Protein A Agarose Magnetic Particles, 10-30 µm
PRODUCT DATA SHEET

Protein A Agarose Magnetic Particles, 10-30 µm

Description

The Protein A Antibody Purification magnetic bead series is a composite particle formed by the NHS-activated superparamagnetic microsphere covalently combined with Protein A. 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 micron magnetic microsphere, which can complete the antibody adsorption process within 15 min and the antibody purification process within 30 min. 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. The affinity between Magrose Protein A magnetic beads and different antibodies is shown in Table 1.

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

Website: www.abvigen.com Phone: +1 929-202-3014 Email: info@abvigenus.com

Characteristics

Particle size	10-30 µm
Concentration	10% (v/v)
Aglycone	Protein A
Medium	Magrose
Antibody binding capacity (mg Human IgG/mL Gel)	40~45
Storage temperature and expiration date	Valid for 2 years at 2~8°C (can be stored or transported for a short time at room temperature)

1378 US-206 Ste 6-126, Skillman, NJ USA Email: info@abvigenus.com
Tel: 1-816-388- 0112 Fax: 1- 888-616-0161

Binding washing solution	PBST (pH 7.2~7.4): 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2.0 mM KH_2PO_4, 0.1% Tween-20
Eluate	100 mM Gly, 0.1% Tween-20, pH 2.0
Neutralizing solution	1.0 M Tris-HCl, pH 9.0
Preservative solution	PBS, 0.1% (v/v) Proclin 300, 0.1% (w/v) BSA

Operation process (Take purified human Serum IgG as an example)

1. Sample treatment: Take 100 µL of human serum to 1.5mL EP tube, then add 900µL combined with washing solution, and mix thoroughly.

2. Magnetic bead pretreatment: The swirl of the antibody purified magnetic bead is oscillated for 30 s, so that the magnetic bead is fully suspended; A 200 µL 10% (V/V) bead suspension was placed in another new 1.5 mL EP tube. Magnetic separation was carried out on the magnetic bead suspension, the supernatant was discarded, and the magnetic bead in the tube could be directly used for antibody separation after being washed with 1 mL combined washing solution twice.

Note: The amount of magnetic beads in this step can be adjusted according to the maximum binding amount of magnetic beads to the target antibody. When the concentration of the target antibody is greater than 150 µg /mL, the user can use 1.2~1.5 times the amount of magnetic beads (calculation method: For example, the target antibody content in 1.5* sample/the maximum binding amount of magnetic beads), if the target antibody concentration is too low, such as less than 70 µg/mL, in order to improve the antibody recovery, the customer can increase the amount of magnetic beads, such as up to 3 times the amount of magnetic beads.

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 min, magnetic separation is carried out, and the superserum is removed.

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

1378 US-206 Ste 6-126, Skillman, NJ USA Email: info@abvigenus.com
Tel: 1-816-388- 0112 Fax: 1- 888-616-0161

5. Antibody elution: Add 0.5-1.0 mL eluent into the EP tube after the magnetic bead washing, 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 turn the EP tube manually, magnetic separation is performed after 10 min of turnover, and the superserum is collected into the new EP tube.

Note: The amount of eluent in this step, it is recommended that the customer make the final eluted antibody concentration control in 0.6~1.2 mg/mL, at this time, more than 95% of the antibodies in the first elution condition will be eluted off; If the amount of eluent is too small, some antibodies will remain on the magnetic bead at the first elution, resulting in lower antibody recovery.

6. Antibody neutralization: A certain amount of neutralizing solution is added to the antibody eluent in Step 5, generally 1/10 of the eluent volume of the antibody, and the pH value of the eluted antibody is ultimately maintained in a neutral environment to help maintain the biological activity of the antibody and avoid the inactivation of the antibody.

7. Post-treatment of magnetic beads: Wash the magnetic beads with eluent twice after use, magnetic separation, discard the supernatant; Then it was washed 3 times with combined washing solution, magnetic separation, discard the supernatant, add 200 μL preservation solution to re-suspension magnetic beads, 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 1 mL of 1% (V/V) Triton X-100 magnetic bead regeneration buffer for every 1 mL 10% (V/V) magnetic bead, shake evenly, and mix in a turning mixer at room temperature or gently by hand. Magnetic separation is carried out after 10 min, and the superliquid is discarded.

3. Immediately add 1 mL of combined washing solution for re-suspension, then magnetic separation, discard the supernatant, repeat the operation for 3 times.

4. Add 1 mL preservation solution to re-suspend magnetic beads and store at 2~8°C.

Notes

1378 US-206 Ste 6-126, Skillman, NJ USA Email: info@abvigenus.com
Tel: 1-816-388- 0112 Fax: 1- 888-616-0161

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.

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 antibody and magnetic beads (30~120 min), increasing the pH value of binding buffer (8~9) and decreasing the ionic strength (25~100 mM NaCl), or selecting ligands with higher affinity with the target antibody (such as Protein G or 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 should be used to avoid irreversible aggregation due to pollution, or aggregation due to drying. 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 washing solution and eluent can effectively prevent the accumulation of magnetic beads. After low pH elution operation, the magnetic beads can be washed with binding washing solution and eluent to neutral, and treated with ultrasonic water bath for 2 min, the magnetic beads

1378 US-206 Ste 6-126, Skillman, NJ USA Email: info@abvigenus.com
Tel: 1-816-388- 0112 Fax: 1- 888-616-0161

can be restored to uniform state, and the above treatment does not affect the antibody binding efficiency of the magnetic beads.

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

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: The affinity of Protein A with antibodies of different sources and types

Pecies	Antibody Classs	Protein A binding
Human	IgA	Changeable
	IgD	-
	IgG_1	++++
	IgG_2	++++
	IgG_3	-
	IgG_4	++++
	IgM	Changeable
Mouse	IgG_1	+
	IgG_{2a}	++++
	IgG_{2b}	+++
	IgG_3	++
	IgM	Changeable
Rat	IgG_1	-

1378 US-206 Ste 6-126, Skillman, NJ USA Email: info@abvigenus.com
Tel: 1-816-388- 0112 Fax: 1- 888-616-0161

	IgG_{2a}	-
	IgG_{2b}	-
	IgG_3	-
Cow	IgG	++
Goat	IgG	-
Sheep	IgG	-
Horse	IgG	++
Rabbit	IgG	++++
Pig	IgG	+++
Guinea Pig	IgG_1	++++
	IgG_2	++++
Hamster	IgG	+
Macaque	IgG	++++
Avian yolk	IgY	-
Dog	IgG	++
Koala	IgG	-
Alpaca	IgG	-

Note: “+” = weak binding ability, “+++” = medium binding ability, “+++++” = strong binding ability, “-” = unable to bind

Ordering Information

Website: www.abvigen.com

Phone: +1 929-202-3014

Email: info@abvigenus.com

1378 US-206 Ste 6-126, Skillman, NJ USA Email: info@abvigenus.com

Tel: 1-816-388- 0112 Fax: 1- 888-616-0161