# NHS Magnetic Beads

**Cat:** M2450

**Storage:** Store at 2-8℃, and it is valid for 1 years.

**Introduction:**

The surface of Mag NHS is modified with NHS groups, which can form stable peptide bonds with proteins and other molecules possessing primary amine groups for the affinity purification of antibodies, antigens, and other biomolecules. In contrast to traditional carboxyl and amino magnetic beads, the magnetic beads with NHS groups on the surface do not need to be activated in advance with EDC/NHS or glutaraldehyde. Merely by simply dissolving the biological ligand containing primary amino groups in the Coupling Buffer provided in the kit, and mixing the protein with NHS magnetic beads at room temperature for 1 - 2 hours, the biological ligand can be covalently coupled to the magnetic beads. This approach boasts advantages such as simple operation, mild coupling conditions, and rapid and efficient coupling of biological ligands. The coupling process of magnetic beads must be conducted in a buffer solvent that contains no amino groups. During manual operation, a magnetic separation rack is utilized to separate the magnetic beads from the solvent. Automated equipment can also be employed for the operation, and it is suitable for the screening of multiple samples.

**Product Information:**

**1. Kit Components**

| Sequence number | Kit Components | Comment | Quantity |
|---|---|---|---|
| 1 | Beads Magnetic NHS | 10mg/mL | 1mL/5mL |
| 2 | Washing Buffer A | Cool to 4°C before use. | 5mL/15mL |
| 3 | Coupling Buffer A | 100 mM 2-morpholinoethanesulfonic acid (MES), pH 4.8 (for the coupling of biomolecules with isoelectric points less than 7) | 5mL/15mL |
| 4 | Coupling Buffer B | 200 mM $NaHCO_3$, pH 8.3 (for the coupling of biomolecules with isoelectric points greater than 7) | 5mL/15mL |
| 5 | Blocking Buffer | 3 M ethanolamine, pH 9.0 | 5mL/15mL |
| 6 | Storage Buffer | 1× PBS, 0.1% proclin-300 can be added as required | Provide For Oneself |

**2. Basic information about magnetic beads**

| Product Information | Mag NHS |
|---|---|
| Particle size | ~2μm |

| | |
|---|---|
| Ability to bind* | ≧30μg rabbit IgG/mg |
| Concentration | 10mg/mL |
| Preservation solution | DMAC |

**Note: The binding ability is related to the characteristics of the biological ligand itself, and the value here is only for reference.*

**Protein coupling operation process and optimization points (*for reference only*) :**

1. Preparation of protein solution
2. Washing of magnetic beads
3. Protein coupling: (optimization)
   (1) Type of Coupling Buffer. The most suitable Coupling Buffer was first determined experimentally.
   (2) After determining the appropriate Coupling Buffer, the concentration of the protein solution was optimized through experiments.
4. Blocking
5. Magnetic bead preservation
6. Note
   (1) The magnetic beads should be washed strictly according to the instructions using the cool Washing Buffer A to prevent the NHS group from hydrolyzing during washing.
   (2) During the protein conjugation process, the appropriate Coupling Buffer should first be determined through experiment (which mainly includes Coupling Buffer A, Coupling Buffer B, 50mM boric acid solution, pH 8.5, 100mM phosphate buffer, 100mM NaCl, pH 7.4).
   (3) After determining the appropriate Coupling Buffer, the suitable protein conjugation concentration can be determined based on it. The higher the protein concentration, the more protein will be conjugated to the magnetic beads (because the NHS group's binding to the protein and the NHS group's hydrolysis are a pair of competitive reactions). Of course, the performance and cost should be comprehensively considered here. Some customers can meet the needs of using a small amount of protein, and then a low concentration of protein can be used, which can reduce the cost.
   (4) Blocking this step can be done with 3 M ethanolamine in the kit or Tris buffer (100 mM Tris-hcl,150 mM NaCl, pH 8.0). The blocking time should not be less than 2 h, if the background is still high after chemical blocking. An additional step of BSA blocking can also be added.

## Protein coupling Procedure (for reference only)

**Prepare before use**

The following procedure was introduced by taking 500μL of magnetic bead sample and using 1.5mL EP tube as an example. Users can adjust the proportion according to their own needs:

**1. Protein solution preparation:**

An appropriate amount of the protein to be coupled was dissolved with Coupling Buffer to prepare protein solutions with concentrations of 0.1-3.0 mg/mL. The protein that has been stored in Buffer needs to be completely removed by dialysis or desalting, and then the protein solution with the concentration of 0.1-3.0 mg/mL is prepared with Coupling Buffer. The prepared protein solution was stored at 4ºC until use.

