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

Elabscience®IP/CoIP Kit (Agarose)

Product code：EA-IP-K007

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 of high quality Protein A/G protein covalently bound with agarose gel and can be used for immunoprecipitation (IP) and co-immunoprecipitation (Co-IP). This product has the characteristics of high binding capacity of protein, fast and convenient operation, strong specificity, low non-specific adsorption and wide binding range.

Performance Index

1. Scope of application

Immunoprecipitation (co-precipitation) of IgG proteins (including most IgG subtypes) from samples of cell lysates, cell secretory supernatants, serums, animal ascites, etc. (See Annex)

2. Binding properties

High purity recombinant Protein A/G protein.

3. Gel properties

Agarose gel granules, average size 50μm.

4. Binding capacity

1mL Sepharose 4B agarose granules are covalently bound to 20mg recombinant Protein A/G protein.

5. Components

0.5 mL of Protein A/G agarose gel stored in 1.5 mL PBS containing preservatives and 50% glycerol.

Product Components

Cat. No.	Component	Code	Specification	Storage
E-IR-IP004	Lysis buffer	L1	30 mL	4°C, 12 months
E-IR-IP001	Centrifugal column	C	50 piece	Room temperature, 12 months
EA-IP-007	Protein A/G Affinity Agarose	G1	2 mL	-20°C, 12 months
E-IR-IP006	Acid elution buffer	E3	1 mL	4°C, 12 months
E-BC-R187	PBS Buffer, pH7.4 (10×)	P10	50 mL	4°C, 12 months
E-IR-R310	PBST Buffer, pH7.4 (10×)	P10T	50 mL	4°C, 12 months
Manual	one copy			

Matters Needing Attention

1. Transportation and storage

The kit is shipped under refrigerated conditions.

After receiving the goods, please remove the purification column **C** and store it at room temperature. The gel should be stored at -20°C.The rest of the kit components are stored at 4°C.

2. Suggestions on reagent use

P10 (PBS Buffer, pH 7.4 (10×)) and **P10T** (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 gel

Do not freeze or dry the gel, do not use sonication for the gel, and acid treatment time of the gel should not exceed 10min.

4. Acid eluent selection

Literature shows that the Arginine-HCL eluent with pH 3.0 provided in this kit can reduce protein denaturation and extend the service life of affinity gels compared to the traditional Glycine-HCL eluent. You can also choose your own acid eluent according to the actual situation.

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

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

Self-Prepared Reagent

1. 1× PBS

Dilute **P10** (PBS Buffer, pH 7.4 (10×)) with deionized water in a ratio of 9:1 for use. For example, add 1mL of **P10** into 9mL of deionized water and mix it to 1× PBS. Prepare when needed.

2. 1× PBST

Dilute **P10T** (PBST Buffer, pH 7.4 (10×)) with deionized water in a ratio of 9:1 for use. For example, add 9mL of deionized water to 1mL **P10T** and mix it to 1× PBST. Prepare when needed.

3. Gel preservation solution

Mix 1x PBS with glycerin at a ratio of 1:1. Prepare when needed.

Note: It is recommended to add a certain concentration of preservatives in the gel preservation solution to prevent the growth of bacteria.

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 dosage of gel suspension should be 40μL (including 10μL gel), and 20ug IgG can be bound from 15μL serum or 100μL cell supernatant. Please adjust the amount of gel 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 **L1** according to the number of cells, and place on the ice for 10-20min after repeated blowing.

Note: Generally, 1mL of cell lysis buffer can process about 0.5–1 x10^7 cells. To avoid degradation of that 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. Take out the supernatant and freeze at −80 °C.

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. Column loading and incubation

1) Preparation of Protein A/G gel

   Fully suspend gel **G1**, take 40 μL gel suspension (containing 10 μL gel), put it in the purification column, add 250 μL 1x PBS, fully suspended, centrifuged at 1000 rpm for 30 seconds, and 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 gel, mix it gently and incubate it at room temperature for 10min on the shaking table.

4) Centrifuge for 30 seconds at 1000 rpm, and take the supernatant into a new centrifuge tube for subsequent use.

5) Add 250 μL 1x PBS to gel, gently mix, wash the gel, centrifuge at 1000 rpm for 30 seconds, and discard the supernatant. Repeat four times. Get antibody gel complex.

3. Binding of target protein to the antibody gel complex

1) Incubation: Add 200 μL prepared sample to the antibody gel complex and incubate at room temperature for 10 min on the shaking table, or at 4°C for 2h or longer.

2) Centrifuge: After incubation, centrifuge for 30 seconds at 1000 rpm, and discard the supernatant. Add 250 μL 1x PBST, mix gently, wash the gel, centrifuge at 1000 rpm for 30 seconds, and discard the supernatant. Repeat four times.

4. Target protein elution

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

1) Denaturation elution method: It is applicable to SDS-PAGE detection.

   Procedure: Transfer the gel to a 1.5ml centrifuge tube, centrifuge, discard the supernatant, and add 2 μL 5x loading buffer to the gel, mix evenly, boil the sample at 95°C for 5 min. Centrifuge the gel, collect supernatant, and perform SDS-PAGE detection.

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

   Procedure: Add 100-200μL acid elution buffer **E3** to the gel, incubate at room temperature for 10 min; replace the collection tube with a new one, centrifuge at 1000 rpm for 30 seconds, collect the flow through into the new collection tube, and immediately add **P10** 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.
4. The conditions recommended in this specification are general. The user can optimize the experimental conditions and select the most appropriate experimental protocol according to the properties of different target proteins.