



## His-tagged Protein Purification Kit

### Product Information

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**Cat**

Kit-0887

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**Common Name**

Protein

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**Cat.No.**

Kit-0887

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**Product Overview**

Ni-NTA Magnetic nano beads are silica beads, with an average diameter of 0.4  $\mu\text{m}$  and a range of 0.2~0.8  $\mu\text{m}$  diameter, that contain magnetic particles and have strongly metal-chelating nitrilotriacetic acid (NTA) groups covalently bound to their surfaces.

They are precharged with nickel and ready to use for capturing 6xHis-tagged proteins under native conditions for protein expression screening programs, as well as smallscale purification of 6xHis-tagged proteins.

Ni-NTA Magnetic nano Beads are supplied as a 10% (v/v) suspension with a binding capacity of 3 mg protein per ml of suspension for 6xHis-tagged DNA polymerase

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**Storage**

Ni-NTA Magnetic nano beads are supplied as a 10% (v/v) suspension in 20% ethanol and should be store at 2~8°C.

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**Kit Components**

Ni-NTA magnetic Nano Beads 5 X 1 mL(10%);

Binding/Washing buffer 100 mL;

Elution buffer 15 mL;

Nd magnet 3 ea;

User's Guide 1 ea;

Manual 1 ea.



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### Features & Benefits

**Flexible:** The protocol may be carried out with a magnet or via centrifugation.

**Excellent performance:** Highly pure proteins are obtained through our exclusive SSMB (Spherical-Shape Magnetic Beads).

**High purity and High yield:** Average binding capacity of 500  $\mu$ g protein per ml of suspension for 6xHis-tagged protein. Purity that is > 90%.

**Reproducibility:** The only hands-on step is the addition of protein extract, maximizing reproducibility.

**Fast process:** The entire purification is complete in about 30 minutes!

### Preparation

1. Inoculate 3 mL of LB medium containing 50  $\mu$ g/mL ampicillin or 20  $\mu$ g/mL kanamycin with a fresh bacterial colony harboring the expression plasmid. Grow at 37 °C, 200 rpm overnight
2. Inoculate 3mL pre-warmed medium (including antibiotics) with 200  $\mu$ L of the overnight cultures, and grow at 37°C for 2 hr, with vigorous shaking, until the OD<sub>600nm</sub> is 0.6~0.8.
3. Induce expression by adding IPTG to a final concentration of 1 mM.
4. Grow the cultures for an additional 4~5 hr, and transfer to micro-centrifuge tubes. Harvest the cells by centrifugation for 1 min at 12,000 rpm, and discard supernatant.
5. Resuspend cells in 500  $\mu$ L Binding/washing buffer. Sonicate on ice a sonicator equipped with a micro-tip.
6. Centrifuge lysate at 12,000rpm for 5~10 min at 4°C to pellet the cellular debris. Save supernatant.

### Assay Protocol

#### 1. Experimental Protocol with magnet

1. Equilibrate the Ni-NTA magnetic nano beads with 1 mL Binding/washing buffer. Place the tube on a Nd magnet for 1 min, and remove supernatant with a pipet. Repeat step 1 one more.
2. Load up to 500  $\mu$ L of the cleared lysate containing the 6xHis-tagged protein on to the pre-equilibrated Ni-NTA magnetic nano beads.
3. Mix by inverting 5 or 10 time (or gentle vortexing). Place the tube on a Nd magnet for 1 min, and collect loading waste.

Note: Save the waste fractions for analysis by SDS-PAGE to check binding efficiency.

4. Remove tube from the magnet, add 1 mL of Binding/ washing buffer, mix the suspension, place

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the tube on a Nd magnet for 1 min, and remove Binding/washing buffer.

Repeat step 4 another 2 times.

Note: Save the wash fractions for analysis by SDS-PAGE to check washing conditions.

- Buffer remaining after the final wash should be removed completely.

5. Remove tube from the magnet, add 500  $\mu$ L of elution buffer, mix the suspension, incubate the tube for 1 min, place for 1 min on Nd magnet, and collect the eluate. Repeat step 5.

Note: Most of the 6xHis-tagged protein will elute in the first elution step. If a more concentrated protein solution is

required, elute in two aliquots of 300  $\mu$ L.

- Magnetic nano beads can be used with MagListo-2 magnetic separation rack

### 2. Experimental protocol with centrifuge

1. Equilibrate the Ni-NTA magnetic nano beads with 1 mL Binding/washing buffer. Centrifuge for 30 sec at 12,000 rpm, and remove supernatant with a pipet. Repeat step 1 another 1 times.

2. Load up to 500  $\mu$ L of the cleared lysate containing the 6x His-tagged protein on to the pre-equilibrated Ni-NTA magnetic nano beads.

3. Mix by inverting 5 or 10 time (or gentle vortexing). Centrifuge for 30 sec at 12,000 rpm, and collect loading waste.

Note: Save the waste fractions for analysis by SDS-PAGE to check binding efficiency.

4. Add 1 mL of Binding/washing buffer, mix the suspension, Centrifuge for 30 sec at 12,000 rpm, and remove Binding/washing buffer. Repeat step 4 another 2 times.

Note: Save the wash fractions for analysis by SDS-PAGE to check washing conditions.

- Buffer remaining after the final wash should be removed completely.

5. Add 500  $\mu$ L of elution buffer, mix the suspension, incubate the tube for 1 min, Centrifuge for 30 sec at 12,000 rpm, and collect the eluate. Repeat step 5.

Note: Most of the 6xHis-tagged protein will elute in the first elution step. If a more concentrated protein solution is required, elute in two aliquots of 300  $\mu$ L.