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NHS Magnetic Beads (200 nm, 10 mg/mL)

1 Packing list

Components	HY-K0227-1 mL	HY-K0227-5 mL	HY-K0227-25 mL
NHS Magnetic Beads (200 nm, 10 mg/mL)	1 mL	5 mL	25 mL

2 Introduction

MCE NHS Magnetic Beads (200 nm, 10 mg/mL) contain N-hydroxysuccinimide (NHS) functional groups, which react with primary amines on proteins or other molecules to form stable amide linkages，can covalently immobilize proteins for the affinity purification of antibodies, antigens and other biomolecules. Compared with the traditional carboxyl and amino magnetic beads, NHS magnetic beads do not need to be activated by EDC/NHS or glutaraldehyde. Simply dissolve the primary amine-containing biological ligands in Coupling Buffer and incubate at room temperature to covalently couple the bioconjugate to the beads, which is easy to operate, mild coupling conditions, fast and efficient. The beads coupling process must be carried out in a buffered solvent without any amine, and automated instruments are especially useful for large screening of multiple samples.

3 Characteristics

Information

Mean Diameter	200 nm
Binding Capacity	≥ 100 μg rabbit IgG/mg of beads
Bead Concentration	10 mg/mL
Storage Solution	ddH_2O

Note: Binding capacity is related to biological ligand properties, the above is for reference only.

Binding capacity

Protein	MW (kDa)	Binding capacity of beads (μg/mg of beads)
IgG	150	80
Streptavidin	53	30
Protein A/G	50	26
Protein G	29	45

Note: Results will vary depending on the number of accessible primary and secondary amines.

4 General Protocol

Recommended Buffers

Washing Buffer	PBS, 0.05% Tween-20, pH 7.4
Coupling Buffer Ⅰ	0.1 M MES，pH 5.0 (For biomolecules with PI < 7)
Coupling Buffer Ⅱ	0.1 M PBS，pH 8.0 (For biomolecules with PI > 7)
Blocking Buffer	0.5 M Ethanolamine, pH 8.3 or 0.1 M Tris-HCl, 150 mM NaCl, pH 8.5
Storage Buffer	PBS, 0.02% (w/v) NaN_3

Protocol

1. Preparation of Protein

   Dissolve the protein in Coupling Buffer to a concentration of 0.5-2.0 mg/mL and store at 4℃.

   Note: Primary amine-containing buffers inhibit coupling of protein to the magnetic beads. Remove primary amine-containing buffer using dialysis or desalting.
2. Preparation of Magnetic Beads

   Resuspend the magnetic beads by repeated inversion, gentle vortexing or using a rotating platform. Then transfer 500uL to a new 1.5mL tube, magnetically separate, and discard the supernatant. Add 1 mL of Washing Buffer and vortex for 15 s to mix. After magnetic separation, discard the supernatant. Repeat this step for 2 times.
3. Magnetic bead coupling

   Add 500 μL of protein solution into the tube and vortex for 30 s. Incubate the tube for 1-2 h at room temperature on a rotator. During the first 30 min of the incubation, vortex the tube for 15 s every 5 min. For the remaining time, vortex the tube for 15 seconds every 15 min until incubation is complete. If required, incubate overnight at 4°C.
3. Blocking

   Collect the beads with a magnetic stand and save the flow-through. Add 1mL of Blocking Buffer to the beads and vortex the tube for 30 s. Incubate the tube for 2 h at room temperature on a rotator.
4. Storage

   Place the tube in a magnet for 2 min and remove the supernatant. Add 1mL of ultrapure water. Place the tube on a magnet for 2 min and remove the supernatant. Repeat the above steps three times. Add 500μL of Storage Buffer to the beads, mix well and store at 4°C until ready for use.

5 Storage

4ºC, 2 years

Do not freeze

6 Precautions

1. Magnetic beads are moisture-sensitive. To protect the beads, cap the bottle immediately after removing the slurry and wrap lab film around the cap before storing at 4°C.
2. For magnetic beads, do not centrifuge, dry, freeze or exposure to a magnetic field for a long time. Bead aggregation and loss of binding activity can result from using these methods.
3. The amount of protein coupled to the magnetic beads cannot be determined by UV because the NHS group has a strong absorption near 280 nm, which will seriously interfere with the results. BCA assay is recommended.
4. For coupling antibodies to magnetic beads, ensure the antibody storage solution does not contain a protein stabilizer (e.g., BSA, gelatin), which inhibits coupling of the antibody to the beads.
5. Primary amine-containing buffers inhibit coupling of protein to the magnetic beads. Remove primary amine-containing buffer using dialysis or desalting.
6. This product needs to be used in conjunction with magnetic separation instruments (e.g., magnetic separation racks, MCE Cat NO. HY-K0200).
7. This product is for R&D use only, not for drug, household, or other uses.
8. For your safety and health, please wear a lab coat and disposable gloves to operate.