(This kit is for in vitro research only, not for clinical diagnosis!)

Elabscience® IP/CoIP Kit (Pro L Agarose)

IP/CoIP Kit (Pro L Agarose)

Cat #：EA-IP-K010

Product specifications：50 Tests

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

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Please refer to the outer packaging label of the kit for the specific shelf life. Please use the kit within the shelf life.

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

Background Information

This product is made of high-quality Protein L covalently conjugated to agarose gel and can be used for immunoprecipitation (IP) and co-immunoprecipitation (Co-IP). This product has the characteristics of high loading capacity, fast and convenient operation, strong specificity, low non-specific adsorption, and wide binding range.

Performance Index

1. Application Scope:

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

Note: If the experimental subject does not belong to the above-mentioned sample, please inquire whether it is within the scope of application.

2. Binding Properties:

Highly pure recombinant Protein L.

3. Gel properties:

Agarose gel particles, average particle size 100μm.

4. Loading Capacity:

1mL Sepharose 4B agarose particles, covalently coupled with 20mg recombinant Protein L.

5. Composition:

0.5mL Protein L agarose gel in 1.5mL PBS with preservative and 50% glycerol.

Kit components

Item No.	Component	Component No.	Specification	Storage
E-IR-IP004	Lysis buffer	L1	30 mL	4℃, 12 months
E-IR-IP001	Centrifugal column	C	50	Room temperature, 12 months
EA-IP-017	Protein L Affinity Agarose	G1	2 mL	-20℃, 12 months
E-IR-IP006	Acid elution buffer	E3	1 mL	4℃, 12 months
E-BC-R187	PBS Buffer, pH7.4 (10×)	P10	50 mL	4℃, 12 months
E-IR-R310	PBST Buffer,pH7.4 (10×)	P10T	50 mL	4℃, 12 months
Manual	One			

Matters Needing Attention

1. Transportation and storage:

This kit is shipped under refrigerated conditions.

After receiving the goods, please take out the purification column C and store it at room temperature; store the gel at -20℃, and store the kit and other components at 4℃.

2. Suggestions for using reagents:

P10 (PBS Buffer, pH7.4 (10×)) and P10T (PBST Buffer, pH7.4 (10×)) need to be diluted with deionized water to 1× working solution before use.

3. Recommendations for using Protein L Agarose:

Do not freeze or dry the gel, do not sonicate the gel, and do not use acid to treat

the gel for more than 10 minutes.

4. **Selection of acidic eluent:**

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

5. **Binding affinity of Protein L to IgG of various species:**

Antibodies (IgG, IgM, IgA, IgD) of various species have different binding affinities to Protein L. Please read the attachment of this instruction manual carefully before use.

Reagent Preparation

1. **1× PBS**

   Dilute P10 (PBS Buffer, pH7.4 (10×)) with deionized water at a ratio of 9:1 and set aside for use. For example: add 9 mL of deionized water to 1 mL of P10 and mix well to obtain 1× PBS. Ready for use.

2. **1× PBST**

   Dilute P10T (PBST Buffer, pH7.4 (10×)) with deionized water at a ratio of 9:1 and set aside for use. For example: add 9mL of deionized water to 1mL of P10T, and mix well to obtain 1× PBST. Ready for use.

3. **Gel preservation solution**

   Mix glycerin and 1× PBS at a ratio of 1:1 and set aside. Ready to use.

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

Instructions

NOTE: All steps should be performed on ice whenever possible to avoid degradation of the target protein. In the following steps, use 40 μL of gel suspension (containing 10 μL of gel), which can bind 20 μg of IgG from 15 μL of serum or 100 μL of cell supernatant. Please adjust the amount of gel according to the amount of antibody to be bound.

1. Cell lysate preparation

1) Cell Collection

   Suspension cells and semi-adherent cells were blown off the cell culture flask and transferred into a centrifuge tube. Centrifuge at 1000 rpm for 5 minutes and discard the supernatant.

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

2) Re-suspend the cells in 1×PBS pre-cooled to 4°C, centrifuge at 1000 rpm for 3 minutes, and discard the supernatant. Repeat once.

