Protein A Immunoprecipitation Kit

Cat: M2410
Size: 20T/100T
Storage: 2-8℃

Introduction:

Protein A Immunoprecipitation Kit series products are intended to use the nano surface technology to enclose Protein A high-density directional precipitation onto the surface of the superdiamagnetic microsphere. Compared with similar products in the current international immunoprecipitation market, the product has more antibody binding sites. With 1ess use of magnetic beads and low non-specific binding rate, immunoprecipitation experiments can be carried out conveniently and efficiently. The ability of binding to Human IgG per milliliter of immunoprecipitation magnetic beads can reach more than 300μg, and a single precipitation reaction can be detected with only 25μL magnetic beads. The large specific surface area provided by micron magnetic beads greatly shortens the balance time required for antibody and antigen adsorption. The antibody adsorption process can be completed within 15min, and the antigen precipitation operation can be completed within 30min. The short operation time avoids the hydrolysis of the target protein caused by long- term operation, and ensures the activity of the target protein and the integrity of the protein complex.

The immunoprecipitation magnetic bead kit is equipped with optimized prefabricated buffer, which provides the best reaction conditions for the immunoprecipitation experiment and enhances the stability of the immunoprecipitation experiment.

This product can be widely used in the immunoprecipitation reaction of antigen in cell lysate, cell secreted supernatant, serum, ascites and other samples. The operator can refer to this operating instruction manual and the data in Schedule 1 to understand the binding ability of antibodies from different species and subtypes with Protein A magnetic beads and Protein A/G.

Product Information:

Product name	20T	100T
Beads Protein A for IP①(particle size: 2μm)	1mL	5mL
IP Binding Buffer ②	30mL	75mL× 2
Phosphate buffer PBS (10 ×, dilute to 1 × before using)	20mL	100mL
IP Washing Buffer ④	20mL	100mL
IP Elution Buffer ⑤	0.5mL	1.25mL×2
IP Neutralization Buffer ⑥	0.2mL	1mL
Storage period	2 years	2 years

Note: Antibody Capacity of magnetic beads to Human IgG is Protein A: 0.4 to 0.5mg/mL: ProteinA/G: 0.5 to 0.6mg/mL .

Protocols:

1. **Antigen sample preparation:** This operation manual provides the following four sample treatment methods, it is recommended that you choose the appropriate way to pretreat the antigen samples from different sources, so that the antigen to be detected is released into the sample solution.

Serum sample treatment: If the target protein abundance is high, it is recommended to dilute the serum sample with binding buffer (②) to the final target protein concentration of 10~ 100μg/ mL, and put it on ice for use (or long-term storage at -20°C).

Suspension cell sample treatment: The cells were collected centrifugally (4°C, 500g, 10 min), the supernatant was discarded and weighed, and washed twice with 1× PBS (③) at a ratio of 50μL per mg of cells; The binding buffer (②) was added at the ratio of 5 to 10μL per mg of cells, and the protease inhibitor (such as PMSF with a final concentration of 1mM) was added, mixed and placed on ice for 10 min; Centrifuge and collect the supermatant (14000 g,10min, 4°C) and place it on ice for future use (or store it at -20°C for long-term storage).

Treatment of adherant cell samples: Remove the medium and wash twice with 1×PBS (③)) at a ratio of 150μL per 1.0× 10^5 cells; The cells were scraped off with a cell scraper and collected in a 1.5mL EP tube. The binding buffer (②) was added at the ratio of 20 to 30 μL per 1.0× 10^5 cells, and the protease inhibitor (such as PMSF with a final concentration of 1mM) was added, mixed and placed on ice for 10 min. The supernatant was collected by centrifugation (14000g,10 min, 4°C) and placed on ice for future use (or long -term storage at -20°C).

E. coli sample treatment: Centrifuge E. coli (4°C, 12000g, 2 min), discard the supemnatant and weigh it, wash it with 1×PBS (③) twice at the ratio of 10 mL per gram (wet weight) bacteria; The binding buffer (②) was added according to the ratio of 5~ 10mL per gram (wet weight) of bacteria, at the same time, protease inhibitors (such as PMSF with a final concentration of 1mM) were added, the bacteria were suspended, the cells were ultrasonically lysed, and the supermatant was collected by centrifuge (17000 g, 10min, 4°C).

