Heparin-28MB Use and Applications

1. Product Introduction

Heparin Magrose Beads feature fast magnetic responsiveness, abundant heparin density, and extremely high physicochemical stability. On one hand, they can serve as a ligand for affinity chromatography, capable of specifically binding with bio-molecules such as growth factors and antithrombin III. On the other hand, due to the presence of a large number of negatively charged sulfate groups on the surface, they can act as a cation exchange medium, exhibiting strong binding capacity with positively charged proteins under certain pH conditions.

Compared to traditional column chromatography purification methods, Magrose Heparin magnetic beads do not require pre-treatment of crude protein samples (such as repeated cumbersome centrifugation, time-consuming filtration operations), and there is no need to control flow rates or column pressure. Expensive chromatography equipment is also not required. For skilled operators, the extraction of high-purity target proteins can be completed in a very short time, and multiple samples can be processed in parallel, achieving high-throughput protein purification.

Suitable for the separation and purification of bio-macromolecules such as anticoagulant factor III, coagulation factors, nucleic acid-binding proteins, lipoproteins, interferons, steroid receptors, thrombin, and thrombin-like enzymes.

2. Product Information

Item	Specification
Appearance	White spherical particles
Magnetic Bead Size Range	30~150μm
Surface Group Content	~3mg Heparin/mL Gel
Protein Binding Capacity[1]	2~3mg Antithrombin III/mL Gel
Storage Solution	20% Ethanol
Suspension Concentration[2]	10% (V/V) Magnetic Bead Suspension
Storage Temperature	2~30°C (For long-term storage, it is recommended to keep at 2~8°C)
Binding Buffer	50 mM Tris-HCl, pH 8.0
Elution Buffer	50 mM Tris-HCl, 1~2 M NaCl, pH 8.0
Shelf Life	Stable storage at 2~8°C, with a shelf life of 2 years

Notes:

1. The protein binding capacity is related to the characteristics of the target protein; the value here is for reference only.
2. 1mL of magnetic bead suspension contains 100μL of magnetic beads.

3. Product Advantages

- Abundant binding sites enhance specific binding with ligands.
- Fast magnetic response reduces operation time.
- The magnetic beads have good dispersibility and resuspendability, improving the convenience of operation.
- The ligand has good physicochemical stability, enhancing the reliability and repeatability of experimental results.

4. Operation Procedure (Using the Purification of Antithrombin III from Human Plasma as an Example)

1). **Sample Treatment:** Take 1 mL of human plasma and add it to a 1.5 mL EP tube. Then, add 500 µL of Binding Buffer and mix thoroughly.

2). **Magnetic Bead Pre-treatment:** Vortex the Magrose Heparin magnetic beads for 30 seconds to fully resuspend them. Take 1 mL of a 10% (V/V) magnetic bead suspension and place it in a new 1.5 mL EP tube. Perform magnetic separation on the bead suspension, discard the supernatant, and wash the magnetic beads twice with 1 mL of Binding Buffer. After magnetic separation, the beads in the tube can be directly used for antibody separation.

3). **Protein Adsorption:** Add the sample solution processed in step 1 to the tube with the magnetic beads pre-treated in step 2. Vortex to evenly mix, and then place the EP tube in a vertical mixer at room temperature (about 25°C) for 15 to 30 minutes to allow the sample and magnetic beads to fully contact and adsorb. After that, perform magnetic separation and discard the supernatant.

4). **Magnetic Bead Washing:** Add 1 mL of Binding Buffer to the EP tube, vortex to resuspend the magnetic beads for 1 minute, then perform magnetic separation and discard the supernatant. Repeat this operation three times.

Note: Depending on the SDS-PAGE profile of the eluted protein, a certain concentration of NaCl can be appropriately added to the Binding Buffer. This step can effectively remove non-specifically adsorbed proteins, allowing the operator to obtain a higher concentration of the target protein.

5). **Protein Elution:** In the EP tube where the magnetic beads have been washed as described above, add 0.2 mL of Elution Buffer. Quickly resuspend by pipetting or vortexing, then place the EP tube in a vertical mixer at room temperature (about 25°C) for 10 to 15 minutes. After mixing, perform magnetic separation and collect the supernatant into a new EP tube.

