.

5.Antigen adsorption: Add 200 μL of the antigen sample prepared in the step 1, and pipette gently the antigen and the magnetic bead-antibody complex until uniformly dispersed. Place the mixture in a vertical mixer at room temperature or gently flip the microcentrifuge tube for 10 min to allow the antigen to fully bind to the antibody. If antigen binding is weak, consider lengthening the time to 1 h or perform this incubation at 4 ° C overnight.

6.Washing and Transfer: Perform magnetic separation of the bead-antibody-antigen complex from last step, collect the supernatant and place it on ice for subsequent detection. Add 200 μL of Wash Buffer （④） to the microcentrifuge tube , pipette gently to evenly disperse the magnetic bead-antibody-antigen complex, then magnetic separate the complex and discard the supernatant. Then remove the microcentrifuge tube from the magnetic rack. Repeat this wash twice. Finally, add 200 μL of Wash Buffer（④）, and transfer the magnetic bead-antibody-antigen complex suspension to a new 1.5 mL tube* with a pipette tip. Perform magnetic separation, then remove the supernatant.
(*: note: Always transfer the beads to a new 1.5 mL tube before eluting the antigen in order to avoid eluting the original non-specifically adsorbed proteins often left on the tube wall.)
Antigen Elution: two antigen elution protocols are provided below. The user can select the antigen elution method according to the needs of the downstream detection.

Denaturing elution method: The sample eluted by this method is suitable for SDS-PAGE detection. Remove the 1.5 mL tube from the magnetic rack, add 25 µL of 1× SDS-PAGE Loading Buffer (not provided), mix well, and heat at 95 ° C for 5 min. Magnetic separation (or centrifuge at 13000 g, 10 min at room temperature) and collect the supernatant for SDS-PAGE detection.

Non-denaturing elution method: The sample eluted by this method retains the original biological activity and can be used for later functional analysis. Remove the 1.5mL tube from the magnetic rack, add 20 µL of elution buffer （⑤） and mix well. Incubate for 10 min at room temperature. Then perform magnetic separation (or centrifuge at 4 ° C, 13000 g for 10 min), collect the supernatant into a new 1.5mL tube and immediately add 1.0 µL of neutralization buffer （⑥） to adjust the pH of the eluted product to neutral for downstream functional analysis.

Notes

1. Be sure to read this user manual carefully before performing an immunoprecipitation procedure.
2. This product must be used with the magnetic separator.
3. Beads should be fully resuspended before use.
4. The beads should be stored in the storage solution to prevent drying and aggregation.
5. Do not freeze or centrifuge the beads to avoid irreversible aggregation.
6. To ensure the best results, select a specific antibody for immunoprecipitation.
7. Antibodies with good binding characteristics, including specificity should be used in immunpreciptation
8. 10×PBS（③ should be diluted in the sterile environment.
9. This product is for research use only

FAQ

Q1：How can I improve the binding efficiency of antibody and magnetic beads?

A1:The binding efficiency of beads and antibody is related to the source and subtype of the antibody . Please check the type of the antibody and affinity of the antibody to the Protein A / G ligand (Table 1). If the affinity is low, the user can increase the incubation time of antibody and magnetic beads (from 30 to 120 min), raise the pH of the binding buffer (from pH 8 to pH 9) and reduce the ionic strength of the binding buffer (from 25 to 100 mM NaCl)

Q2: How can I improve the specificity of magnetic beads in immunoprecipitation?

A2: Antibodies can be incubated with the sample to form an antibody-antigen complex first, and use the protein A/G beads to capture the complex later. This method can improve the efficiency of antibody and antigen binding, and reduce the mixing time between the beads and the sample, thereby enhancing the specificity of the precipitated product. This method is also recommended for protein/nucleic acid coprecipitation or chromatin immunoprecipitation.

Q3: How can I avoid the aggregation of the beads in the storage or process of the use?

A3: Magnetic beads should be stored at 2 ~ 8 ℃. Magnetic beads tend to aggregate in the low pH elution buffer, but this aggregation should not affect the normal use. The addition of non-ionic detergents (such as NP-40, Tween-20 or Triton X-100) with a final concentration of 0.1% (v / v) in the Binding buffer and Elution buffer can effectively prevent the accumulation of beads. The beads subjected to low pH elution can be washed to neutral in pH with binding buffer and then suspended in a Tris buffer (pH 7.5) containing 0.1% (v / v) Tween-20. The beads can be treated with ultrasonic bath 2 min, to help restore them to a uniform state. The above treatment does not affect the antibody binding efficiency of magnetic beads.

Q4： How can I solve the problem of the beads easily adhere to the tube wall?

A4: It is recommended to use low-adsorption tips and tubes for magnetic bead operation. In addition, a nonionic detergent can be added to the buffer solution ot 0.01% to 0.1% (v / v) (Such as NP-40, Tween-20 or Triton X-100) to effectively reduce the adhesion of the beads to the consumables.

Q5: How can I solve the problem of agglomeration of magnetic beads during usage?

A5: If beads are left in the magnetic field for too long, they will firmly bond together. These aggregates are difficult to break down by just vortexing. One solution is to put the tube of beads in an ultrasonic bath for 2 minutes. This can efficiently disperse and redistribute the beads; however, this treatment will also shear the antibody off the beads, so this method should not be used either after adding sample or before the elution process.

Table 1: Comparison of Antibody Affinity between Protein A and Protein A / G with Different Sources and Types

Species	Antibody Classs	Protein A/G	Protein A
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 IgY	-	-

Note: “+”weak binding， “+++”medium binding， “+++++”strong binding， “-”no binding

GB-Magic ™ Beads for Protein A (or A/G) Immunoprecipitation				
Cat No.	**Prodcuts Name**	**Size**	**Diameter**	**human IgG capability**
22202-20	GB-Magic BeadsTM Protein A/G	20rnxs	2 μm	0.5~0.6 mg/mL
22202-100	GB-Magic BeadsTM Protein A/G	100 rnxs		
22203-20	GB-Magic BeadsTM Protein A	20 rnxs	2 μm	0.4~0.5 mg/mL
22203-100	GB-Magic BeadsTM Protein A	100 rnxs		

Cat No	Magnetic Separator Stand	size	Remark
60201	Magnetic Separator Stand 2/15	2/15ml	Suitable for 1.5 mL, 2 mL EP tube and 15 mL centrifuge tube
60203	Magnetic Separator Stand 50	50ml	Suitable for regular 50 mL centrifuge tubes
60302	Magnetic Separator Stand 96-I	96-I	Suitable for conventional 96-well plate, PCR plate, 8-well or 12-well PCR tube etc.
60303	Magnetic Separator Stand 96-II	96-II	Suitable for 96 200 μL experimental system) -well PCR plates (20 to 200 μL experimental system)

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