# BeaverBeads™ Magrose Protein A (or Protein G) Antibody Purification

## Product Introduction

BeaverBeadsTM Protein A(or Protein G) antibody purification beads are NHS activated superparamagnetic beads covalent binding Protein A (or Protein G). Compared with the similar products in the international market, the product has higher antibody binding capacity and lower protein non-specific adsorption rate, and the elution conditions are more homogeneous. The antibody can be separated from the serum sample by one-step purification, and the purity of the antibody is more than 90%.

This product is micron magnetic beads, skilled operation can complete the antibody adsorption process within 15 min, and complete the process of antibody purification within 30 min. This product can be used repeatedly, which is suitable for the purification of antibodies in plasma, ascites, tissue culture supernatant and other samples. It can also be used for antibody fixation and other related studies. By referring the attached Table 1 users can select the categories of magnetic beads according to the source and subtype of the target antibody, and compare the affinity of Magrose Protein A and Magrose Protein G beads with different antibodies.

## Product Information

| Product name | Magrose Protein A | Magrose Protein G |
|---|---|---|
| **Bead size** | 30~150 μm | 30~150 μm |
| **Beads Concentration** | 10% (v/v) | 10%(v/v) |
| **Ligand** | Protein A | Protein G |
| **Medium** | Magrose | Magrose |
| **Antibody binding quantity** | 25~30 mg Human IgG/mL Gel | 25~30 mg Human IgG/mL Gel |
| **Storage Temperature** | 2~8℃ | 2~8℃ |
| **Binding/Washing buffer** | 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 | |
| **Elution buffer** | 100 mM Gly, 0.1% Tween-20, pH 2.5 | |
| **Neutrilization buffer** | 1.0 M Tris-HCl, pH 9.0 | |
| **Storage buffer** | PBST (includes 0.05% $NaN_3$) | |
| **Shelf life** | 12 months | |

## Operational Process

1. **Sample processing：** add 100 μL Human serum to 1.5 mL EP tube, then add 900 μL Binding/Washing buffer, fully mix.
2. **Beaverbeads pretreatment:** vortex oscillating 30 s antibody purification beads to make beads fully resuspend; take 200 μL 10% (v/v) magnetic suspension to a new 1.5 mL EP tube. Magnetic separate the beads suspension, then discard supernatant, washed with 1 mL Binding/Washing buffer for 2 times, and magnetic separate. Magnetic beads in the tube can be directly used for antibody separation.
   **Note:** Beads amount can be adjusted according to binding capacity of the target antibody, when the target concentration of antibody is more than 150 μg/mL, users can take 1.2~1.5 times of magnetic beads (calculation method: 1.5* samples' target antibody content / binding capacity), if the target antibody concentration is low, such as less than 70 μg/mL, in order to improve the recovery rate of antibody, users can increase the amount of beads, such as increased to 3 times the amount of magnetic beads.
3. **Antibody adsorption:** add step 1 sample solution to step 2 tube, vortex oscillate evenly, put it on the flipping mixing instrument or manual gently flip the EP tube at room temperature (about 25℃) , make full mix, magnetic separate after flipping for about 15 min, then discard supernatant.
4. **BeaverBeads washing:** add 1 mL Binding/Washing buffer to the EP tube, oscillate and resuspend the magnetic beads then magnetic separate, and discard the supernatant; repeat 3 times of this step.
5. **Antibody elution:** add 0.5~1.0 mL Elution Buffer to the EP tube of step 4, quickly resuspend by blowing a pipeater or vortex oscillating, then at room temperature (about 25℃) place it on the flipping mixing instrument or manual gently flip the EP tube, magnetic separate after 10 min, collect the supernatant to a new EP tube.
   **Note：** Recommend users to control the final antibody elution concentration in between 0.6~1.2 mg/mL, in that case, more than 95% of antibody will be eluted ; if the amount of Elution Buffer is too few, it can lead to some of the antibody remain with the magnetic beads, which leads to lower recovery of antibody.
6. **Neutralization of antibodies:** add a certain amount of Neutralization Buffer into the antibody eluent in step 5, which is usually 1/10 of antibody elution volume, and finally the pH value of the eluted antibody is kept in a neutral environment to maintain the biological activity of the antibody and avoid the inactivation of antibody.
7. **Follow-up processing of Beaverbeads:** wash 2 times of magnetic beads with the Elution Buffer, magnetic separate, then discard the supernatant; and then use the Binding/Washing buffer washing 3 times, magnetic separate, discard the supernatant, add 200 μL Storage buffer to resuspend beads, and store at 2~8℃.

