INSTRUCTIONS

EmerTher Ni-NTA (His-tag Affinity) Magnetic Beads

Catalog Number: AE04001 AE04002

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【Introduction】

EmerTher™ 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, EmerTher™ 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 crude cell lysate and His-tagged fusion protein binds to the beads. After washing unbound proteins off, the target His-fusion protein is eluted from the beads for downstream experiments. The process can be completed manually or fully automated for high throughput applications.

【Product Specifications】

Diameter： 500 nm

Magnetic response rate: >30 emu/g

pH stability： pH 3-12

Solvent： 1 mM $NiSO_4$, 50 mM Tris-HCl (pH 8.0), containing 20% ethanol

Binding capacity: 10-60 μg His-tagged fusion protein per mg magnetic beads

【Product Content】

Catalog Number	Conc. (mg/ml)	Volume (ml)	Amount of Beads (mg)
AE04001	50	1	50
AE04002	50	4	200

【Purification Protocol】

The following protocol provides general guidelines for purification of His-tagged fusion proteins using EmerTher™ Ni-NTA Magnetic Beads and may be modified by 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 EmerTher™ Ni-NTA Magnetic Beads does not depend on the 3-dimensional structure of the protein or His tag. Therefore, EmerTher™ 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. EmerTher™ 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.
6. If a His-tagged protein is unstable at room temperature, it is recommended to perform the purification process (binding, washing and elution steps) at 4°C and extend the time for each step as needed.
7. Avoid freezing, drying and centrifugation at a high speed during use and storage of beads. Otherwise, this may decrease the binding capacity of the beads.

B. Additional materials recommended:

1. Buffers recommended for purification under native conditions:
   1) Binding Buffer: 1 mM imidazole，0.5 M NaCl，20 mM Tris-HCl，pH 8.0
   2) Washing Buffer: 5 mM imidazole，0.25 M NaCl，0.05% Tween-20，10 mM Tris-HCl，pH 8.0
   3) Elution Buffer: 500 mM imidazole，0.25 M NaCl，10 mM Tris-HCl，pH 8.0

2. Buffers recommended for purification under denaturing conditions:
   1) Binding Buffer: 1 mM imidazole，0.25 M NaCl，6 M guanidine-HCl, 0.05% Tween-20, 20 mM Tris-HCl，pH 8.0
   2) Washing Buffer: 5 mM imidazole，0.25 M NaCl，6 M guanidine-HCl，0.05% Tween-20, 10 mM Tris-HCl，pH 8.0
   3) Elution Buffer: 500 mM imidazole，0.25 M NaCl，6 M guanidine-HCl，10 mM Tris-HCl，pH 8.0

3. Bead regeneration and storage:
   1) Strip Buffer: 0.5 M NaCl，100 mM EDTA，20 mM Tris-HCl，pH 8.0
   2) Charge Buffer: 50 mM $NiSO_4$
   3) Storage buffer after regeneration of the beads: 20% ethanol

4. A magnetic stand or a 96-well magnetic bead automatic processor

C. Purification procedure:

Two sets of buffers are recommended for purification in Section B. If His-tagged proteins have been released into cell lysis and are soluble, buffers recommended for native conditions can be used. If overexpressed His-tagged proteins are present in inclusion bodies, the proteins may be insoluble and thus it is necessary to purify proteins under denaturing conditions, i.e. add denaturants, such as 6 M guanidine-HCl, to enhance protein solubility.

1. Gently mix the magnetic beads thoroughly before use by shaking.
2. Place 20 µl of magnetic beads (1 mg) into a 2 ml sterile microcentrifuge tube.
3. Place the tube on a magnetic stand, collect the beads and discard the supernatant.
4. Wash the beads with 500 µl Binding Buffer by magnetic separation, and re-suspend the beads in 200-750µl of Binding Buffer.
5. Add the same volume (200-750 µl) of cell lysate to the bead suspension; mix thoroughly and incubate at room temperature on a rotator for 30 min.
6. Collect the beads with a magnet and save the supernatant for analysis if desired.
7. Wash the protein-coupled beads 3-5 times with Washing Buffer (1ml each time), incubate for 5 min each time before applying magnet to separate beads from buffer.
8. Suspend the beads in 100 µl of Elution Buffer, 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 elution steps.
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), incubate for 5 min 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 and discard the supernatant.
3. Add 1ml of Charge Buffer to the beads and incubate for 30 min. Collect the beads with a magnet.
4. Re-suspend the beads in Storage Buffer. Stored at 2-8°C until ready for use.

【Storage】

Shipped at room temperature. Stored at 2-8 °C, 2 years

【Troubleshooting】

A. Low recovery of His-fusion proteins after the purification using the beads
   1. Proteins may degrade. Add protease inhibitors to cell lysate preparations or perform the purification process at 4°C.
   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.
   2. Increase the number of wash steps or the volume of the washing buffer.

C. Non-specific proteins are observed in eluted sample
   1. Proteins may degrade. Add protease inhibitors to cell lysate preparations or perform the purification process at 4°C.
   2. Nonspecific proteins bind to the beads. Adjust the recommended imidazole and detergent concentrations in Binding, Washing and Elution Buffers.

D. Beads tend to aggregate during binding and are difficult to re-suspend
   1. Insufficient detergent concentration. Increase detergent concentration to 0.1%.
   2. Protein concentration is too high. Reduce protein concentration.

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