**Note:**

(1) In order to achieve better performance, protein concentration ≥2.0 mg/mL, so that the coupling efficiency will be higher, the cost and use requirements need to be comprehensively considered here;

(2) The protein solution should not contain components with primary amino groups, such as Tris, glycine, gelatin, BSA, etc.

**2. Magnetic bead cleaning:**

(1) 500μL magnetic beads were placed in a 1.5mL EP tube.

Note: The magnetic beads should be inverted repeatedly before sampling and mixed evenly using a vortex oscillator to ensure the identity of the experiment.

(2) The EP tube was placed in a magnetic separator to enrich the magnetic beads and remove the supernatant.

(3) Add 1mL Washing Buffer A pre-cooled at 2-8 ºC to a 1.5mL EP tube and vortex for 15s to make the magnetic beads mixed evenly.

(4) Put the EP tube in the magnetic separation rack, enrich the magnetic beads, and remove the supernatant.

**3. Biological ligand fixation:**

(1) Add 500μL protein solution into EP tube and swirl for 30s to make it evenly mixed.
Note: Add the protein solution immediately after washing the magnetic beads.

(2) The EP tube was vortexed for 15s, placed on a vertical mixer, and mixed for 1 to 2 h at room temperature. If the vertical mixing was not uniform, the EP tube was removed and vortexed and mixed at 5min intervals for 15 seconds 30min before the reaction. Thereafter, at 15 min intervals, the EP tube was removed and vortexed for 15s.
Note: Overnight reaction at 4ºC if necessary.

(3) The magnetic separation frame is used to enrich the magnetic beads and preserve the flow through the liquid.

**4. Magnetic bead sealing:**

(1) 500μL Blocking Buffer was added to the EP tube, vortexed for 30s, and the EP tube was placed in the magnetic separation frame to enrich the magnetic beads, and the supernatant was discarded.

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

(2) Repeat step (1) four times.

(3) 500μL Blocking Buffer was added to the EP tube, vortexed for 30 s, and the EP tube was placed in a vertical mixer for 2 h at room temperature.

(4) The EP tube was placed in a magnetic separation frame, the magnetic beads were enriched, and the supernatant was discarded.

(5) Add 1mL ultrapure water to EP tube, mix thoroughly, concentrate magnetic beads with magnetic holder, discard supernatant.

**5. Save:**

(1) Add 1 ml Storage Buffer (customer's own, such as PBS buffer containing 0.05% sodium azide or 0.1%proclin-300, or customer's choice of suitable storage solution according to their actual needs) in EP tube, mix well, and concentrate magnetic beads with magnetic holder. The supernatant was discarded. The procedure was repeated two times.

(2) Add 0.5mL Storage Buffer into EP tube, mix thoroughly, and store at 4ºC until use.

Note: The magnetic bead concentration of the final conjugated protein was 10mg/mL.

**Note:**

1. Magnetic beads are sensitive to moisture. To ensure product quality, the bottle should be capped immediately after sampling, sealed with a sealing film, and stored at 4 ℃.
2. Do not dry or freeze the magnetic beads. Drying and freezing operations may lead to aggregation of magnetic beads and thus loss of binding activity.
3. The protein content coupled to the surface of magnetic beads can be detected by indirect method of protein content before and after reaction or direct method (such as BCA). It is not recommended to use the wavelength around 280nm to determine the protein content, because the NHS group has strong absorption near 280nm wavelength, which will seriously interfere with the detection results.
4. Substances containing primary amine in the buffer will inhibit the coupling of proteins to the surface of magnetic beads, and the removal of primary amine substances can be carried out by dialysis and desalting.
5. Protein stabilizers (such as BSA and gelatin) can inhibit the binding of antibodies to magnetic beads. Therefore, in the process of antibody coupling with magnetic beads, it is necessary to ensure that there is no protein stabilizers containing primary amino groups in the antibody preservation system.
6. NHS groups are easily hydrolyzed, so when Washing with Washing Buffer A, we must refer to the instructions.
7. The protein solution should be prepared in advance. After Washing Buffer A, the protein solution should be added immediately for coupling reaction.
8. The coupling efficiency of proteins and magnetic beads varies according to the protein species and properties. In general, protein concentration of 0.1-3.0 mg/mL is conducive to protein coupling. However, the concentration needs to be optimized for different proteins.