ISO 9001
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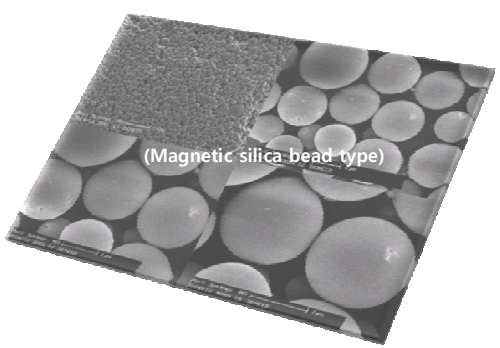
Ver. 1.0

User's Guide

Global Genomics Partner

AccuPrep®
His-tagged Protein Purification kit

Cat. No. K-7200



BIONEER
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Safety Warnings and Precautions

AccuPrep® His-tagged protein purification kit is developed and sold for research purposes only. It is not recommended for human or animal diagnostic use, unless cleared for such purposes by the appropriate regulatory authorities in the country of use.

Wear appropriate protection when handling any irritant or harmful reagents. The use of a laboratory coat, protective gloves and safety goggles are highly recommended. For more information please consult the appropriate Material Safety Data Sheets (MSDS).

Warranty and Liability

All BIONEER products undergo extensive Quality Control testing and validation. BIONEER guarantees quality during the warranty period as specified, when following the appropriate protocol as supplied with the product. It is the responsibility of the purchaser to determine the suitability of the product for its particular use. Liability is conditional upon the customer providing full details of the problem to BIONEER within 30 days.

Quality Management System ISO 9001 Certified

Every aspect of Bioneer's quality management system from product development to production to quality assurance and supplier qualification meets or exceeds the world-class quality standards.

Trademarks

AccuPrep® is trademark of Bioneer Corporation in Korea.

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I. Description

Ni-NTA Magnetic Silica Resins are silica beads, with an average diameter of 1.29 μm and a range of 1~5 μm diameter, that contain magnetic particles and have strongly metal-chelating nitrilotriacetic acid (NTA) groups covalently bound to their surfaces (Figure 1).
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 500 μg protein per ml of suspension for 6xHis-tagged Phosphatase.

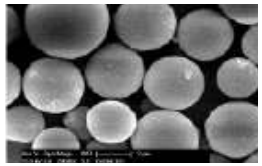

Figure 1. Ni-NTA Magnetic Silica Resins

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	3ea
User's Guide	1ea

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.

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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 3 mL prewarmed medium (including antibiotics) with 200 μLof the overnight cultures, and grow at 37℃ for 2 hr, with vigorous shaking, until the OD_{600nm} is 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 microcentrifuge tubes. Harvest the cells by centrifugation for 1 min at 12,000 rpm, and discard supernatants.

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

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.

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

Quality of Protein after purification

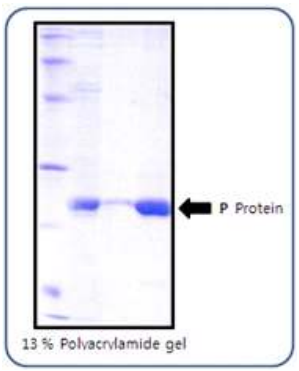

Figure 2. A : P protein was purified for *AccuPrep*® His-tagged Protein Purification kit.

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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.