Ni-NTA (His-tag Affinity) Magnetic Beads

Catalog No. MBPP-04001, MBPP-04002, MBPP-04003

【Introduction】

Ni-NTA Magnetic Beads are nano-superparamagnetic beads coupled with nickel-charged nitrilotriacetic acid (Ni-NTA). With a fast magnetic response rate, high protein binding capacity and low non-specific binding, Ni-NTA Magnetic Beads provide a rapid and efficient method to purify His-tagged fusion proteins from crude cell lysates. The beads are simply added to the crude cell lysate and His-tagged fusion proteins will bind to the beads. After washing the unbound proteins off, the target His-fusion proteins can be eluted for downstream experiments. The process can be completed manually or fully automated for high throughput applications.

【Product Specifications】

- **Diameter:** 500nm
- **pH stability:** pH 3-12
- **30min sedimentation rate:** <0.1%
- **Magnetic response rate:** >30emu/g
- **Solvent:** 1mM $NiSO_4$, 50mM Tris-HCl (pH8.0), containing 20% ethanol
- **Binding capacity:** 10-60μg His-tagged fusion proteins per mg magnetic beads

【Product Content】

Catalog No.	*Conc. (mg/ml)*	*Volume (ml)*	*Amount of Beads (mg)*
MBPP-04001	50	1	50
MBPP-04002	50	4	200
MBPP-04003	50	20	1000

【Purification Protocol】

The following protocol provides general guidelines for purification of His-tagged fusion proteins using Ni-NTA Magnetic Beads and may be modified by the user for specific applications. The protocol is scalable.

A. Note

1. NTA has four chelation sites for nickel ions and thus binds nickel more tightly than other metal-chelating purification systems that only have three sites available for interaction with metal ions. The extra chelation site prevents nickel ion leaching and results in a greater binding capacity and higher purity of the extracted protein than those obtained using other metal-chelating purification systems.
2. Purification of His-tagged proteins using Ni-NTA Magnetic Beads does not depend on the 3-dimensional structure of the protein or His tag. Therefore, Ni-NTA Magnetic beads can be used to purify His-tagged proteins from different expression systems under native or denaturing conditions.
3. Avoid using protease inhibitors or other additives that contain strong reducing agents (e.g. DTT or β-mercaptoethanol) or chelators (e.g. EDTA), which will disrupt the function of Ni-NTA magnetic beads.
4. Ni-NTA Magnetic Beads can be re-used after bead regeneration with EDTA. However, a very low level of protein may remain on the beads after regeneration. For best quality, it is recommended to use new Ni-NTA magnetic beads for purification.
5. Boiling the beads in SDS-PAGE reducing sample buffer will cause loss of binding activity of the beads, in which case the beads cannot be re-used.

B. Additional materials recommended

1. Binding Buffer: 5mM imidazole, 0.5M NaCl, 20mM Tris-HCl, pH 8.0
2. Washing Buffer: 20mM imidazole, 0.25M NaCl, 10mM Tris-HCl, pH 8.0
3. Elution Buffer: 500mM imidazole, 0.25M NaCl, 10mM Tris-HCl, pH 8.0
4. Strip Buffer: 0.5M NaCl, 100mM EDTA, 20mM Tris-HCl, pH 8.0
5. Charge Buffer: 50mM $NiSO_4$
6. Storage buffer after regeneration of the beads: 20% ethanol
7. A magnetic stand or a 96-well magnetic bead automation processor

C. Purification procedure

1. Gently mix the magnetic beads thoroughly before use by repeated inversion.
2. Place 50µl of magnetic beads (2.5mg) into a 1.5ml sterile microcentrifuge tube.
3. Place the tube on a magnetic stand, collect the beads and discard the supernatant.
4. Wash the beads three times with Binding Buffer (500µl each time) by magnetic separation, and re-suspend the beads in 200-750µl of Binding Buffer.
5. Add the same volume (200-750µl) of cell lysate to the bead suspension, mix thoroughly and incubate for 30min at room temperature on a rotator.
6. Collect the beads with a magnet and save the supernatant for analysis if desired.
7. Wash the protein-coupled beads three times with Washing Buffer (1ml each time), and incubate for 3min each time before applying magnet to separate beads from buffer.
8. Suspend the beads in 100µl of Elution Buffer, and incubate for 15 min. Apply magnet and transfer the supernatant to a clean microcentrifuge tube. Repeat this step once if desired. Combine the eluates from multiple elutions.
9. The purified protein is ready for use (e.g. determination of protein content or analysis by SDS-PAGE).

D. Regeneration of the Ni-NTA magnetic beads

1. After elution of the protein, wash the beads three times with Strip Buffer (500µl each time), and incubate for 5min each time before applying magnet to separate beads from buffer.
2. Wash the beads three times with deionized water (500µl each time) by magnetic separation.
3. Add 1ml of Charge Buffer to the beads and incubate for 30min on a rotator. Collect the beads with a magnet.
4. Re-suspend the beads in Storage Buffer. Store at 2-8 º C until ready for use.

【Storage】

Stored at 2-8°C, 2 years.

【Manufacturer】

Avanbio, ABA-04001, ABA-04002, ABA-04003

【Troubleshooting】

A. Low recovery of His-fusion proteins after the purification using the beads
   1. Protein may degrade. Add protease inhibitors to cell lysate preparations.
   2. The protein level is high. Increase the amount of magnetic beads accordingly.
   3. Insufficient target His-fusion protein is present in sample. Optimize expression conditions or increase the amount of sample.

B. Poor protein purity
   1. Insufficient washing. Adjust imidazole concentration in the Binding and/or Washing Buffer. Wash the beads at least two more times.

C. Non-specific proteins are observed in eluted sample
   1. Nonspecific proteins bind to the beads. Adjust the recommended imidazole concentration in Binding, Washing and Elution Buffers.