BeaverBeads™ Magrose NHS

Product Introduction

The NHS magnetic beads are superparamagnetic beads with activated groups of N_Hydroxysuccinimide on the surface, which can react with primary amines on desired ligands and bind them covalently with amide linkage to the beads. The ligand can be various molecules including antigen, antibody and other proteins. Compared with the traditional carboxyl, amino magnetic beads, the NHS group of magnetic beads don't need to activate EDC/NHS or Glutaraldehyde, simply dissolving the amino-containing bio-ligand in the coupling buffer, at room temperature, mixing with proteins and the NHS magnetic beads for only 1-2 hours, the bio-ligand can be coupled to the magnetic beads. The advantages include easy operation, mild coupling conditions and coupling fast and efficient. The magnetic bead coupling process must be carried out in a buffer solvent free of any amino. This method can be operated manually or automatically.

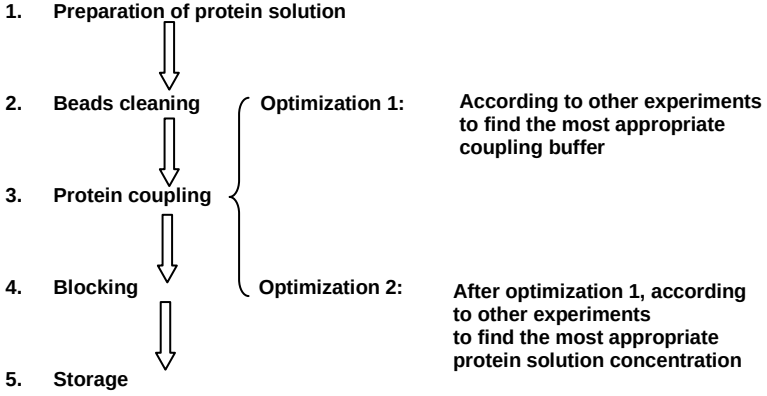
Product Information

1. Product basic information

Product information	BeaverBeads™ Magrose NHS
Beads Material	Magnetic agrose microspheres
Beads Size	30-150 μm
Ligand	N - hydroxysuccinimide
Ligand Density	20-30 μmol/mL beads
Binding Capacity	20-30 mg rabbit IgG/mL beads
Suspension Concentration	20%(v/v)
Preservative	Anhydrous isopropyl alcohol
Shelf Life	12 months at 2-8 ºc

Note 1: The binding ability is related to the characteristics of the bio-ligand itself, values here are only for reference
Note 2:1 mL beads suspension includes 200 μL beads.

The flow chart and optimization of protein coupling operation



1. Beads cleaning should be strictly following the instructions: a fast washing with the cooling washing Buffer are needed, in order to prevent magnetic beads NHS group hydrolysis in the washing process;
2. In the process of protein coupling, first need to find the appropriate Coupling Buffer (mainly include Coupling Buffer A, Coupling Buffer B, 50 mM Boric acid Solution, 100 mM NaCl, pH 7.4 etc.)
3. Based on Step 2's Coupling Buffer, then need to find the appropriate protein coupling concentration, Because of the higher protein concentration, the amount of protein coupled to the beads will be greater (This is because the NHS group with the protein coupling and hydrolysis of the NHS group itself is a competition.) Of course, here we must consider the use of performance and cost. If only needs to couple a small amount of protein, use the low concentration protein to reduce the cost.
4. Blocking step can use 3 M ethanolamine along with the kits. Tris buffer can also be used(100 mM Tris-HCl, 150 mM NaCl, pH 8.0) Blocking time shall not be less than 2 h, If the chemistry blocking is closed, the background is still high. Additional BSA blocking can be added as well.

Protein coupling operation steps

Reagents to be prepared:

Serial number	Reagent name	Remarks
①	Washing Buffer A	1 mM hydrochloric acid, cool to 4ºC before use.
②	Coupling Buffer A	100 mM 2-morpholinoethanesulfonic acid MES), pH 4.8 (for coupling of biomolecules with an isoelectric point of less than 7)
③	Coupling Buffer B	200 mM NaHCO3, pH 8.3 (for conjugation of biomolecules with isoelectric point greater than 7)
④	Blocking Buffer	3 M ethanolamine, pH 9.0
⑤	Storage Buffer	Containing 0.05% sodium azide in PBS buffer or according to the needs with other buffers

The following procedure are taking beads sample 500 μL, using 1.5 mL EP tube as an example. Users can adjust amount according to the needs:

1. Protein solution preparation:
Take the appropriate amount of the protein to dissolve with Coupling Buffer, to make the concentration of ≥3.0 mg / mL protein solution. Proteins that have been stored in buffer need to be thoroughly dialyzed or desalted to remove any primary amine group-containing substances in the original buffer and then formulated into a protein solution at a concentration of ≥3.0 mg / mL using a Coupling Buffer. The protein solution is stored at 4ºC for use.
Note: (1) In order to achieve better performance, when the protein concentration ≥ 3 mg / mL, the coupling efficiency will be higher at this time, but according to the cost and use requirements need to be considered.
(2) Protein solution can not contain primary amino-containing ingredients, such as Tris, glycine, gelatin, BSA, etc.

