# HisPur™ Ni-NTA Spin Columns

Catalog Numbers 88224, 88225, and 88226

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**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

## Contents and storage

| Item | Catalog Number | Storage |
|---|---|---|
| HisPur™ Ni-NTA Spin Columns, 0.2 mL resin bed | 88224 | Store at 4°C |
| HisPur™ Ni-NTA Spin Columns, 1.0 mL resin bed, 5 each | 88225 | |
| HisPur™ Ni-NTA Spin Columns, 3.0 mL resin bed, 5 each | 88226 | |
| Binding Capacity: ≤60 mg of a 28 kDa 6x His-tagged protein from a bacterial source per milliliter of settled resin<br>Resin: Crosslinked 6% agarose supplied as a 50% slurry in a 20% ethanol solution | | |

## Product description

The Thermo Scientific™ HisPur™ Ni-NTA Spin Columns enable effective immobilized metal affinity chromatography (IMAC) purification of polyhistidine-tagged proteins from a soluble protein extract. These spin columns contain nickel-charged nitrilotriacetic acid (NTA) chelate immobilized onto 6% crosslinked agarose resin. The Ni-NTA resin is compatible with native or denaturing conditions and can be used in multiple formats, including conventional gravity-flow chromatography, spin column, and fast protein liquid chromatography (FPLC). Ni-NTA resins are commonly chosen for His-tagged protein purification because of the 4 metal-binding sites on the chelate, which allow for high-binding capacity and low-metal ion leaching.

## Required materials not provided

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

**For native conditions prepare the following buffers:**

- Equilibration Buffer: 20 mM sodium phosphate, 300 mM sodium chloride (PBS) with 10 mM imidazole; pH 7.4
- Wash Buffer: PBS with 25 mM imidazole; pH 7.4
- Elution Buffer: PBS with 250 mM imidazole; pH 7.4

**For denaturing conditions prepare the following buffers:**

- Equilibration Buffer: PBS with 6M guanidine-HCl and 10 mM imidazole; pH 7.4
- Wash Buffer: PBS with 6M guanidine-HCl and 25 mM imidazole; pH 7.4
- Elution Buffer: PBS with 6M guanidine-HCl and 250 mM imidazole; pH 7.4

**For resin regeneration prepare the following buffer:**

- MES Buffer: 20 mM 2-(*N*-morpholine)-ethanesulfonic acid, 0.1 M sodium chloride; pH 5.0

## Important product information

- 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 before attempting a large-scale purification. 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 (Cat. 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 (Cat. No. 78441), to protect proteins from degradation.
- Sometimes overexpressed proteins are sequestered in inclusion bodies. Inclusion bodies of His-tagged proteins can be solubilized in 8 M urea, 6 M guanidine or Thermo Scientific™ Inclusion Body Solubilization Reagent (Cat. No. 78115) and purified with the Ni-NTA resin, but a denaturant must be added to buffers so the protein remains soluble throughout the procedure.
- These instructions are effective for many types of samples; however, optimization might 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 nickel resin.

- When using the Thermo Scientific™ Coomassie Plus™ (Bradford)™ Assay (Cat. No. 23238) or Thermo Scientific™ Pierce™ 660 nm Protein Assay (Cat. No. 22660) to monitor protein concentration in the elution fractions, dilute the samples at least 1:2 before performing the protein assay.

## Procedure for spin purification of His-Tagged proteins

**Note:** The total volume of the 0.2-, 1-, and 3-mL columns are 1 mL, 8 mL, and 22 mL, respectively. If a sample volume is greater than the column, perform multiple applications and centrifugations until the entire sample has been processed. Be careful not to exceed the resin's binding capacity. The HisPur™ Ni-NTA Spin Columns also may be used for gravity-flow purifications.

1. Equilibrate column(s) to working temperature. Perform purifications at room temperature or 4°C.
2. Prepare sample by mixing the protein extract with an equal volume of Equilibration Buffer. Use the Equilibration Buffer to adjust the total volume to be ≥2 resin-bed volumes.
3. Remove the bottom closure from the HisPur™ Ni-NTA Spin Column by gently twisting (SAVE closure for later use). Place column into a centrifuge tube.

