Beads Magrose Protein A/ G Antibody Purification

Cat： M2380、M2390
Specification： 5ml
Storage： Store at 2-8℃, and it is valid for 2 years.

Introduction：

The Protein A / G antibody purification magnetic beads series products are composite particles formed by the covalent binding of NHS-activated superparamagnetic microspheres and Protein A / G. The product has higher antibody binding ability and lower protein non-specific adsorption rate, and the elution conditions are more uniform. Antibodies with purity greater than 90 % can be isolated from serum samples by one-step purification.

This product is a micron-sized magnetic microsphere. The antibody adsorption process can be completed within 15 min and the antibody purification process can be completed within 30 min. Users can select the type of magnetic beads according to the species source and subtype of the target antibody. The affinity comparison of Magrose Protein A and Magrose Protein G magnetic beads with different antibodies is shown in Table 1.

Product Characteristics：

product name	Magrose Protein A	Magrose Protein G
Particle size	30~150μm	30~150μm
Beads concentration	10% (v/v)	10% (v/v)
Ligand	Protein A	Protein G
The medium	Magrose	Magrose
Antibody binding ability	25~30mg Human IgG/mL Gel	25~30mg Human IgG/mL Gel
Storage temperature	2~8℃	2~8℃
Binding/ Washing buffer	PBST (pH7.2~7.4), 137mM NaCl, 2.7mM KCl,10mM Na_2HPO_4, 2.0mM KH_2PO_4, 0.1% Tween-20	
Elution buffer	100mM Gly, 0.1% Tween-20, pH 2.5	
Neutrilization buffer	1.0 M Tris-HCl, pH 9.0	
Storage buffer	PBST，0.1%(v/v) Proclin 300	

Scope of application :

It is suitable for antibody purification in plasma, ascites, tissue culture supernatant and other samples. It can also be used for antibody immobilization and other related research.

Operation steps (e.g. purified human serum IgG, for reference) :

1.Sample treatment : 100μL of human serum was taken into a 1.5 mL EP tube, followed by

adding 900μL Binding / Washing buffer and fully mixed.

2.Magnetic bead pretreatment : The antibody purified magnetic beads were vortexed for 30 s to make the magnetic beads fully re-suspended ; 200 μL of 10 % (v / v) magnetic bead suspension was placed in another new 1.5 mL EP tube. The magnetic beads suspension was magnetically separated, and the supernatant was discarded, washed twice with 1 mL Binding / Washing buffer, and magnetically separated. The magnetic beads in the tube can be directly used for antibody separation.

Note : The amount of magnetic beads in this step can be adjusted according to the maximum binding amount of the magnetic beads to the target antibody. When the concentration of the target antibody is greater than the binding amount), if the concentration of the target antibody is too low, such as less than 70μg / mL, the customer can increase the amount of magnetic beads in order to improve the antibody recovery rate, such as increasing to 3 times the amount of magnetic beads.

3.Antibody adsorption : The sample solution treated in step 1 was added to the magnetic bead tube pretreated in step 2, and the vortex oscillated evenly. At room temperature (about 25 ° C), it was placed in a flip mixer or manually gently flip the EP tube to promote the sample and the magnetic bead to fully contact and adsorb. After about 15 min of flipping, magnetic separation was performed and the supernatant was discarded.

4.Magnetic beads washing : 1 mL Binding / Washing buffer was added to the EP tube, and the magnetic separation was performed after the magnetic beads were oscillated and re-suspended, and the supernatant was removed ; the operation was repeated 3 times.

5.Antibody elution : 0.5-1.0 mL Elution buffer was added to the above-mentioned EP tube that was washed with magnetic beads, and quickly resuspended with a pipette or vortex shaker, and then placed in a flip mixer at room temperature (about 25 ° C) or manually gently flip the EP tube. After flipping for 10 min, magnetic separation was performed, and the supernatant was collected to a new EP tube.

Note : In this step, the amount of Elution buffer is recommended to the customer to control the final elution antibody concentration at 0.6 ~ 1.2mg / mL, and more than 95 % of the antibody in the first elution condition will be eluted. If the amount of Elution buffer is too small, some antibodies will remain on the magnetic beads during the first elution, resulting in a decrease in antibody recovery.

6.Antibody neutralization : A certain amount of Neutrilization buffer was added to the antibody eluent in step 5, which was generally 1/10 of the antibody elution volume. Finally, the pH value of the eluted antibody was kept in a neutral environment, which was beneficial to maintain the biological activity of the antibody and avoid antibody inactivation.

7.Magnetic beads post-treatment : The used magnetic beads were washed twice with Elution buffer, magnetically separated, and the supernatant was discarded ; then washed with Binding / Washing buffer for 3 times, magnetically separated, discarded the supernatant, added 200μL Storage buffer to re-suspend the magnetic beads, and stored 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-specifically adsorbed to the magnetic beads. In order to ensure the efficiency of the use of magnetic beads, it is recommended to use the magnetic beads for regeneration after 5 times.
2. Add 1mL 1 % (v/v) Triron X-100 magnetic beads regeneration buffer to 1mL 10 % (v / v) magnetic beads according to about 1mL 10 % (v / v) magnetic beads, oscillate evenly, and place it at room temperature. Flip the mixer or manually gently flip the mixture, 10 minutes later, magnetic separation is performed, and the supernatant is discarded.
3. Immediately add 1mL Binding / Washing buffer for resuspension, and then magnetic separation, discard the supernatant, repeat the operation for 3 times.
4. Add 1mL Storage buffer to re-suspend magnetic beads and store at 2 ~ 8 °C.

Notes

1. Before the antibody purification operation, please be sure to read this instruction carefully.
2. This product must be used with magnetic separator.
3. Before using magnetic beads, they should be fully oscillating and uniform.
4. Magnetic beads should be stored in the storage solution to prevent drying.
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 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 antibody affinity between immunomagnetic beads Protein A and Protein G and different sources and types

Species	Antibody Classs	Protein A	Protein G
Human	IgA	Variable	-
	IgD	-	-
	IgG1	++++	++++
	IgG2	++++	++++
	IgG3	-	++++
	IgG4	++++	++++
	IgM	Variable	-
Mouse	IgG1	+	++++
	IgG2a	++++	++++
	IgG2b	+++	+++
	IgG3	++	+++
	IgM	Variable	-

Species	Antibody Classs	Protein A	Protein G
Rat	IgG1	-	+
	IgG2a	-	++++
	IgG2b	-	++
	IgG3	-	++
Cow	IgG	++	++++
Goats	IgG	-	++
Sheep	IgG	-	++
Horse	IgG	++	++++
Rabbit	IgG	++++	+++
Pigs	IgG	+++	+++
Guinea pig	IgG1	++++	++
	IgG2	++++	++
Hamster	IgG	+	++
Rhesus monkey	IgG	++++	++++
Poultry egg yolk	IgY	-	-
Dog	IgG	++	+
Koala	IgG	-	+
Alpaca	IgG	-	+

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