INSTRUCTIONS

# HisPur™ Ni-NTA Magnetic Beads

88831 88832 2385.1

| Number | Description |
|---|---|
| **88831** | **HisPur Ni-NTA Magnetic Beads,** 2mL, supplied at 12.5mg/mL in 20% ethanol |
| **88832** | **HisPur Ni-NTA Magnetic Beads,** 10mL, supplied at 12.5mg/mL in 20% ethanol |

**Storage:** Upon receipt store at 4°C. Product is shipped with an ice pack.

## Table of Contents



## Introduction

The Thermo Scientific™ HisPur™ Ni-NTA Magnetic Beads enable effective immobilized metal affinity chromatography (IMAC) purification of polyhistidine-tagged proteins from a soluble protein extract. The beads contain nickel-charged nitrilotriacetic acid (Ni-NTA) chelate immobilized onto a blocked magnetic surface. The Ni-NTA Magnetic Beads are compatible with native or denaturing conditions and can be used in manual applications with a magnetic stand or automated applications with an instrument such as the Thermo Scientific KingFisher Flex System. Ni-NTA Magnetic Beads are chosen for His-tagged-protein purification because of the four metal-binding sites on the chelate, which allow for high-binding capacity and low-metal ion leaching.

**Table 1. Characteristics of the Thermo Scientific HisPur Ni-NTA Magnetic Beads.**

| | |
|---|---|
| **Composition:** | Nickel on nitrilotriacetic acid covalently coupled on a blocked magnetic bead surface |
| **Magnetization:** | Superparamagnetic (no magnetic memory) |
| **Mean Diameter:** | 1µm (nominal) |
| **Density:** | 2.0g/cm$^3$ |
| **Bead Concentration:** | 12.5mg/mL in 20% ethanol |
| **Binding Capacity:** | ≥ 40µg green fluorescent protein (GFP)/mg of bead |


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## Important Product Information

- Do not centrifuge, dry or freeze the HisPur Magnetic Beads. Handling the beads in this way will cause the beads to aggregate and lose binding capacity.
- Protein yield and purity are dependent upon the expression level, conformation and solubility characteristics of the recombinant fusion protein; therefore, it is important to optimize these parameters. For best results, perform a small-scale test to estimate the expression level and determine the solubility of each His-tagged protein.
- Optimization of the lysis procedure is critical for maximizing protein yield. Some methods for protein extraction include using commercially available detergent-based reagents, such as Thermo Scientific™ B-PER™ Bacterial Protein Extraction Reagent with Enzymes (Product No. 90078), and mechanical methods, such as freeze/thaw cycles, sonication or French press. Add EDTA-free protease inhibitors, such as Thermo Scientific™ Halt™ Protease and Phosphatase Inhibitor Cocktail, EDTA-free (Product No. 78441), to protect proteins from degradation.
- These instructions are effective for many types of samples; however, optimization may be required to further reduce nonspecific binding. To optimize conditions, adjust the recommended imidazole concentration in the Equilibration, Wash and Elution Buffers.
- Avoid using protease inhibitors or other additives that contain chelators, such as EDTA, or strong reducing agents, such as DTT or β-mercaptoethanol, which will disrupt the function of the HisPur Ni-NTA Magnetic Beads.
- Concentration of proteins in the eluted fractions can be determined by using the Thermo Scientific™ Pierce™ 660nm Protein Assay Kit (Product No. 22662).
- When scaling up, use 2-3 volumes of Equilibration, Wash and Elution Buffers per volume of bead slurry.
- Do not regenerate the HisPur Ni-NTA Magnetic Beads.

## Additional Materials Required

**Note:** The buffers listed below are recommendations. To decrease nonspecific binding and increase yield, adjustments to the imidazole concentration may be required for specific proteins.

- Vary the imidazole concentration in the Elution Buffer from 250mM to 500mM.
- Vary the imidazole concentration in the Equilibration Buffer from 5mM to 50mM and in the Wash Buffers from 10mM to 50mM.
- Purification of Protein L from cell lysate is optimal with 10mM imidazole in the Equilibration Buffer and 25mM imidazole in the Wash Buffer.
- Purification of GFP and β-galactosidase from cell lysate are optimal with 30mM imidazole in the Equilibration Buffer and 50mM imidazole in the Wash Buffer.

