# Protein A Matrix Antibody Purification Kit

**Cat:** M2360

**Specification:** 1 mL/5 mL

**Storage:** 2-8℃,valid for 1 year

**Product content:**

The antibody-purified magnetic bead series products use 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 purity greater than 90 % can be isolated from serum samples by one-step purification.

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 information:**

| product name | specifications |
|---|---|
| Protein A (orA / G) for Antibody Purification① | 5 mL |
| Antibody Binding Buffer ② | 200 mL |
| Antibody Elution Buffer I (pH4.5) ③ | 100 mL |
| Antibody Elution Buffer II (pH2.0) ④ | 100 mL |
| Antibody Neutrilization buffer ⑤ | 50 mL |
| Beads Washing Buffer ⑥ | 50 mL |
| Beads Storage Buffer ⑦ | 50 mL |
| Beads Regeneration Buffer ⑧ | 50 mL |

**Scope of application:**

This product can be reused for antibody purification in samples such as plasma, ascites, and tissue culture supernatants. It can also be used for antibody immobilization and other related research.

**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.

**Operation steps ( *just 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, based on the antibody binding ability of the selected immunomagnetic beads (see list of immunomagnetic beads related products or Antibody Capacity data of product composition), Calculate the approximate amount of the immunomagnetic beads, Too much or too little of immunomagnetic beads affects the effect of antibody purification, It is recommended that the maximum load of immunomagnetic beads should be 80%~100% of the sample antibody content.

The following steps are described in detail according to the purified antibody, taking 100 μL Protein A antibody purified magnetic beads as an example. The operator can adjust the amount of each component in the actual operation proportionally by referring to the amount of various buffers and the size of the container in the following steps.

**1. Sample processing:**

Place samples containing about 0.1~0.15 mg in a new 1.5 mL EP tube, add antibody binding buffer (②) to a total volume of 500 μL (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 μL magnetic bead suspension in another 1.5 mL EP tube. The magnetic bead suspension is magnetically separated (placing the EP tube on the magnetic separator) for the magnetic bead adsorption to the tube wall until the solution is clarified; this operation is omitted 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 magnetic bead tube pretreatment 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 three 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 adjusted with antibody elution buffer for antibody concentration determination. 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 (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 μ L magnetic beads storage buffer (⑦) in 2~8℃.

**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. Magnetic bead washing buffer (⑥) was added to about 1 mL of 5 mL for resuspension, then magnetic separation was performed, the supernatant was discarded, and the above operation was repeated twice.

4. Add an appropriate amount of magnetic beads storage buffer (⑦) at the ratio of about 1 mL of magnetic beads and store them 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 (Supplementary Table 1), If the antibody subtype has a low affinity with Protein A, 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 reducing the ionic strength (25~100 mM NaCl), Or select a matching medium with a higher affinity to the target antibody (such as 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 can be improved by reducing the pH value of the elution buffer (1.9~2.5), increasing the ionic strength of the elution buffer (2~3 M 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 to the pH to neutral with alkaline buffer agents (eg.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. Adding non-ionic scale remover (eg. NP-40, Tween-20, or Triton X-100) to Binding Buffer and Elution Buffer at 0.1% (v/v) can effectively prevent magnetic bead aggregation. The magnetic beads after low pH elution operation can be washed to neutral with binding buffer, and then resuspended with Tris Buffer (pH7.5) containing 0.1% (v/v) Tween-20 and treated with an ultrasonic water bath for 2 min to restore the magnetic beads. The above treatment does 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% or Triton X-100) (v/v) to the buffer can effectively reduce the adhesion of magnetic beads to consumables.

**Q5: Did the magnetic beads block during use?**

A5: If the magnetic beads are used, it is generally difficult to vibrate and disperse, 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 ultrasonic water bath for 2 min can disperse the magnetic beads to disperse them, but it should be noted that the ultrasonic treatment will also cause the antibody captured by the magnetic beads in the sample solution to fall off, so this method should not be used before the magnetic beads washout after addition.

**Schedule 1: Comparison of antibody affinity of immunomagnetic beads Protein A and Protein A / G with different sources and types**

| Species | 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 | +++ | +++++ |
| 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**