Protein A/G Affinity Agarose

Cat. No: EA-IP-007 **Size: 2 mL**

Note: *Do not centrifuge and use after mixing gently.*

Performance metrics

Scope of application	Immuno (co) precipitation of IgG proteins from cell lysate, supernatant of cell secretory fluid, serum, animal ascites and other samples of multiple species, covering most IgG subtypes.
Antibody properties	High purity recombinant Protein A/G protein.
Gel properties	Agarose gel granules, average size 100~200 μm.
Binding capacity	1mL Sepharose 4B agarose granules are covalently coupled with 20mg recombinant Protein A/G protein.
Components	0.5mL Protein A/G agrose gel, stored in 1.5mL PBS containing preservative and 50% glycerol.

Matters Needing Attention

1.The product can be stored for 1 year at - 2 0 °C and transported under refrigerated conditions.

2.This product is only used for scientific research by professionals and cannot be used for clinical diagnosis or treatment.

3.For your safety and health, please wear lab clothes and disposable gloves.

4.This product provides affinity gel in the form of gel suspension. The content of affinity gel in gel suspension is 25%. Before use, gently resuspend the gel suspension, and then take it as required.

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

6.Do not dry t h e gel, and do not use ultrasonic treatment of gel. Do not allow the acid treatment time of gel to exceed 10min.

7.Related reagents for supporting use shall be prepared by the laboratory itself.

Method of Application

Note: All steps shall be carried out on ice as far as possible to avoid degradation of target protein. In the following steps, the dosage of gel suspension is 40μL (including 10μL gel), and 20μg 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 target protein sample

1) Treatment of serum and secreted target protein samples

Collect serum or supernatant of culture medium, and detect the concentration of target protein. If the target protein concentration is high, it is recommended to dilute with 1xPBS until the final protein concentration is 10-100 μg/mL for subsequent experiments.

2) Preparation of cell lysate

a) 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.

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

c) Add the corresponding volume of cell lysate according to the amount of cells, and place it on the ice for 10-20 min 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.

d) 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 for subsequent experiments.

2. Column installation and incubation

1) Protein A/G gel preparation

Fully suspend the gel, take 40 μL gel suspension (containing 10 μL gel) in a centrifuge tube, add 250 μL1xPBS, fully suspend, centrifuge 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 1xPBS to prepare an antibody working solution. Or adjust the total volume of antibody to 500 μL. Put it on ice for later use.

3) Add the diluted antibody to the pre-washed gel, mix it gently and incubate it at room temperature for 30 min 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 1xPBS to the gel, mix gently, wash the gel, centrifuge at 1000 rpm for 30 seconds, and discard the supernatant. Repeat this step four times. Get antibody gel complex.

3. Binding of target protein to the antibody gel complex

1) Incubation: Add 200 μL prepared sample（see step **1**） to the antibody gel complex and incubate at room temperature for 30 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, clean 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 different target protein elution methods 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 20μL 1xPBS and 5μL 5x loading buffer into 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 eluent to the gel, incubate at room temperature for 10 min; replace the collection pipe with a new one, centrifuge at 1000 rpm for 30 seconds, collect the supernatant into the new collection pipe, and immediately add 10×PBST Buffer of 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.

Background

This product is made by covalently coupling high-quality Protein A/G protein 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 and wide combination range.

Storage

-20°C for 12 months.

Annex

Protein A/G Affinity to IgG binding of various 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	+++++