3) Add the corresponding volume of cell lysis buffer according to the amount of cells, pipet repeatedly and place on ice for 10-20 minutes.
   Note: Generally, 1mL of cell lysis buffer can process about 0.5~1×10^7 cells. To avoid degradation of your target protein, you can add protease inhibitors.

4) Use a sonicator to treat the cell lysate until the cell lysate is transparent and no longer viscous. After placing on ice for 30 minutes, centrifuge at 12,000 rpm and 4°C for 10 minutes. 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, there is no need for the above treatment. The culture supernatant can be collected directly, and after concentration, the following steps can be performed. If the target protein content is high, it is recommended to dilute the sample with 1×PBS to a final target protein concentration of 10-100 μg/mL.

2. Loading and incubating the column

1) Preparation of Protein L Agarose:
   Fully suspend gel G1, use a pipette tip with the end cut off or cut off to absorb 40 μL of gel suspension (containing 10 μL of gel), place it in a purification column, add 250 μL of 1×PBS, fully suspend, and centrifuge at 1000 rpm for 30 seconds. , discard the centrifuge. Repeat this washing step 2 times.
2) Antibody preparation: According to the IP dilution ratio recommended in the antibody instruction manual, dilute the antibody with 1×PBS to prepare an antibody working solution. Or adjust the total antibody volume to 500 μL. Place on ice and set aside.
3) Add the diluted antibody to the pre-washed gel, mix gently, and incubate on a shaker at room temperature for 30 minutes.
4) Centrifuge at 1000 rpm for 30 seconds and transfer the supernatant to a new centrifuge tube for subsequent use.
5) Add 250 μL 1×PBS to the gel, mix gently, wash the gel, centrifuge at 1000 rpm for 30 seconds, and discard the supernatant. Repeat four times. Obtain antibody-gel complex.

3. Target protein binds to antibody-gel complex

1) Incubation: Add 200 μL of the prepared sample to the antibody-gel complex, and incubate on a shaker at room temperature for 30 minutes. It can also be incubated at 4°C for 2 hours or longer.
2) Centrifugal separation: After incubation, centrifuge at 1000 rpm for 30

seconds and discard the centrifuge. Add 250 μL 1×PBST, mix gently, wash the gel, centrifuge at 1000 rpm for 30 seconds, and discard the centrifuge. Repeat four times.

4. Target protein elution

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

1) Denaturing elution method: The purpose of this method is to apply to SDS-PAGE detection.

   Steps: Move the gel to a 1.5ml centrifuge tube, centrifuge, discard the supernatant, add 2 μL of 5× loading buffer to the gel, mix evenly, and cook the sample at 95°C for 5 minutes. Centrifuge the gel, collect the supernatant, and perform SDS-PAGE detection.

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

   Steps: Add 100-200 μL of acidic eluent E3 to the gel and incubate at room temperature for 10 minutes; replace with a new collection tube, centrifuge at 1000 rpm for 30 seconds, collect the centrifuged liquid into a new collection tube, and immediately drop 1/10 of the total volume. Neutralize with P10, adjust the pH of the elution product to neutral, and the sample can be used for later functional analysis.

Annex

Protein L Binding affinity 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	-
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	/
Chicken	Total IgY	-
Monkey	Total IgG	/

"+" indicates the relative strength of affinity, "-" indicates no affinity, and "/" indicates that there is no reliable data for this item.

Declaration

1. This product is limited to scientific research by professionals.
2. Please pay attention to safety precautions and comply with laboratory reagent operating specifications.
3. The lysis buffer provided by this kit is a formula that has been repeatedly optimized over a long period of time and has been verified by a large number of experiments. When processing cells, it is recommended to use the lysis buffer provided by this kit. Lysis buffers provided by other manufacturers may affect the results of protein co-precipitation or subsequent IP experiments.
4. The conditions recommended in this manual are universal. Users can optimize experimental conditions and choose the most appropriate experimental plan based on the properties of different target proteins.