Beads Protein A（or A/G)Antibody Purification Kit

Cat： M2370
Specification： 1ml/5ml
Storage： Store at 2-8℃, and it is valid for 1 year.

Introduction：

The BeadsProtein A (orA / G) antibody purified magnetic bead series uses protein coupling technology to covalently wrap the Protein A (orA / G) to the surface of a superparamagnetic microsphere activated by the NHS. The product has higher antibody binding ability and lower non-specific adsorption rate of protein, the elution conditions are more uniform, and antibodies with more than 90% purity from serum samples can be isolated by purification in a one step.

This product is a nanoscale magnetic microsphere, with a large specific surface area, which can greatly shorten the time required for antibody adsorption. killed operation can complete the antibody adsorption process within 15 min and complete the antibody purification process within 30 min. The antibody purification magnetic bead kit with optimized precast buffer provided the optimal reaction conditions for antibody purification experiments and enhances the stability of the antibody purification experiments.

Product Characteristics：

product name	Beads Protein A（or A/G）Antibody
Antibody-purified magnetic beads Protein A (orA / 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

Note：The magnetic bead binding Human IgG capability (Antibody Capacity) is: Protein A: 1.4 ~ 1.6 mg/mL; Protein A / G: 1.8~2.0 mg / mL.

Application ranges

It is 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.

Operation steps (for reference) :

Different classes of immunomagnetic beads have different antibody-binding capabilities,

Before performing the antibody purification operation, The operator shall first estimate the antibody content in the sample to be purified (the antibody content in the general serum samples is about 2~8 mg / mL, The antibody concentration in the cell cultures varies greatly due to different expression levels); Then, according to the antibody binding capacity of the selected immunomagnetic beads (see the list of immunomagnetic beads related products or the Antibody Capacity data of product composition), the approximate amount of immunomagnetic beads was calculated. Too much or too little amount of immunomagnetic beads affects the effect of antibody purification. It is suggested that the maximum load of immunomagnetic beads should be 80 % ~ 100 % of the sample antibody content.

The following procedure follows purified antibody and magnetic beads with 100 μL Protein A antibody. The operator may adjust the amount of each component in the actual operation in proportion according to the amount of various buffers and the container size in the following steps.

1、**Sample processing**: Place samples containing about 0.1 to 0.15 mg in a new 1.5 mL EP tube, add antibody binding buffer (②) to a total volume of 500 μ L (If the sample volume is greater than 500 μL, there is no need to add) and mix well.

2、**Magnetic bead pretreatment:** vortex antibody purified magnetic bead (①) for 30s to resuspend the beads; place 100 μ L magnetic bead suspension in another 1.5 mL EP tube. The magnetic bead suspension is magnetically separated (place the EP tube on the magnetic separator) for the magnetic bead adsorption to the tube wall until the solution is clarified; this operation description is omitted below and the supernatant is discarded. Remove the EP tube from the magnetic separator. Washed twice with antibody binding buffer (②), discard the supernatant, and the magnetic beads in the tube can be directly used for antibody separation).

3、**Antibody adsorption:** Add the sample solution processed in step 1 to the pretreatment magnetic bead tube in step 2, vortex oscillation evenly, at room temperature (about 25℃) in the flip mixing instrument or manually gently flip the EP tube, to make the sample and the magnetic bead fully contact and adsorption, flip about 15 min for magnetic separation, and discard the supernatant.

4、**Magnetic bead washing**: add 1 mL antibody binding buffer (②) to the EP tube, resuspend the magnetic beads for magnetic separation and discard the supernatant; this operation was repeated 3 times.

5、**Antibody elution (Method 1-High salt weak acid elution):**

1) Antibody elution: Add 0.5~1.0 mL antibody elution buffer (③) to the EP tube of magnetic bead washing in step 4, resuspend with pipette or vortex shock, and then at room temperature (about 25℃) or manually flip the EP tube, flip 10 min after magnetic separation, collect the supernatant to a new EP tube;

2) Antibody dialysis: Because the antibody elution buffer (③) contains a high concentration of salt, the collected antibody solution cannot be directly used for SDS-PAGE detection, but, the antibody concentration can be determined by zeroing the antibody elution buffer. The antibody elution buffer is slightly acidic (pH 4.5). The operator is recommended to immediately dialysis the collected antibody solution with a self-prepared neutral low salt solution (i. e., dialysate) to reduce the antibody inactivation rate and obtain high active and stable antibody solution.

6、**Antibody elution (Method 2-low pH elution):**

1) Antibody elution: Add 0.5~1.0 mL antibody elution buffer (④) to the EP tube of magnetic bead washing in step 4, resuspend with pipette or vortex shock, and then at room temperature (about 25℃) or manually flip the EP tube, flip 10 min after magnetic separation, collect the supernatant to a new EP tube;

2) Antibody neutralization: Due to the low pH value of antibody elution buffer (④), the collected antibody solution needs to immediately add a certain amount of antibody neutralization buffer (⑤), generally collected 1 / 20~1 / 10 antibody solution volume, the pH of the eluted antibody solution remain neutral environment, to reduce the antibody inactivation rate, obtain high activity and stability of the antibody solution. The collected antibodies can be directly used for SDS-PAGE detection and the determination of antibody concentration.

