MagListo™ His-tagged Protein Purification kit

I. Description

Ni-NTA Magnetic Silica Resins 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 small-scale purification of 6xHis-tagged proteins.
Ni-NTA Magnetic silica 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.

II. KIT COMPONENTS

His-tagged Protein Purification kit(Cat. No. K-7200)	
Ni-NTA magnetic silica resin	5X1 ml(10%)
Binding/Washing buffer	100 ml
Elution buffer	15 ml
Nd magnet	3 ea
User's Guide	1 ea

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

III. Before you begin

i. 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 ℃, 200 rpm overnight

ii. Inoculate 3mL pre-warmed medium (including antibiotics) with 200 μLof the overnight cultures, and grow at 37℃for 2 hr, with vigorous shaking, until the OD600nmis 0.6~0.8.

iii. Induce expression by adding IPTG to a final concentration of 1 mM.

iv. 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.

v. Resuspend cells in 500 μL Binding/washing buffer. Sonicate on ice a sonicator equipped with a micro-tip.

vi. Centrifuge lysate at 12,000rpm for 5~10 min at 4℃to pellet the cellular debris. Save supernatant.

IV. Experimental protocol for magnetic purification

1. **Equilibrate the Ni-NTA magnetic silica resin 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 another 1 times.

2. **Load up to 500 μL of the cleared lysate containing the 6xHis-tagged protein on to the pre-equilibrated Ni-NTA magnetic silica resin.**

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.**
   - 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.**
   - Repeat step 4 another 2 times.
   - 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.
   - 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.

Note: *Magnetic beads can be used with MagListo™-2 magnetic separation rack.*

V. Experimental protocol for centrifuge purification

1. **Equilibrate the Ni-NTA magnetic silica resin 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 silica resin.**

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

VI. Troubleshooting

6xHis-tagged protein elutes in the wash buffer
Lower the concentration of imidazole or increase the pH slightly.
Reduce wash stringency. Purify under denaturing conditions.
Check pH and composition of the wash buffer.
6xHis-tagged protein does not elute
Elute with decreased pH or increased imidazole concentration.
Try EDTA, but bear in mind that elution will be as a 6x His-tagged protein–Ni complex.

VII. Supplement

Purification of 6xHis-tagged protein.

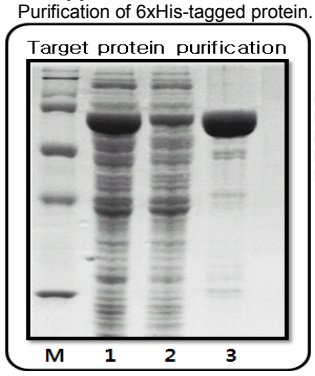

Figure 1.
Purification of target protein (94 kDa).
M: Protein size marker
Lane 1: loaded sample
Lane 2: unbound sample
Lane 3: eluted sample

VIII. References

1. Porath, J., et al. (1975), Nature 258, 598-599.
2. Hochuli, E. (1989), *Biologically Active Molecules*. 217-239.
3. Hochuli, E. (1990), *Setlow, J.K.*, ed. 12, 87-98.

IX. Ordering Information

Cat. No.	Product Description
K-7200	*MagListo*™ His-tagged Protein Purification kit (5 x 1 mL(10%))
TS-1070-1	Ni-NTA Magnetic beads, size 400 nm (0.5 g / 25 mL)
TM-1010	MagListo™-2 magnetic separation rack
TM-1020	MagListo™-15 magnetic separation rack
TM-1030	MagListo™-50 magnetic separation rack
TM-1040	MagListo™-96 magnetic separation rack

Trademarks