(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS!)

Elabscience® IP/CoIP Kit (Magnetic bead)

Product code：EA-IP-K007M

Product size：50 T

Please read the instructions carefully before use. If you have any questions, please contact us at:

Tel: 240-252-7368(USA)
Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please refer to the outer package label of the kit for the specific shelf life and use the kit within its shelf life.

Please provide the product cat number (see kit label) when contacting so that we can serve you more efficiently.

Background Information

This product is made by covalently binding high-quality Protein A/G protein with magnetic beads, and can be used for immunoprecipitation (IP) and immuno-co-precipitation (Co IP). This product has the characteristics of high binding capacity of protein, fast and convenient operation, strong specificity, low non-specific adsorption, wide combination range, etc.

Performance Index

1. Scope of application

Immunoprecipitation (co-precipitation) of IgG proteins (including most IgG subtypes) of multiple species from cell lysate, supernatant of cell secretion, serum, animal ascites and other samples (see appendix).

2. Binding properties

High purity recombinant Protein A/G protein.

3. Magnetic bead properties

Magnetic bead particles, average particle size 300nm.

4. Binding capacity

1mL superparamagnetic beads, covalently bound with 20mg recombinant Protein A/G protein.

5. Components

0.5mL Protein A/G immunomagnetic beads, stored in 1.5mL PBS containing preservatives.

Product Components

Component	Code	Specification	Storage
Lysis buffer	E-IR-IP004	30 mL	4°C, 12 months
Magnetic frame	E-IR-IP003	1 piece	Room temperature, 12 months
ProteinA/G magnetic beads	EA-IP-007M	1mL*2支	4°C, 12 months
Acid elution buffer	E-IR-IP006	2 mL	4°C, 12 months
PBS Buffer, pH7.4 (10×)	E-BC-R187	50 mL	4°C, 12 months
PBST Buffer,pH7.4 (10×)	E-IR-R310	50 mL	4°C, 12 months
Manual	one copy		

Matters Needing Attention

1. Transportation and storage

The kit is transported under refrigeration.

After receiving the goods, please take out the magnetic frame **C** and store it at room temperature. Other components of the kit were stored at 4°C。

2. Suggestions on reagent use

10×PBS (PBS Buffer, pH 7.4 (10×)) and 10×PBST (PBST Buffer, pH 7.4 (10×)) should be diluted into 1x working solution with deionized water before use.

3. Suggestions for using Protein A/G magnetic beads

When mixing the magnetic beads, please use soft vortex, upside down, shaking table mixing and other methods. Do not centrifuge and dry the magnetic beads, do not use sonication for the magnetic beads, and do not use acid to treat the magnetic beads for more than 10min.

4. Acid eluent selection

Literature shows that compared with traditional Glycine HCL eluent, Arginine HCL with pH 3.0 provided in this kit can reduce protein denaturation. You can also choose acid eluent according to the actual situation.

5. Binding affinity of Protein A/G to IgG of various species

Antibodies (IgG, IgM, IgA, IgD) of various species have different binding affinities with Protein A/G. Please read the appendix of this manual carefully before use.

Self-Prepared Reagent

1. 1× PBS

Dilute 10×PBS(PBS Buffer, pH 7.4 (10×)) with deionized water in a ratio of 9: 1 foruse. For example, add 1mL of 10×PBS into 9mL of deionized water and mix it to 1×PBS. Prepare when needed.

2. 1× PBST

Dilute 10×PBST (PBST Buffer, pH 7.4 (10×)) with deionized water in a ratio of 9:1for use. For example, add 9mL of deionized water to 1mL 10×PBST and mix it to 1×PBST. Prepare when needed.

Method of Application

Note: All steps must be performed on ice as much as possible to avoid degradation of the target protein. In the following steps, the amount of magnetic bead suspension is 40μL (including 10μL magnetic bead), and 20ug IgG can be bound from 15μL serum or 100μL cell supernatant. Please adjust the amount of magnetic bead according to the amount of antibody to be bound.

1. Preparation of cell lysate

1) Collecting cells

Blow the suspended cells and semi-adherent cells off the cell culture flask and transfer them into a centrifuge tube, centrifuge at 1000rpm for 5min, and discard the supernatant.

Gently scrape the adherent cells off the bottle wall with a cell scraper, transfer them into a centrifuge tube together with the culture medium, centrifuge at 1000rpm for 5min, and discard the supernatant.