2. **Pre-treatment of magnetic beads:** Swirl the immunoprecipitated magnetic beads (①) for 1 min, so that the magnetic beads are fully oscillated and re-suspended; 25~ 50μL magnetic bead suspension was placed ina 1.5mL EP tube. Add 200μL combined buffer (②) for washing and magnetic separation [Place EP tube on magnetic separator so that magnetic bead is adsorb on tube wall until solution is clarified; this operation description is omitted below], discard superliquant, remove EP tube from magnetic separator and repeat washing. Finally, 200uL of binding buffer (②) was added to re-suspend the magnetic bead for reserve.

3. Antibody binding reaction:

Preparation of antibody working liquid: Dilute the antibody sample with binding buffer (②), prepare the antibody working liquid with a final concentration of 5- 50μg/ mL, and put it on ice for use.

Antibody adsorption: The magnetic bead suspension pretreated in step 2 was magnetically separated, and the supernatant was discarded; Add 200μL of antibody working liquid, quickly resuspend and place in a turning mixer at room temperature or gently tum the EP tube manually. Magnetic separation is performed 15 min later, and the superserum is collected and placed on ice for subsequent detection.

Washing: 200μL binding buffer (②) was added to the EP tube for washing, and the magnetic beads-antibody complex was evenly dispersed by gently blowing with a pipette, then magnetic separation was carried out, the supernatant was discarded, and the EP tube was removed from the magnetic separator. 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 together, please ignore this step and proceed to Step 5. This step is for tests where the operator needs to elute the target antigen separately.

5. Antigen precipitation reaction

Antigen adsorption: Add 200μL of the antigen sample prepared in step 1 and gently blow it with a pipette to evenly disperse the antigen and the bead-antibody complex. Place the sample in the turnover mixer at room temperature or gently turn the EP tube manually for 10 min, so that the antigen and antibody can be fully combined, if the binding force is weak, it can react at room temperature for 1 h or at 4℃ overnight.

Washing and transfer: the magnetic bead-antibody-antigen complex that has completed antigen adsorption is magnetically separated, and the supernatant is collected and placed on ice for subsequent detection. 200μL washing buffer was added to the EP tube for washing (④), and the magnetic beads-antibody-antigen complex was evenly dispersed by gently blowing with a pipette, then magnetic separation was carried out and the supernatant was discarded; Remove the EP tube from the magnetic separator and repeat washing twice. Finally, 200uL washing buffer (④) was added, and the magnetic beads-antibody-antigen complex suspension was transferred to the new 1.5mL EP tube* with a pipette, magnetic separation was performed, and the supernatant was removed.

(*** Note:** Before antigen elution, the magnetic beads must be transferred to the new EP tube to avoid elution of the original non-specific adsorbed proteins on the tube wall.)

6. **Antigen elution:** This operation manual provides the following two antigen elution schemes. The operator can choose different antigen elution methods according to the needs of late detection.

Denatured elution method: The sample eluted by this method is suitable for SDS -PAGE detection. Remove the EP tube from the magnetic separator, add 25μL 1× SDS-PAGE Loading Buffer (**prepared by yourself**) into it, mix well, and heat at 95℃ for 5 min. Then magnetic separation was performed [or centrifugation could be used (13000g at room temperature, 10 min)] to collect the supernatant for SDS-PAGE detection.

Non-denaturing elution method: The eluted sample in this method retains the original biological activity and can be used for later functional analysis. Remove the EP tube from the magnetic separator, add 20μL of elution buffer (⑤) into it, mix well, and incubate at room temperature for 10min. Then magnetic separation was performed [or centrifugation could be used (4℃, 13000g, 10 min)]. the supematant was collected into the new EP tube, and 1.0μL neutralizing buffer (⑥) was immediately added to adjust the pH of the elution product to neutral for later functional analysis.

