Protein A/G Antibody Purification Kit
PRODUCT DATA SHEET

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Description

The Protein A/G Antibody purification magnetic bead series utilizes Protein coupling technology to covalently package Protein A/G onto the surface of NHS-activated superparamagnetic microspheres. Compared with similar products in the current international immuno-magnetic bead market, the product has higher antibody binding capacity and lower non-specific protein adsorption rate, and the elution conditions are more uniform. One step purification can separate the antibody with a purity greater than 90% from the serum sample. This product is a nano-scale magnetic microsphere with a large specific surface area, which can greatly shorten the time required for antibody adsorption. Skilled operation can complete the antibody adsorption process within 15 min and the antibody purification process within 30 min. Antibody purification magnetic bead kit

It is equipped with optimized prefabricated buffer, which provides the best reaction conditions for antibody purification experiment and enhances the stability of antibody purification experiment.

This product can be reused, suitable for antibody purification in plasma, ascites, tissue culture supernatant and other samples, and can also be used for antibody fixation and other related studies. Users can select the type of magnetic beads according to the species source and subtype of the target antibody, and the affinity between Protein A/G magnetic beads and different antibodies is shown in Table 1.

For custom sizes, formulations or bulk quantities please contact our customer service department.

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Characteristics

Antibody purification magnetic beads Protein A/G for Antibody Purification ①	1 mL
Antibody binding buffer ②	100 mL
Antibody Elution Buffer I (pH4.5) ③	50 mL
Antibody Elution Buffer II (pH2.0) ④	50 mL
Antibody Neutrilization buffer ⑤	10 mL

Beads Washing Buffer ⑥	10 mL
Beads Storage Buffer ⑦	10 mL
Beads Regeneration Buffer ⑧	10 mL
Storage condition	Store at 2~8°C
Shelf life	2 years

Note: Ability of magnetic beads to bind Human IgG (Antibody Capacity) is 1.8 ~ 2.0 mg/mL

Advantages

1. It has high antibody binding capacity and ultra-low non-specific adsorption performance
2. The operation is time-saving, simple and gentle
3. High product stability
4. The antibody eluting system is more neutral
5. Very low Protein A ligand shedding rate
6. Reusable

Operation process

Different kinds of immunomagnetic beads have different antibody binding abilities. Before performing antibody purification operation, the operator should first estimate the antibody content in the sample to be purified (the antibody content in the general serum sample is about 2~8 mg/mL, and the antibody concentration in cell culture varies greatly due to different expression levels). Then, according to the Antibody binding Capacity of the selected immunomagnetic beads (see the Antibody Capacity data of immunomagnetic beads related products or product components), the approximate dosage of immunomagnetic beads is calculated. Too much or too little immunomagnetic beads will affect the effect of antibody purification. It is recommended that the maximum load of the dosage of immunomagnetic beads should be 80% to 100% of the antibody content of the sample.

The following steps are described in detail as an example of purifying magnetic beads with 100 µL Protein A antibody. The operator can refer to the dosage of various buffers and the size of containers in the following steps to adjust the dosage of each component in the actual operation.

1. Sample treatment: Take a sample with an antibody content of about 0.1-0.15 mg and place it in a new 1.5 mL EP tube, add the antibody binding buffer (②) until the total volume is 500 µL (if the sample volume is greater than 500 µL, there is no need to add it), and mix it evenly.

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2. Magnetic bead pretreatment: The antibody purified magnetic bead (①) swirls for 30 s, so that the magnetic bead is fully suspended; The 100 μL magnetic bead suspension was placed in another new 1.5 mL EP tube. Magnetic separation of the magnetic bead suspension (EP tube is placed on the magnetic separator), so that the magnetic bead is adsorbed on the tube wall until the solution is clarified; The operation description is omitted below, discard the supernatant. Remove EP tube from magnetic separator. Wash with antibody binding buffer (②) twice, discard the supernatant, and the magnetic beads in the tube can be directly used for antibody separation.