2) Re-suspend the cells with 1x PBS pre-cooled at 4 °C, centrifuge at 1000rpm for 3min, and discard the supernatant. Repeat.

3) Add the corresponding volume of lysis buffer according to the number of cells, and place on the ice for 10-20min after repeated blowing.

Note: Generally, 1mL of lysis buffer can process about 0.5–1 x10^7 cells. To avoid degradation of target protein, you may add protease inhibitor.

4) Treat cell lysate with ultrasonic crusher until cell lysate is clear and no longer viscous. After 30 min on ice, centrifuge at 12000 rpm for 10 min at 4 °C. The supernatant is taken as the protein sample. It is recommended to proceed to the next step of the experiment immediately. If time does not allow, store the protein sample at -80 ℃.

5) If the target protein is secreted and expressed, the above treatment is not required, the supernatant of the medium can be directly collected and the

following steps can be performed after concentration. If the target protein content is high, it is recommended to dilute the sample with 1xPBS until the final target protein concentration is 10-100μg/mL.

2. Antibody incubation with magnetic beads

1) Preparation of Protein A/G magnetic beads:

Fully suspend the magnetic beads, take 40 μL magnetic beads suspension (including 10 μL magnetic bead), put it in the centrifuge tube, add 500 μL 1x PBS, fully suspended the suspension and place on the magnetic rack for magnetic separation, discard the supernatant; Repeat this washing step twice.

2) Antibody preparation: According to the IP dilution ratio recommended in the antibody manual, dilute the antibody with 1x PBS to prepare an antibody working solution or adjust the total volume of antibody to 500 μL. Set aside on ice.

3) Add the diluted antibody to the pre washed magnetic beads, mix gently and evenly, and incubate on the shaking table at room temperature for 30min.

4) Magnetic separation, transfer the supernatant to a new centrifugal tube for subsequent use.

5) Add 500 μL 1x PBS to the magnetic beads, gently mix, clean the magnetic beads, magnetic separation, and discard the supernatant. Repeat four times. Get antibody magnetic bead complex.

3. Binding of target protein to the magnetic bead complex

1) Incubation: Add 400 μL prepared sample to the antibody magnetic bead complex and incubate at room temperature for 30min on the shaking table, or at 4°C for 2h or longer.

2) After incubation, perform magnetic separation, and take the supernatant into a new centrifuge tube for subsequent use.

3) Add 500 μL 1x PBST, gently mix, clean magnetic beads, perform magnetic separation, and discard the supernatant. Repeat four times.

4. Target protein elution

This manual provides the following two target protein elution schemes. Please select different target protein elution methods according to the needs of later detection.

1) Denaturation elution method: the sample eluted by this method is applicable to SDS-PAGE detection.

   Procedure: Separate the magnetic beads, discard the supernatant, and add 40 μL 1x PBS and 10 μL 5x loading buffer to the magnetic beads, mix evenly, boil the sample at 95°C for 5 min. Separate magnetic beads, collect supernatant and conduct SDS-PAGE detection.

2) Acid elution method: The target protein eluted by this method can be used for later functional analysis.

   Procedure: Separate the magnetic beads, discard the supernatant, add 50 ~ 100 μL acid elution buffer to the magnetic beads and incubate for 10 minutes at room temperature on the shaking table; separate the magnetic beads, collect the supernatant into a new centrifuge tube, and immediately add 10×PBS 1/10th of the total volume of the supernatant for neutralization, adjust the pH of the eluted product to neutral, and the sample can be used for later functional analysis.

Annex

Binding affinity of Protein A/G to IgG of each species

Human	Total IgG	+++++
	IgG1	+++++
	IgG2	+++++
	IgG3	+++++
	IgG4	+++++
	IgM	+
	IgD	-
	IgA	+
	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	+++++
Shhep	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	+++++
Chicken	Total IgY	-
Monkey	Total IgG	+++++

Declaration

1. This product is limited to the scientific use of a professional.
2. Please pay attention to safety matters, and follow the laboratory reagent operation specification.
3. The lysis buffer provided by the kit is a formula repeatedly optimized for a long time and verified by a large number of experiments. When treating cells, it is recommended to use the lysis buffer matching with this kit, and the lysis buffer provided by other manufacturers may affect protein co-precipitation or the results of subsequent IP experiments.