ProteinIso® Protein A/G Resin

Cat. No. DP501

Storage: at 2-8°C (20% ethanol) for two years

Description

ProteinIso® Protein A/G Resin is an affinity chromatography medium with recombinant protein A/G ligands immobilized to cross-linked agarose support, which can efficiently and specifically bind to the Fc regions of immunoglobulins. It combines the immunoglobulin binding profiles of both Protein A and Protein G and shows better binding capacities for immunoglobulins from different sources and subclasses. *ProteinIso*® Protein A/G Resin is suitable for the isolation of immune complexes, such as IP, Co-IP, etc.

Resin Specifications

Support	4% cross-linked agarose
Ligand	Protein A/G
Shape	Sphere
Average particle size	90 μm (45-165)
Ligand density	5 mg Protein A/G/ml wet gel
Dynamic binding capacity	35-40 mg h-IgG/ml wet gel
Maximum Linear Velocity (25°C)	300 cm/h
Recommended Linear Velocity	<150 cm/h
Maximum pressure	0.3 Mpa
pH stability	3~10

Procedures

Immunoprecipitation (IP) procedures (e.g. 2-5×10^7 cells):

1. Wash the cells with 3-5 ml of pre-cooled PBS, and discard the supernatant. Repeat this step twice.
2. Add 1 ml of pre-cooled IP lysis buffer (e.g. 25 mM Tris-HCl, 150 mM NaCl, 1% NP-40, 1 mM EDTA, 1 mM PMSF, and 1 mM protease inhibitor cocktail, pH 7.4. Components of IP lysis buffer vary with the proteins to be tested.). Incubate on ice for 20 minutes. Transfer the lysate to a 1.5 ml microcentrifuge tube and centrifuge at 14,000 ×g for 10 minutes at 2-8°C. Transfer the supernatant to a new tube.
3. Pretreatment (optional): Add 20 μl of *ProteinIso*® Protein A/G Resin to the supernatant (*ProteinIso*® Protein A/G Resin should be mixed thoroughly before use. Then carefully pipette 20 μl and resuspended in 500 μl IP lysis buffer. Centrifuge at 1000 ×g for 5 minutes at 2-8°C, and discard the supernatant. Repeat this step three times.). Mix and incubate at 2-8°C for 30-60 minutes. Centrifuge at 1000 ×g for 5 minutes at 2-8°C. Transfer the supernatant to a new tube.
4. Add 0.5-2 μg of antibodies targeting the proteins to the pre-cleared supernatant. Incubate for 2-4 hours or overnight at 2-8°C.
5. Add 20-50 μl of *ProteinIso*® Protein A/G Resin. Mix and incubate for 1-2 hours or overnight at 2-8°C.
6. Centrifuge at 1,000 ×g for 5 minutes at 2-8°C. Discard the supernatant.
7. Wash the resin with 500 μl of pre-cooled IP lysis buffer. Centrifuge at 1000×g for 5 minutes at 2-8°C. Discard the supernatant as thoroughly as possible. Repeat this step three times.
8. Add 1× protein loading buffer to resuspend the resin. Boil for 5 minutes. Detect the protein by Western Blot.

Comparison between Protein A/G, Protein A, and Protein G

Agarose affinity medium immobilized with Protein A and G can both be used in immunoprecipitation. The affinity of Protein A and protein G for immunoglobulins varies with different sources and subclasses, while Protein A/G has better binding capacities for immunoglobulins from different sources and subclasses. The following table compares the IgG binding capacities of protein A, G, and A/G for reference.

Affinity of Protein A/G for IgG Types

Sources	IgG Subclasses	Affinity for Protein A	Affinity for Protein G	Affinity of Protein A/G
Human	IgG_1	++++	++++	++++
	IgG_2	++++	++++	++++
	IgG_3	-	++++	++++
	IgG_4	++++	++++	++++
Mouse	IgG_1	+	++++	++++
	IgG_{2a}	++++	++++	++++
	IgG_{2b}	+++	+++	+++
	IgG_3	++	+++	+++
Rabbit	IgG	++++	+++	++++
Goat	IgG	-	++	++
Horse	IgG	++	++++	++++
Gog	IgG	++	+	++
Bovine	IgG	++	++++	++++
Porcine	IgG	+++	+++	+++
Monkey	IgG	++++	++++	++++

Notes

In order to avoid cross-contamination, it is not recommended to reuse the resin for IP and Co-IP experiments.

FOR RESEARCH USE ONLY