3. Antibody adsorption: Add the sample solution treated in Step 1 to the pre-treated magnetic bead tube in Step 2, swirl and oscillate evenly, place the sample in the turnover mixer at room temperature (about 25°C) or gently flip the EP tube manually, so as to make the sample and magnetic bead fully contact and adsorb, flip for about 15 minutes, magnetic separation is carried out, and the superserum is removed.

4. Magnetic bead washing: Add 1 mL of antibody-binding buffer (②) to the EP tube, oscillate and re-suspend the magnetic bead for magnetic separation, and remove the supernatant; Repeat this operation three times.

5. Antibody elution (Method 1- high salt weak acid elution):

1) Antibody elution: Add 0.5~1.0 mL antibody eluting buffer (③) into the EP tube that has been washed with magnetic beads in Step 4, blow with a pipette or quickly re-suspend under vortex oscillation, and then place the EP tube in the turnover mixer at room temperature (about 25°C) or gently flip the EP tube manually, and magnetic separation is performed after 10 min of turnover. Collect the supernatant to the new EP tube;

2) Antibody dialysis: Because the antibody eluting buffer (③) contains relatively high concentration of salt, the collected antibody solution cannot be directly used for SDSPAGE detection, but the antibody concentration can be determined by zeroing the antibody eluting buffer. The antibody eluting buffer is slightly acidic (pH 4.5), it is recommended that the operator immediately dialysis the collected antibody solution with a neutral low-salt solution (that is, dialysate) prepared by himself, in order to reduce the antibody inactivation rate and obtain a more active and stable antibody solution.

6. Antibody elution (Method 2- low pH elution):

1) Antibody elution: Add 0.5-1.0 mL antibody eluting buffer (④) into the EP tube that has been washed with magnetic beads in Step 4, blow with a pipette or quickly re-suspend under vortex oscillation, and then place the EP tube in the turnover mixer at room temperature (about 25°C) or

gently flip the EP tube manually, and magnetic separation is performed after 10 min of turnover. Collect the supernatant to the new EP tube;

2) Antibody neutralization: Due to the low pH value of the antibody eluting buffer (④), the collected antibody solution needs to be immediately added to a certain amount of antibody neutralizing buffer (⑤), generally 1/20 to 1/10 of the volume of the collected antibody solution, and ultimately the pH value of the eluted antibody solution is maintained in a neutral environment, so as to reduce the antibody inactivation rate and obtain a more active and stable antibody solution. The collected antibodies can be directly used for SDSPAGE detection and antibody concentration determination.

7. Magnetic bead post-treatment: The magnetic bead after use is re-suspended with magnetic bead washing buffer (⑥), and then magnetic separation is performed, the supernatant is abandoned, and the operation is repeated once; Add 100 μL magnetic bead storage buffer (7) and store at 2~8°C.

Magnetic bead regeneration

1. After repeated use of magnetic beads, precipitated proteins, strong hydrophobic proteins, lipoproteins and other impurities will be non-specific adsorbed to the magnetic beads. In order to ensure the use efficiency of magnetic beads, it is recommended to carry out magnetic bead regeneration treatment after continuous use for 5 times.

2. Add the regeneration buffer of magnetic beads (⑧) at a ratio of about 1 mL magnetic beads to 5 mL, shake evenly, place in the turnover mixer at room temperature or gently flip the mixture manually, magnetic separation is carried out after 10 min, and the superliquid is discarded.

3. Add magnetic bead washing buffer (⑥) at a ratio of about 1 mL magnetic bead 5 mL for re-suspension, then perform magnetic separation, discard the superliquid, and repeat the above operation twice.

4. Add an appropriate amount of magnetic bead storage buffer (7) according to the ratio of about 1 mL magnetic bead 1 mL, and store it at 2~8°C.

Notes

1. Before performing antibody purification, please read this operation manual carefully.

2. This product must be used with a magnetic separator.

3. Magnetic beads should be fully oscillated and uniform before use.

4. Magnetic beads should be kept in storage solution to prevent drying.

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5. Do not freeze or centrifuge the magnetic beads to avoid irreversible aggregation.

6. This product is for research use only.

Frequently Asked Questions and Answers (FAQ)

Q1: How to improve the binding efficiency of antibody and magnetic beads?