## Regeneration of Beads

1. After using the magnetic beads many times, the proteins such as precipitation protein, strong hydrophobic protein and lipoprotein can be absorbed onto the magnetic beads non-specifically. In order to ensure the use efficiency of magnetic beads, it is recommended to do magnetic bead regeneration treatment after 5 times of continuous use.
2. Add 1 mL 1% (v/v) Triron X-100 magnetic regeneration buffer for every 1 mL 10% (v/v) beads, oscillate evenly at room temperature, then place it on the flipping mixing instrument or manual gently flip to mix, magnetic separate after 10 min, then discard the supernatant.
3. Immediately add 1 mL Binding/Washing Buffer to resuspend, then magnetic separate, discard the supernatant, repeat the step 3 times.
4. Add 1 mL Storage Buffer to resuspend beads, store at 2~8℃.

## Note

1. Please be sure to read the instructions carefully before operating the antibody purification.
2. This product must be worked with magnetic separator.
3. Magnetic beads should be fully vibrated before use.
4. Magnetic beads should be stored in the storage solution to prevent drying.
5. Do not freeze or centrifuge magnetic beads so as to avoid irreversible aggregation.
6. This product is for research use only.

## Common problems and Solutions(FAQ)

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

**A1:** The binding efficiency of antibody with magnetic beads is related to the origin of the antibody and its subtype, refer to the type of antibody and the affinity efficiency of Protein A ligand antibody. (See attached table 1 ). If the antibody subtype has low affinity with Protein A , users can increase incubation time between antibody and beads (30~120 min), increase pH value (8~9) of the combination buffer and decrease ionic strength (25~100 mM NaCl) to increase affinity efficiency, or select the target antibodies with higher affinity ligands (such as Protein G or Protein A/G).

**Q2: How to improve the efficiency of antibody elution?**

**A2:** When the high affinity between antibody and Protein A ligand result in antibody low elution efficiency, users can avoid this situation by reducing the pH value (1.9~2.5) of elution buffer, increasing the ionic strength of elution buffer (2~3 M $MgCl_2$) or prolonging the elution time. But pay attention that antibody could easily aggregate under low pH conditions. Users can adjust the pH to neutral immediately with alkaline buffers (such as Tris, HEPES, etc.).

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

**A3:** Beads should be stored at 2~8℃, and avoid for irreversible aggregation due to contamination or drying. Magnetic beads aggregation at low pH elution buffer is a normal phenomenon, and it does not affect the normal use of magnetic beads. In Binding/Washing buffer and Elution buffer adding with the final concentration of 0.1% (v/v) nonionic detergent (such as NP-40, Tween-20 or Triton X-100) can effectively prevent the beads aggregation. The magnetic beads in low pH elution can be washed to neutral with Binding/Washing buffer and Elution buffer, and process with 2 min ultrasonic water bath. It can restore the magnetic beads to homogeneity, and the above treatments had no effect on the antibody binding efficiency with magnetic beads.

**Q4: How to solve magnetic bead adhere to tube wall?**

**A4:** It is recommended to use the consumables with low adsorption rate. In addition, adding 0.01%~0.1%(v/v) nonionic detergent (such as NP-40, Tween-20 or Triton X-100) to the buffer solution can effectively reduce the adhesion of beads on tube wall.

**Q5: Magnetic beads appear agglomeration in the process of using?**

**A5:** The agglomeration phenomenon is generally difficult to shake and break, which easily lead to uneven distribution. The reason is mainly because the magnetic beads are placed in the magnetic field for too long, the magnetic beads firmly binding together. The magnetic beads can be dispersed by 2 min ultrasonic water bath, but it should be noted that the antibody captured in the sample solution can lost from ultrasonic treatment, so it's not recommended to use this method until elution.