6). **Magnetic Bead Regeneration:** Add 1 mL of purified water to the EP tube, vortex to resuspend the magnetic beads, then perform magnetic separation and discard the supernatant. Repeat this operation three times. Then, wash the magnetic beads three times with Binding Buffer. After multiple uses, precipitated proteins, strongly hydrophobic proteins, lipoproteins, and other impurities may non-specifically adsorb onto the magnetic beads. To ensure the efficiency of the magnetic beads, it is recommended to perform in-place cleaning (CIP).

7). **In-Place Cleaning (CIP):** Sequentially wash the magnetic beads twice with 0.1 M NaOH, 8 M urea, purified water, and Binding Buffer. Finally, add 1 mL of Storage Buffer (20% ethanol) to resuspend the

magnetic beads and store at 2~8°C.

5. Precautions

1). This product should not be subjected to freezing, drying, or centrifugation. Operations such as freezing, drying, and centrifugation can cause the magnetic beads to aggregate, making them difficult to resuspend and disperse, and affecting the chemical activity of the functional groups on the surface of the magnetic beads.

2). Before using this product, ensure to thoroughly vortex or ultrasonicate the magnetic beads to maintain a uniform suspension.

3). During the usage, as needed, wash the magnetic beads 2 to 3 times with purified water or buffer to remove ethanol from the storage solution.

4). This product must be used in conjunction with magnetic separation equipment.

5). Both salt concentration and pH value will affect the binding and elution of specific proteins. Customers need to explore the binding and elution conditions for different proteins to ensure the quantity and purity of protein purification.

6). This product is for research use only.

6. Examples of Product Applications

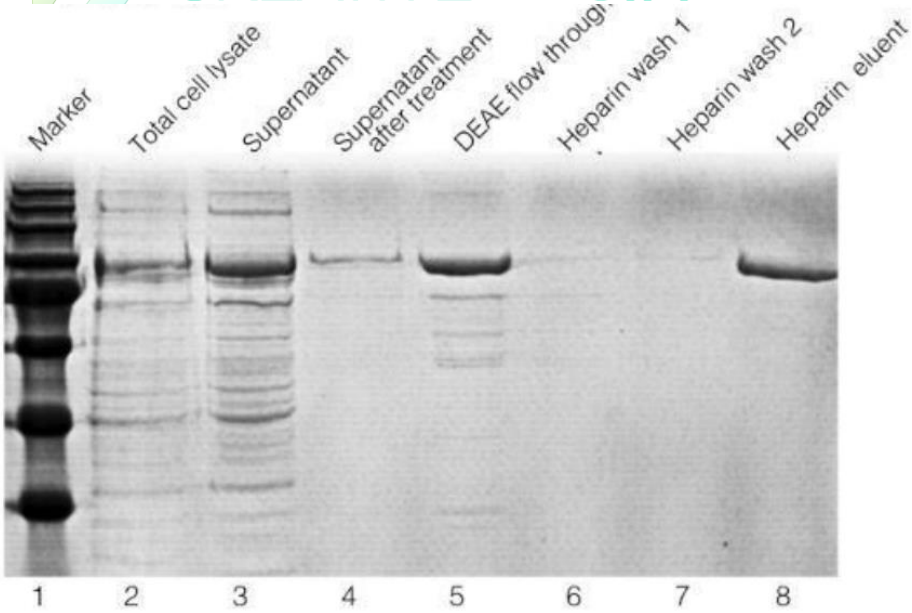

Figure: SDS-PAGE gel electrophoresis result for the purification of nucleic acid-related proteins using a two-step purification method involving Heparin-28MB.

As shown in Figure: Heparin-28MB can specifically bind to nucleic acid-related proteins. Combined with DEAE ion exchange chromatography, high-purity target proteins (lane 8) can be obtained, and the purification recovery rate is relatively high, significantly higher than that of nucleic acid-related proteins obtained by heat treatment (lane 4).