A1: The binding efficiency of magnetic beads and antibodies is related to the species source and subtype of antibodies. Please confirm the affinity efficiency of the antibody type and Protein A ligand (Table 1). If the affinity between the antibody subtype and Protein A is low, The affinity efficiency can be improved by increasing the incubation time of the antibody with the magnetic beads (30-120 min), increasing the pH value of the binding buffer (8-9) and reducing the ionic strength (25-100 mM NaCl), or selecting ligands with higher affinity with the target antibody (such as Protein A/G).

Q2: How to improve the efficiency of antibody elution?

A2: The antibody and Protein A ligand affinity is too high, resulting in low antibody elution efficiency, you can reduce the pH value of the elution buffer (1.9~2.5), increase the ionic strength of the elution buffer (2~3 M $MgCl_2$ can be selected) or prolong the elution time, improve the elution efficiency of the antibody. However, it should be noted that antibodies are easy to form aggregates under low pH conditions, and the elution products of antibodies should be adjusted to neutral pH immediately with alkaline buffers (such as Tris, HEPES, etc.).

Q3: How to avoid the accumulation of magnetic beads that may occur during storage or use?

A3: Magnetic beads should be kept at 2~8°C, and irreversible aggregation caused by pollution or aggregation caused by drying should be avoided when used. The aggregation of magnetic beads in low pH eluting buffer is a normal phenomenon and does not affect the normal use of magnetic beads. The addition of a non-ionic detergent (such as NP-40, Tween-20, or Triton X-100) with a final concentration of 0.1% (V/V) to the Binding Buffer and the Elution Buffer effectively prevents the accumulation of magnetic beads. After low pH elution, the magnetic beads can be washed with binding Buffer to neutral, and then the magnetic beads can be oscillated with Tris Buffer (pH 7.5) containing 0.1% (V/V) Tweene-20 and treated with ultrasonic water bath for 2 min to restore the uniform state of the magnetic beads. The above treatments did not affect the antibody binding efficiency of magnetic beads.

Q4: How to solve the phenomenon that magnetic beads are easy to adhere to the tube wall?

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A4: It is recommended to use consumables with low adsorption rate for magnetic bead operation. In addition, the addition of 0.01%~0.1% (V/V) of non-ionic detergent (such as NP-40, Tween-20 or Triton X-100) to the buffer can effectively reduce the adhesion of magnetic beads to consumables.

Q5: Does the magnetic bead caking occur during use?

A5: If the phenomenon of agglomeration occurs when the magnetic beads are used, it is generally difficult to oscillate and break up, which is easy to lead to uneven distribution. The reason for this problem is that the magnetic beads are placed in the magnetic field for too long and the magnetic beads are firmly combined together. The magnetic beads can be dispersed by using ultrasonic water bath for 2 min to re-disperse them, but it should be noted that ultrasonic treatment will also make the antibodies captured by the magnetic beads in the sample solution fall off, so the magnetic beads should not be used before elution after sample addition.

Table 1: Affinity of immunomagnetic beads Protein A/G with different sources and types of antibodies

Pecies	Antibody Classs	Protein A
Human	Total IgG	+++++
	IgG_1, IgG_2	+++++
	IgG_3	+++++
	IgG_4	+++++
	IgM	-
	IgD	-
	IgA	+
	IgA_1, IgA_2	+
	IgE	+++
	Fab	-
	ScFv	-
Mouse	Total IgG	+++++
	IgM	-
	IgG_1	+++
	IgG_{2a}	+++

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	IgG_{2b}	+++
	IgG_3	+++
Rat	Total IgG	+++
	IgG_1	+++
	IgG_{2a}	+++++
	IgG_{2b}	+
	IgG_{2c}	+++++
Cow	Total IgG	+++++
	IgG_1	+++++
	IgG_2	+++++
Goat	Total IgG	+++++
	IgG_1	+++++
	IgG_2	+++++
Sheep	Total IgG	+++++
	IgG_1	+++++
	IgG_2	+++++
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	+++++
Monkey	Total IgG	+++++
Chicken	Total IgY	-

Note: “+” = weak binding, “+++” = medium binding, “+++++” = strong binding, “-” = no binding

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