7. Post-processing of magnetic beads: use the magnetic beads were resuspended with magnetic bead washing buffer (⑥), then perform magnetic separation, discard the supernatant and repeat once; add 100 μL magnetic beads storage buffer (⑦), stored at 2 ~ 8 ° C.

Magnetic bead regeneration

1. After using the magnetic beads for many times, impurities such as precipitated proteins, strong hydrophobic proteins and lipoproteins will unspecifically adsorb to the magnetic beads. In order to ensure the use efficiency of the magnetic beads, it is recommended to regenerate the magnetic beads after continuous use for 5 times.
2. Add magnetic bead regeneration buffer (⑧) according to the ratio of about 1 mL magnetic beads and 5 mL, shake evenly, put it in the flip mixing instrument or gently flip mix at room temperature, 10 min, magnetic separation, and discard the supernatant.
3. Add magnetic beads washing buffer (6) at a ratio of about 1 mL magnetic beads 5 mL for resuspension, and then perform magnetic separation and discard.
4. Add an appropriate amount of magnetic beads storage buffer (⑦) at the ratio of about 1 mL magnetic beads to 1 mL, and store it in 2~8℃.

Note

1、Be sure to read this instruction carefully before antibody purification.

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

3、The magnetic beads should be fully oscillevenly before use.

4、The magnetic beads should be stored in the storage solution to prevent drying.

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

6、This product is intended for study 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 the magnetic beads and the antibody is related to the species origin and subtype of the antibody, Please confirm the affinity efficiency of the type of antibody and the Protein A ligands (table), For example, if the affinity of the antibody subtype and Protein A is low, the affinity efficiency can be improved by increasing the incubation time of the antibody and the magnetic beads (30~120 min), increasing the pH value of the binding buffer (8~9), and reducing the ionic strength (25~100mM NaCl), Or select one with higher affinity to the target antibody (e. g., Protein G or Protein A / G).

Q2: How to improve the antibody elution efficiency?

A2: The high affinity of antibody and Protein A leads to low antibody elution efficiency. The elution efficiency of antibody can be improved by reducing the pH value of the elution buffer (1.9~2.5), increasing the ionic strength of the elution buffer (choose 2~3M MgCl2) or extending the elution time. However, it should be noted that antibodies tend to form aggregates under low pH conditions, and the antibody elution products should be immediately adjusted from pH to neutral with alkaline buffer agents (such as Tris, HEPES, etc.).

Q3: How to avoid the possible aggregation of magnetic beads during storage or use?

A3: The magnetic beads shall be stored at 2~8℃, and irreversible aggregation due to contamination or drying shall be avoided. The aggregation of magnetic beads in the elution buffer with low pH is a normal phenomenon and does not affect the normal use of magnetic beads. The addition of non-ionic detergent (e. g. NP-40, Tween-20 or Triton X-100) (0.1%) to Binding / Washing buffer and Elution buffer can effectively prevent magnetic bead aggregation. The magnetic beads with low pH elution operation can be washed to neutral with Binding / Washing buffer and Elution buffer and treated with ultrasonic water bath for 2 min to restore the magnetic beads to a uniform state. None of the above treatments can not affect the antibody binding efficiency of the magnetic beads.

Q4: How to solve the phenomenon of magnetic beads easy to adhere to the pipe wall?

A4: Recommend using low adsorption rate consumables for magnetic bead operation. In addition, the addition of 0.01% to 0.1% (v / v) non-ionic scale removal agents such as NP-40, Tween-20 or Triton X-100) to the buffer can effectively reduce the adhesion of magnetic beads to the consumables.

Q5: How to deal with the agglomeration phenomenon of magnetic beads during use?

A5: If magnetic beads clump during use, they are generally difficult to shake and disperse, which can easily lead to uneven distribution. The reason for this problem is that the magnetic beads are placed in a magnetic field for too long, causing them to firmly bond together. Ultrasonic water bath treatment for 2 minutes can break up the magnetic beads and disperse them again, but it should be noted that ultrasonic treatment can also cause the antibodies captured by the magnetic beads in the sample solution to fall off. Therefore, this method should not be used before elution of the magnetic beads after sample addition.

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

Pecies	Antibody Classs	Protein A/G	Protein A
Human	Total IgG	+++++	+++++
	IgG1，IgG2	+++++	+++++
	IgG3	+++++	+
	IgG4	+++++	+++++
	IgM	-	-
	IgD	-	-
	IgA	+	+
	IgA1，IgA2	+	+
	IgE	+++	+++
	Fab	-	-
	ScFv	-	-
Mouse	Total IgG	+++++	+++++
	IgM	-	-
	IgG1	+++	+
	IgG2a	+++	+++
	IgG2b	+++	+
	IgG3	+++	+++++

Pecies	Antibody Classs	Protein A/G	Protein A

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	+++++	+++++
Monkey	Total IgG	+++++	+++++
Chicken	Total IgY	-	-

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