Note:

1. Before performing the immunoprecipitation procedure, be sure to read this operation manual carefully.
2. This product must be used in conjunction with a magnetic separator.
3. Magnetic beads should be fully oscillated and uniform before use.
4. Magnetic beads should be kept in storage solution to prevent drying.
5. Do not freeze or centrifuge the magnetic beads to avoid causing irreversible aggregation.
6. 10×PBS (③) should be diluted under sterile conditions and discontinue use of the solution as soon as contamination is detected.
7. To ensure the best experimental results, please select a strong specific antibody for immunoprecipitation reaction.
8. The operator can use the supernatant collected in the steps of antibody binding reaction and antigen binding reaction to detect the combination of antibody, antigen and magnetic beads according to the actual needs.
9. For IP experiment, different types of antibody and antigen binding affinity is different, antibody and antigen binding will also be affected by binding buffer and washing buffer, therefore, if the operator can not use the buffer system provided by this kit to obtain good experimental results, you can screen and prepare buffer for the experiment.
10. The recombinant Protein A coated on the surface of the magnetic bead has very low protein shedding under extreme conditions (such as low pH, heating treatment), but it is still not recommended for the operator to use the immunoprecipitation experiment of the target protein with a molecular weight of about 130 kD.
11. This product is for research use only.

Frequently Asked Questions and Answers (FAQ):

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

A1: The binding efficiency of magnetic beads and antibodies is related to the species source and subtype of antibodies. Please confirm the affinity efficiency of the antibody type and Protein A ligand (Schedule 1). If the affinity between the antibody subtype and Protein A is low, the affinity efficiency can be improved by increasing the incubation time of the antibody and the magnetic bead (30 ~ 120 min), increasing the pH value of the binding buffer (8 ~ 9) and reducing the ionic strength (25 ~ 100mM NaCl).

Q2: How to improve the specificity of magnetic beads in immunoprecipitation reaction?

A2: The antibody can be incubated with the sample to form an antibody-antigen complex, and then Protein A magnetic beads can capture the complex. This method can improve the binding efficiency of the antibody to the antigen, and reduce the contact time between the magnetic bead and the sample, thus improving the specificity of the precipitated product. This method is also recommended for protein/nucleic acid co-precipitation or chromatin immunoprecipitation.

Q3: How to avoid the accumulation of magnetic beads that may occur during storage or use?

A3: Magnetic beads should be kept at 2 ~ 8℃, and irreversible aggregation caused by pollution or aggregation caused by drying should be avoided when used. The aggregation of magnetic beads in the eluting buffer of low pH is a normal phenomenon, and does not affect the normal use of magnetic beads. Add a non-ionic detergent with a final concentration of 0.1% (v/v) to the Binding buffer and Elution buffer (e.g. NP-40, Tween-20 or Triton X-100) Effective in preventing magnetic beads from gathering. Magnetic beads that have undergone a 1ow pH elution operation can be washed to neutral with a binding buffer and then washed with a containing 0.1% (v/v) Tween-20 Tris buffer (pH7.5) oscillating the re-suspended magnetic beads and treating them with ultrasonic water bath for 2 min can restore the uniform state of the magnetic beads, and the above treatment does not affect the antibody binding efficiency of the magnetic beads.

Q4: How to solve the phenomenon that the magnetic bead is easy to adhere to the tube wall?

A4: It is recommended to use consumables with 1ow adsorption rate for magnetic bead operation. In addition, adding 0.01% to 0.1% (v/v) of non-ionic detergent to the buffer (such as NP-40, Tween-20 or Triton X-100) can effectively reduce the adhesion of magnetic beads to the consumables).

Q5: Do magnetic beads caking during use?

A5: If the phenomenon of agglomeration occurs when the magnetic beads are in use, it is generally difficult to oscillate and break up, which is easy to lead to uneven distribution. The reason for this problem is that the magnetic beads are placed in the magnetic field for too long and the magnetic beads are firmly combined together. The ultrasonic water bath can be used to disperse the magnetic beads for 2min to re-disperse them, but it should be noted that the ultrasonic treatment will also make the magnetic beads in the sample solution captured antibodies off, so the magnetic beads in the sample after elution should not be used before the method.

Schedule 1: Comparison of affinity between immunomagnetic beads Protein A and Protein A/G and antibodies from different sources and types

Pecies	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