Protein A Immunomagnetic beads

Catalog Number: EA-IP-0015M

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

Performance metrics

Scope of application	Used in experiments such as immunoprecipitation of antibody-bound target proteins or protein complexes.
Conjugating protein:	Highly pure recombinant Protein A.
Magnetic beads properties	Agarose coated superparamagnetic beads with an average particle size of 3 μm.
Binding capacity	1mL magnetic bead suspension contains approximately 20mg magnetic beads, covalently Conjugated to ⩾ 0.6mg recombinant Protein A, and can bind ⩾0.7mg IgG.
Components	0.25mL Protein A agarose gel in 0.75mL PBS with preservative and 50% glycerol.

Matters Needing Attention

1. This product is limited to scientific research by professionals and cannot be used for clinical diagnosis or treatment.
2. For your safety and health, please wear laboratory clothes and disposable gloves for operation.
3. This product is in the form of gel suspension, and the content of affinity gel is 50%. Before use, gently re-suspend the gel suspension, and then use it as required.
4. It is best to prepare and use the IP-WB sample on site to avoid affecting the experimental results.
5. Do not dry the gel, do not sonicate the gel, and do not allow the acid treatment of gel to exceed 10 minutes.
6. The amount of gel mentioned in the method is the demonstration amount prepared in small quantities, and the specific amount should be adjusted according to the actual situation.

Method of Application

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 magnetic bead suspension (containing 10 μL of magnetic beads). You can combine 20 μg of IgG from 15 μL of serum or 100 μL of cell supernatant. Please adjust the amount of magnetic beads according to the amount of antibody to be bound.

1. Sample Preparation of Target Proteins

1) Sample processing serum and recombinant proteins

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

2) Sample processing of target protein for intracellular expression

a. Blow off in case of adherent cells or take suspension cells from the cell culture flask and transfer them to a centrifuge tube, centrifuge at 1000 rpm for 5 min, and discard the supernatant.

b. Re-suspend cells in 1× PBS pre-cooled at 4 °C, centrifuge at 1,000 rpm for 3 min, and discard the supernatant. Repeat once.

c. Add the corresponding volume of cell lysate according to the amount of cells, and place on ice for 10~20 min after repeated pipetting

Note: Generally, 1mL of cell lysate can process about 0.5~1×10^7 cells. To avoid degradation of the target protein, you can add a protease inhibitor.

d. 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. Take the supernatant for subsequent experiments。

2. Column Installation and Incubation

1) Protein A Immunomagnetic beads preparation

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a. Gently re-suspend the Protein A magnetic beads, mix evenly, and add 40 μL of magnetic bead suspension (containing approximately 10 μL of magnetic beads) into a centrifuge tube.

b. Add 500 μL of 1×PBS and gently re-suspend and wash magnetic beads. After standing on the magnetic stand for 10 seconds, discard the supernatant and repeat the above steps twice.

Note: For multiple samples, the magnetic beads can be re-suspended and divided into several reaction tubes for separate reactions.

2) Binding of antibodies to Protein A magnetic beads

a. 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 and place it on ice for later use.

b. Add the diluted antibody to the pre-washed magnetic beads, mix gently, and incubate on a shaker at room temperature for 30 minutes.

c. Perform magnetic separation, transfer the supernatant to a new centrifuge tube for subsequent use.

d. Add 500 μL 1×PBS to the magnetic beads, mix gently, wash the magnetic beads, magnetically separate, and discard the supernatant. Repeat 4 times. Obtain antibody-magnetic bead complex.

3) Binding of target protein to antibody-magnetic bead complex

a. Incubation: Add 500 μL of the prepared sample to the antibody-magnetic bead 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.

b. Magnetic separation: After incubation, perform magnetic separation and discard the supernatant. Add 500 μL 1×PBST, mix gently, wash the magnetic beads, magnetically separate, and discard the supernatant. Repeat 4 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.

Denaturing elution method

This method is only suitable for SDS-PAGE detection.

a. Add 20 μL 1×PBS and 5 μL 5× loading buffer, boil the sample for 5 minutes, cool it down room temperature and centrifuge.

b. Take the supernatant and run the SDS-PAGE in preparation for subsequent Western Blot detection.

Acid elution method

Acidic elution method has low cost, short operation time, generally does not cause protein denaturation, and facilitates subsequent analysis and detection of proteins.

a. Add pre-cooled acid eluent pH 3.0, 0.5 mL or 20 times the volume of magnetic beads, to the above precipitation, suspend the magnetic beads, and incubate at room temperature for 5 minutes.

Note: Acidic environment will shorten the service life of immunomagnetic beads. The contact time between magnetic beads and acidic eluent should be shortened as much as possible. It is recommended not to exceed 10 minutes.

b. After the incubation, magnetically separate, transfer the supernatant to a new centrifuge tube, and immediately add 1/10 volume of pH 8.0 neutralizing solution and mix well.

c. Process and store proteins according to subsequent experimental needs.

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Background

Protein A immunomagnetic beads are made of high-quality recombinant Protein A covalently conjugated to magnetic beads. They can specifically bind the corresponding antibodies and are mainly used for immunoprecipitation, co-immunoprecipitation or chromatin immunoprecipitation.

Storage

4°C for 12 months.

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Annex

Protein A 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	+++
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	+++

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