**Table 1: Comparison of antibody affinity between Protein A & Protein G with different sources and types.**

| Species | Subclass | Protein A binding | Protein G binding |
|---|---|---|---|
| Human | IgA | Variable | - |
| | IgD | - | - |
| | $IgG_1$ | ++++ | ++++ |
| | $IgG_2$ | ++++ | ++++ |
| | $IgG_3$ | - | ++++ |
| | $IgG_4$ | ++++ | ++++ |
| | IgM | Variable | - |
| Mouse | $IgG_1$ | + | ++++ |
| | $IgG_{2a}$ | ++++ | ++++ |
| | $IgG_{2b}$ | +++ | +++ |
| | $IgG_3$ | ++ | +++ |
| | IgM | Variable | - |
| Rat | $IgG_1$ | - | + |
| | $IgG_{2a}$ | - | ++++ |
| | $IgG_{2b}$ | - | ++ |
| | $IgG_3$ | - | ++ |
| Cow | IgG | ++ | ++++ |
| Goat | IgG | - | ++ |
| Sheep | IgG | - | ++ |
| Horse | IgG | ++ | ++++ |
| Rabbit | IgG | ++++ | +++ |
| Pig | IgG | +++ | +++ |
| Guinea Pig | $IgG_1$ | ++++ | ++ |
| | $IgG_2$ | ++++ | ++ |
| Hamster | IgG | + | ++ |
| Monkey (rhesus) | IgG | ++++ | ++++ |
| Avian egg yolk | IgY | - | - |
| Dog | IgG | ++ | + |
| Koala | IgG | - | + |
| LIama | IgG | - | + |

**Note:** "+"=weak binding, "+++"=medium binding, "+++++"=strong binding, "-"=no binding

**The related product list of antibody purified magnetic beads**

| NO. | Product | Specification | Size | Binding capacity with Human IgG |
|---|---|---|---|---|
| 70804-5 | BeaverBeads™ Magrose Protein A Antibody Purification | 5 mL | 30~150 μm | 25~30 mg /mL Gel |
| 70804-100 | BeaverBeads™ Magrose Protein A Antibody Purification | 2×50 mL | | |
| 70804-500 | BeaverBeads™ Magrose Protein A Antibody Purification | 2×250 mL | | |
| 70805-5 | BeaverBeads™ Magrose Protein G Antibody Purification | 5 mL | 30~150 μm | 25~30 mg /mL Gel |
| 70805-100 | BeaverBeads™ Magrose Protein G Antibody Purification | 2×50 mL | | |
| 70805-500 | BeaverBeads™ Magrose Protein G Antibody Purification | 2×250 mL | | |
| 20102-1 | BeaverBeads™ Protein A Antibody Purification Kit | 1 mL | 2 μm | 1.2~1.5 mg /mL |
| 20102-5 | BeaverBeads™ Protein A Antibody Purification Kit | 5 mL | | |
| 20102-25 | BeaverBeads™ Protein A Antibody Purification Kit | 25 mL | | |
| 20202-1 | BeaverBeads™ Protein A/G Antibody Purification Kit | 1 mL | 2 μm | 1.2~1.5 mg /mL |
| 20202-5 | BeaverBeads™ Protein A/G Antibody Purification Kit | 5 mL | | |
| 2020-25 | BeaverBeads™ Protein A/G Antibody Purification Kit | 25 mL | | |

| NO. | Magnetic Separator | Specification | Note |
|---|---|---|---|
| 60201 | Magnetic Separator Stand 2/15 | 2/15 mL | 1.5 mL, 2 mL EP tube and 15 mL centrifuge tube |
| 60203 | Magnetic Separator Stand 50 | 50 mL | for conventional 50 mL centrifuge tube |
| 60302 | Magnetic Separator Stand 96-I | 96-I | for regular 96-well microporous plate, PCR plate, 8-well or 12-well PCR article tube. |
| 60303 | Magnetic Separator Stand 96-II | 96-II | for 96-well PCR plate (20~200 μL experimental system) |



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