CREATIVE BIOMART®
Assay Kit

His-tagged Protein Purification Kit

Product Information

Cat

Kit-0887

Common Name

Protein

Cat.No. Kit-0887

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

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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 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 μLof the overnight cultures, and grow at 37°C for 2 hr, with vigorous shaking, until the OD600nmis 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 buffer.

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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 μL 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 μL.
- 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 μL 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 μL 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 μL.