ProteinIso® Protein A Resin

Please read the manual carefully before use.
Cat. No. DP301
Version No. Version 1.1
Storage: at 2-8°C (20% ethanol) for two years

Description

ProteinIso® Protein A Resin is an affinity chromatography medium with Protein A as the ligand and agarose as the matrix, which can efficiently and specifically combine with the Fc segment of antibody for the separation and purification of monoclonal antibody and polyclonal antibody. *ProteinIso*® ProteinA Resin is also suitable for the separation of immunology complex, such as IP and Co-IP, because only Fc segment of antibody is involved in binding Protein A, while Fab segment can still bind antigen.

Resin Specifications

Parameter	Standard
Resin	Cross-linked 4% agarose
Ligand	r-Protein A
Shape	Sphere
Pore size	90 μm (45-165)
Ligand density	6 mg Protein A/ml wet gel
Dynamic binding capacity	40-50 mg h-IgG /ml wet gel
Maximum flow rate(25°C)	300 cm/h
Recommended flow rate	<150 cm/h
Highest resistance of atmospheric pressure	0.3 Mpa
pH stability	3~10

Procedures

A. Procedures of antibody purification

The procedures of antibody separation and purification with *ProteinIso*® Protein A Resin usually include: packing column, balance, sample loading, washing, elution and regeneration.

1. Packing column: Suspension medium, add appropriate amount of medium into the chromatographic column according to the amount of protein to be purified, and leave it standing.
2. Balance: Balance the chromatographic column with 5-10 times the volume of the equilibrium buffer (20 mMPB, 0.15 MKCl, pH17.0) until the outflow conductance and pH remain unchanged (consistent with the equilibrium buffer).
3. Sample loading: The sample buffer shall be consistent with the balance buffer as far as possible. To avoid clogging the chromatographic column, the sample should be centrifuged or microfiltered (0.45 μm).
4. Washing: After sample loading, wash the chromatographic column with a balanced buffer of 5-10 times column volume, and collect the effluent.
5. Elution: Elution with elution buffer (20 mM citric acid, pH 3.0-4.0; or 0.1 M glycine, pH 3.0; or 20 mM sodium acetate, pH 3.0-4.0), and collect the effluent. The specific elution conditions are closely related to the binding strength and stability of the antibody, and the elution buffer should be optimized if necessary. After elution, neutralize the collected antibodies with an alkaline buffer (e.g. 1 mtris-HC1, pH 9.0) immediately.
6. Regeneration: After the medium is used for several times (the specific times are related to the type and source of raw materials and experimental requirements), the combining ability will be reduced, and the medium needs to be regenerated.

(1) Wash the column/resin with 3~5 times column volume of 0.1 M citric acid or 0.1 M citric acid /20% ethanol and then immediately balance with 5 times column volume neutral PBS buffer to neutral.

(2) Or wash with 3~5 times column volume of 0.05 M NaOH/1 M NaCl or 6 M GuHCl, wash with 3~5 times column pure water, and then immediately balance with 5 times column volume neutral PBS buffer to neutral.

B. Procedures of immunoprecipitation (IP) (2-5×10^7 cells to be tested as an example)

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 Resin or *ProteinIso*® Protein G Resin to the supernatant (Before use, *ProteinIso*® Protein A Resin or *ProteinIso*® Protein G Resin should be mixed thoroughly. Then carefully pipette 20 µl and resuspend in 500 µl IP lysis buffer. Centrifuge at 500 ×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 500 ×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 Resin or *ProteinIso*® Protein G Resin. Mix and incubate for 1-2 hours or overnight at 2-8 °C.
6. Centrifuge at 500 ×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 500×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. Boil for 5 minutes. Detect the protein by Western Blot.

Protein A and Protein G Selection Guide

Agarose affinity medium immobilized with Protein A and G can both be used in antibody purification . The affinity of Protein A and protein G for immunoglobulins varies with different sources and subclasses. The following table compares the IgG binding capacities of protein A and G for reference.
It should be noted that the strength of antibody binding ability does not directly reflect the quality of antibody purification effect.

Sources	IgG Subtype	Affinity for Protein A	Affinity for Protein 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	++	++++
Dog	IgG	++	+
Bovine	IgG	++	++++
Porcine	IgG	+++	+++
Monkey	IgG	++++	++++

Notes

- Samples (especially bacterial lysates) should be filtrated with 0.45 µm filter before loading.
- After repeated use of the medium, the change of its binding ability is related to the purified sample. To avoid cross-contamination, it is not recommended to use the same medium to purify different antibodies.

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