Protein A/G Magnetic Beads

Catalog Number: EA-IP-007M

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

Performance metrics

Scope of application	Immuno (co) precipitation of IgG proteins from multiple species of cell lysate, supernatant of cell secretion, serum, animal ascites and other samples, covering most IgG subtypes.
Binding properties	High purity recombinant Protein A/G protein.
Magnetic beads properties	Agarose coated superparamagnetic beads with an average particle size of 3 μm.
Binding capacity	1mL superparamagnetic beads are covalently coupled with 20mg recombinant Protein A/G protein.
Components	0.25mL Protein A/G immune magnetic beads were stored in 0.75mL PBS containing preservatives.

Matters Needing Attention

1.The product can be stored for 1 year at 4° 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 magnetic beads in the form of suspension. The content of magnetic beads in the suspension is 25%. Before use, warm and re-suspend the magnetic bead suspension, and then use it as required.

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

6.When mixing the magnetic beads, please use the pipette to gently blow, use soft vortex, turn upside down, shaking table mixing and other methods. Do not centrifuge and dry the magnetic beads, do not use ultrasonic treatment to treat the magnetic beads, and do not allow the acid treatment time of the magnetic beads 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 magnetic beads suspension is 40μL (including 10μL magnetic beads), and 20μg IgG can be bound from 15μL serum or 100μL cell supernatant. Please adjust the amount of magnetic beads 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) Preparation of ProteinA/G magnetic beads
Fully suspend the magnetic beads, take 40 μL magnetic bead suspension (including 10 μL magnetic beads), put it in the centrifuge tube, add 500 μL1xPBS, fully suspended, place on the magnetic frame, perform 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 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 magnetic beads, mix gently and evenly, and incubate on the shaking table at room temperature for 10min.
4) Perform magnetic separation, take the supernatant into a new centrifuge tube for subsequent use.
5) Add 500 μL 1xPBS to the magnetic beads, mix gently, clean the magnetic beads, perform magnetic separation, and discard the supernatant. Repeat this step four times. Get antibody magnetic bead complex.

3. Binding of target protein to the antibody magnetic bead complex

1) Incubation: add 400 μL prepared sample (see step **1**) to the antibody magnetic bead complex, mix gently and incubate at room temperature for 10min on the shaking table, or at 4 °C for 2h or longer.
2) After incubation, perform magnetic separation, and take supernatant into a new centrifuge tube for subsequent use.
3) Add 500 μL 1xPBST, gently mix, wash magnetic beads, perform magnetic separation, discard 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: Separate the magnetic beads, discard the supernatant, and add 20 μL 1xPBS and 5 μL 5x loading buffer solution to the magnetic bead, mix evenly, and boil the sample at 95 ℃ for 5min. perform magnetic separation, collect supernatant, perform 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, and add 50-100 μL acid eluent to the magnetic bead, incubate at room temperature for 10min; Separate the magnetic beads, collect the supernatant into a new centrifuge tube, and immediately add 10×PBST Buffer, 1/10 of the total volume 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 magnetic beads, and can be used for immunoprecipitation (IP) and 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

4°C for 12 months.

For Research Use Only

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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	+++++

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