

His-tagged Protein Purification Kit

Cat. No. Kit-0887 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Ni-NTA Magnetic nano beads are silica beads, with an average diameter of 0.4 μm and a range of 0.2~0.8 μm 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
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.
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.
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 μg protein per ml of

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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 µg/mL ampicillin or 20 µg/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 µL of the overnight cultures, and grow at 37°C for 2 hr, with vigorous shaking, until the OD_{600nm} 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 µL 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 µL 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 the tube on a Nd magnet for 1 min, and remove Binding/washing

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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 μ 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 μ 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 μ 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 μ 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 μ L.

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