   **Note:** Use 2.0-, 15-, or 50-mL centrifuge tubes for the 0.2 mL, 1 mL, and 3 mL spin columns, respectively.
4. Centrifuge column at 700 x *g* for 2 minutes to remove storage buffer.
5. Equilibrate column with two resin-bed volumes of Equilibration Buffer. Allow buffer to enter the resin bed.
6. Centrifuge column at 700 x *g* for 2 minutes to remove buffer.
7. Place the bottom closure in the column and add the prepared protein extract. Mix on an orbital shaker or end-over-end mixer for 30 minutes at room temperature or 4°C.
8. Remove the bottom closure. Centrifuge the column at 700 x *g* for 2 minutes and collect the flowthrough in a centrifuge tube.
9. Wash resin with two resin-bed volumes of Wash Buffer. Centrifuge at 700 x *g* for 2 minutes and collect fraction in a centrifuge tube. Repeat this step 2 more times collecting each fraction in a separate centrifuge tube.
10. Elute His-tagged proteins from the resin by adding one resin-bed volume of Elution Buffer. Centrifuge at 700 x *g* for 2 minutes. Repeat this step 2 more times, collecting each fraction in a separate tube.
11. Monitor protein elution by measuring the absorbance of the fractions at 280 nm or by Coomassie Plus™ (Bradford)™ Assay Reagent (Cat. No. 23238). The eluted protein can be directly analyzed by SDS-PAGE.

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

## Procedure for Ni-NTA resin regeneration

The Ni-NTA resin may be used at least five times without affecting protein yield or purity. Between each use, perform the procedure as described below to remove residual imidazole and any nonspecifically adsorbed protein. To prevent cross-contamination of samples, designate a given column to one specific fusion protein.

1. Wash resin with 10 resin-bed volumes of MES Buffer.
2. Wash resin with 10 resin-bed volumes of ultrapure water.
3. Store resin as a 50% slurry in 20% ethanol.
4. Cap the column bottom by inverting the snap-off closure and applying to the column with gentle pressure.

## 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 (for example, Inclusion Body Solubilization Reagent, Product No. 78115). |
| | Insufficient cell lysis and extraction. | Optimize cell lysis protocol. |
| | Fusion™ protein did not bind to the column. | Verify the sequence or perform an ELISA or western blot using an antibody against the His tag to make sure the His tag is present. |
| Poor protein purity | Insufficient washing. | Wash resin additional times or modify imidazole concentration and pH of the Equilibration and/or Wash Buffer. |
| Slow column flow | Column is overloaded. | Apply less protein extract onto the column and make sure the extract is not too viscous or highly particulate. |

## Related products

| Product | Catalog Number |
|---|---|
| HisPur™ Ni-NTA Resin, 10 mL settled resin | 88221 |
| HisPur™ Ni-NTA Purification Kit, 0.2 mL, 25 each | 88227 |
| Pierce™ High Capacity Endotoxin Removal Resin | 88270 |
| Pierce™ LAL Chromogenic Endotoxin Quantitation Kit | 88282 |
| HisPur™ Cobalt Spin Columns, 0.2 mL, 25 each | 89967 |
| HisPur™ Cobalt Spin Columns, 1 mL, 5 each | 89968 |
| HisPur™ Cobalt Spin Columns, 3 mL, 5 each | 89969 |
| Guanidine-HCl, 500 g | 24110 |
| **B-PER™ Protein Extraction Reagent with Enzymes**, 250 mL | 90078 |
| Halt™ Protease Inhibitor Cocktail (100X), EDTA-free, 1 mL | 87785 |
| Halt™ Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X), 1 mL | 78441 |
| Coomassie Plus™ (Bradford)™ Assay Reagent, 300 mL | 23238 |
| Pierce™ 660 nm Protein Assay Reagent, 750 mL | 22660 |
| Inclusion Body Solubilization Reagent, 100 mL | 78115 |
| Zeba™ Spin Desalting Columns, 7K MWCO, 2 mL, 25 columns, for 200-700 µL samples | 89890 |
| Zeba™ Spin Desalting Columns, 7K MWCO, 5 mL, 25 columns, for 500-2000 µL samples | 89892 |
| Zeba™ Spin Desalting Columns, 7K MWCO, 10 mL, 25 columns, for 1500-4000 µL samples | 89894 |
| Zeba™ Spin Desalting Columns, 40K MWCO, 2 mL, 25 columns, for 200-900 µL samples | 87769 |
| Zeba™ Spin Desalting Columns, 40K MWCO, 5 mL, 25 columns, for 300-2000 µL samples | 87771 |
| Zeba™ Spin Desalting Columns, 40K MWCO, 10 mL, 25 columns, for 1000-4000 µL samples | 87773 |
| Slide-A-Lyzer™ G2 Dialysis Cassettes, 10K MWCO, 3 mL, 10 cassettes | 87730 |
| Slide-A-Lyzer™ G2 Dialysis Cassettes, 10K MWCO, 15 mL, 8 cassettes | 87731 |

Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

**Revision history:** Pub. No. MAN0011699 C

| Revision | Date | Description |
|---|---|---|
| C | 13 May 2024 | Updated spin column closures. |
| B.0 | 3 December 2021 | The buffer components for native conditions and denaturing conditions were updated. . |
| A.0 | 17 October 2015 | New document for HisPur™ Ni-NTA Spin Columns. |