2. Take 500 μL 20% suspension to put into a 1.5 mL EP tube.
Note: Before sampling, magnetic beads need to repeatedly reverse, or use vortex shaker or vertical mixer to mix well to ensure the identity of the experiment. Need to mix uniformly for every sampling.

3. Put the EP tube in a magnetic separator, magnetically aggregate the magnetic beads, then remove the supernatant.

4. Add 1 mL of 2～8ºC Washing Buffer A into a centrifuge tube and vortex shaking for 15s to mix the magnetic beads.

5. Put the EP tube in a magnetic separator, magnetically aggregate the magnetic beads, then remove the supernatant.

6. Add 200 μL protein solution into EP tube and vortex shaking for 30 s to mix well.

7. Vortex shaking the tube for 15 s, place in a vertical mixer, mix at room temperature for 2～4h. If vertical mixing is not uniform, start 30 min before each reaction, remove EP tube to vortex shaker for 15s every 5 min. Thereafter, every 15 min, remove the EP tube to vortex shaker for 15 s. If necessary, the tube can be incubated overnight at 4 ºC.

8. Use magnetic separator to magnetically aggregate magnetic beads, store the flow of liquid.

9. Add 1 mL Blocking Buffer into the EP tube, vortex shaking for 30 s, place the EP tube in magnetic

separator, to magnetically aggregate the magnetic beads, then discard the supernatant.

Note: Blocking Buffer In addition to the 3 M ethanolamine provided in the kit, other blocking reagents such as 100 mM Tris-HCl, 150 mM NaCl, pH 8.0, etc., may also be used.

10. Repeat "Step 9" four times.
11. Add 1 mL Blocking Buffer into the EP tube and vortex shaking for 30 s. Place the EP tube into a vertical mixer at room temperature for 2 h.
12. Put the EP tube in a magnetic separator, magnetically aggregate the magnetic beads, then remove the supernatant.
13. Add 1 mL of ultrapure water in a EP tube and mix thoroughly. Place the EP tube in magnetic separator, to magnetically aggregate the magnetic beads, then discard the supernatant.
14. Add 1 mL of Storage Buffer (eg PBS buffer containing 0.05% sodium azide or choose a suitable preservative solution) in a EP tube and mix well. Place the EP tube in magnetic separator, to magnetically aggregate the magnetic beads, then discard the supernatant. Repeat this operation 2 times.
15. Add 500 μL Storage Buffer into EP tube and mix thoroughly and store at 4ºC.

Note: The concentration of beads after the coupled protein was 10% (v/v).

Precautions

1. Magnetic beads are sensitive to water. In order to ensure product quality, after sampling immediately cover the bottle caps and seal with a sealing tape, store at 4 ℃.
2. Beads are not allowed to dry or freeze. Drying and freezing operations may result in the aggregation of magnetic beads and thus loss of binding activity.
3. Pre- and post-reaction protein content can be determined by indirect methods (e.g., Thermo Scientific ™ Pierce ™ 660 nm Protein Assay, Product No. 22660 and 22662) or by direct methods (e.g., use the Thermo Scientific ™ Pierce ™ Micro BCA Protein Assay, Product No. 23235) to detect the surface of the magnetic beads coupled with the protein content. Using wavelengths around 280 nm to determine protein levels is not advisable. Because NHS groups strongly absorb near 280nm, they can seriously interfere with the detection.
4. Protein stabilizers (such as BSA, gelatin) inhibit the binding of antibodies to magnetic beads. Therefore, during the bead-coupled antibody process, it is necessary to ensure that no primary amino-containing protein stabilizer is present in the antibody storage system.
5. The presence of a primary amine-containing substance in the buffer inhibits protein coupled with the beads. Removal of primary amine materials can use dialysis and desalination methods.
6. NHS group is easy to hydrolysis in washing buffer A, be sure to refer to the instructions.
7. Prepare protein solution in advance, after washing Washing Buffer A, needs to add the protein solution immediately for coupling reaction.
8. The efficiency of coupling proteins and magnetic beads varies, depending on the type and nature of the protein. In general, a protein concentration of ≥3.0 mg / mL facilitates protein coupling. However, its concentration needs to be optimized for different proteins.

Product List

Item No.	Product Name	Specification	Concentration
70702－1	Magrose NHS	1mL	20% (v/v)
70702－5		5mL	20% (v/v)
70702-50		50mL	20% (v/v)