**A. For native conditions, prepare the following buffers:**

- Equilibration Buffer: Phosphate-buffered saline (PBS; 100mM sodium phosphate, 600mM sodium chloride), 0.05% Tween™-20 Detergent, 30mM imidazole; pH 8.0
- Wash Buffer: PBS, 0.05% Tween-20 Detergent, 50mM imidazole; pH 8.0
- Elution Buffer: PBS, 250mM imidazole; pH 8.0

**B. For denaturing conditions, prepare the following buffers:**

- Equlibration Buffer: PBS, 6M guanidine•HCl, 0.05% Tween-20 Detergent, 30mM imidazole; pH 8.0
- Wash Buffer: PBS, 6M guanidine•HCl, 0.05% Tween-20 Detergent, 50mM imidazole; pH 8.0
- Elution Buffer: PBS, 6M guanidine•HCl, 250mM imidazole; pH 8.0

## Procedure for Manual Purification of His-tagged Proteins

### A. Additional Materials Required

- 1.5mL microcentrifuge tubes
- Sample containing His-tagged protein
- Magnetic stand (e.g., Thermo Scientific MagnaBind Magnet for 6 × 1.5mL microcentrifuge tubes; Product No. 21359)

### B. Purification Using a Magnetic Stand

**Note:** To ensure homogeneity, mix the beads thoroughly before use by repeated inversion, gentle vortexing or slow mixing using a rotating platform.

1. Place 40µL (0.5mg) of HisPur Ni-NTA Magnetic Beads into a 1.5mL microcentrifuge tube.

   **Note:** This procedure can be scaled up to accommodate higher volumes of beads (see the Important Product Information Section).
2. Add 160µL of Equilibration Buffer to the beads and vortex for 10 seconds to mix.
3. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.
4. Add 400µL of Equilibration Buffer to the tube. Vortex the beads for 10 seconds and collect the beads with a magnetic stand. Remove and discard the supernatant.
5. Prepare sample by diluting the protein extract with an equal volume of Equilibration Buffer.
6. Add 400µL of prepared protein extract to the tube, vortex for 10 seconds and then mix on an end-over-end rotator for 30 minutes.
7. Collect the beads by placing the tube on a magnetic stand. If desired, save the supernatant (flow-through) for downstream analysis.
8. Add 400µL of Wash Buffer to the tube and mix well. Collect the beads with a magnetic stand, then remove and discard the supernatant.
9. Repeat wash step once.
10. Add 25µL of Elution Buffer to the tube and vortex for 15 seconds. If needed, centrifuge the tube for 1 minute at 700 × *g* to ensure all of the beads are submerged in the Elution Buffer. Incubate the beads for 15 minutes on a rotating platform. Alternatively, vortex the tube for 15 seconds every 5 minutes.
11. Collect the beads on a magnetic stand. Carefully remove and save the supernatant containing the His-tagged protein.
12. Repeat the elution step once using 25µL of Elution Buffer. Incubate the beads for 10 minutes. Combine the two eluates, if desired.
13. Monitor the elution for protein content using the Pierce 660nm Protein Assay Kit (Product No. 22662). Eluted protein can also be directly analyzed by SDS-PAGE.

    **Note:** To remove imidazole for downstream applications, use gel filtration (e.g., Thermo Scientific™ Zeba™ Spin Desalting Columns) or dialysis (e.g., Thermo Scientific™ Slide-A-Lyzer™ Dialysis Cassettes). Samples containing 6M guanidine•HCl must be dialyzed against a buffer containing 8M urea before SDS-PAGE analysis to be compatible and retain proteins in solution. The Thermo Scientific™ Pierce™ SDS-PAGE Sample Prep Kit (Product No. 89888) may also be used to remove guanidine.

## Procedure for Automated Purification of His-tagged Proteins

### A. Additional Materials Required

- KingFisher Flex System with 96 deep well head (Product No. 5400630)
- Thermo Scientific™ Microtiter Deep Well 96 Plate, V-bottom, polypropylene (100-1000µL; Product No. 95040450)
- KingFisher Flex 96 Tip Comb for Deep Well Magnets (Product No. 97002534)

## B. Instrument Preparation and Plate Set-up

**Note:** The following protocol is designed for use with the KingFisher Flex Instrument. The protocol can be modified according to your needs using the Thermo Scientific™ BindIt™ Software provided with the instrument.

1. Download the "His_Tag_Protein_Purification" protocol from the Thermo Fisher Scientific website (http://www.thermoscientific.com/bindit-protocols) into the BindIt Software on an external computer.
2. Transfer the protocol to the KingFisher Flex Instrument from an external computer. See the BindIt Software User Manual for detailed instructions on importing protocols.
3. Set up plates according to Table 2.

**Table 2. Pipetting instructions for the His-tagged Protein Purification protocol using the Thermo Scientific Microtiter Deep Well 96 Plates.**

| Plate # | Plate Name | Content | Volume | Time/Speed |
|---|---|---|---|---|
| 1 | Beads | Beads | 40µL | 15 seconds |
| | | Equilibration Buffer | 160µL | |
| 2 | Bead Equilibration | Equilibration Buffer | 400µL | 30 seconds/Medium |
| 3 | Bind | Protein in Equilibration Buffer | 400µL | 30 minutes/Slow |
| 4 | Wash 1 | Wash Buffer | 400µL | 15 seconds/Slow |
| 5 | Wash 2 | Wash Buffer | 400µL | 15 seconds/Slow |
| 6 | Elution 1 | Elution Buffer | 100µL | 15 minutes/Medium |
| 7 | Elution 2 | Elution Buffer | 100µL | 10 minutes/Medium |
| 8 | Tip Plate | KingFisher Flex 96 Tip Comb for Deep Well Magnets | - | 10 seconds/Fast |

## C. Automated His-tagged Protein Purification Protocol

1. Select the protocol using the arrow keys on the instrument keypad and press Start. See the KingFisher Flex Instrument User Manual for detailed information.
2. Slide open the door of the instrument's protective cover.
3. Load plates into the instrument according to the protocol requests, placing each plate in the same orientation. Confirm each action by pressing Start.
4. After sample processing, remove the plates as instructed by the instrument's display. Press Start after each plate. Press Stop after removing all of the plates.

**Notes:**

- If fewer than 96 wells are used, fill the same wells in each plate. For example, if using wells A1 through A12, use these same wells in all plates.
- Combine the Tip Comb with a Deep Well 96 Plate. See the instrument user manual for detailed instructions.
- A minimum volume of 100µL is required for efficient elution of bound protein.

## Troubleshooting

| Problem | Possible Cause | Solution |
|---|---|---|
| Low protein yield | Poor expression of soluble protein | Optimize expression conditions |
| | His-tagged protein formed inclusion bodies | Alter growth conditions to minimize inclusion body formation and maximize soluble protein yield; alternatively, solubilize inclusion bodies and perform the purification with a compatible denaturant (e.g., Thermo Scientific™ Inclusion Body Solubilization Reagent, Product No. 78115) |
| | Insufficient cell lysis and extraction | Optimize the cell lysis protocol |
| | Fusion protein did not bind to the magnetic beads | Verify the sequence |
| | | Perform an ELISA or Western blot using an antibody against the His-tagged protein to ensure the His-tagged protein is present |
| Poor protein purity | Insufficient washing | Wash beads a minimum of two additional times |
| | | Adjust imidazole concentration of the Equilibration and/or Wash Buffer |
| Beads aggregate during the binding step | Detergent missing or insufficient in the Equilibration Buffer | Vortex beads periodically during the binding step (e.g., every 10 minutes) |
| | | Increase Equilibration Buffer detergent concentration (e.g., increase detergent concentration from 0.05% to 0.1%) |

## Additional Information Available on Our Website

- Tech Tip #43: Protein stability and storage
- Visit *www.thermoscientific.com/kingfisher* for information on the KingFisher Products
- In the U.S.A., purchase KingFisher Supplies from Fisher Scientific. Outside the U.S.A., contact your local Thermo Fisher Scientific office to purchase KingFisher Supplies.

## Related Thermo Scientific Products

**88826-7** **Pierce NHS-Activated Magnetic Beads**
**88828** **Pierce Direct Magnetic IP/Co-IP Kit**
**88802-3** **Pierce Protein A/G Magnetic Beads**
**88804** **Pierce Classic Magnetic IP/Co-IP Kit**
**88805** **Pierce Crosslink Magnetic IP/Co-IP Kit**
**88816-7** **Pierce Streptavidin Magnetic Beads**
**88821-2** **Pierce Glutathione Magnetic Beads**
**88836-7** **Pierce Anti-HA Magnetic Beads**
**8838** **Pierce HA-Tag Magnetic IP/Co-IP Kit**
**22660** **Pierce 660nm Protein Assay Reagent**
**22662** **Pierce 660nm Protein Assay Kit**
**88221-6** **HisPur Ni-NTA Resin, Spin Columns**
**88227-9** **HisPur Ni-NTA Spin Purification Kits**
**89964-9** **HisPur Cobalt Resin and Spin Columns**
**90090-2** **HisPur Cobalt Purification Kits**
**28320** **Surfact-Amps™ Tween-20 Detergent Solution**

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