

# HisPur™ Ni-NTA Resin

88221 88222 88223

2204.2

Number	Description
88221	<b>HisPur Ni-NTA Resin</b> , 10mL settled resin
88222	<b>HisPur Ni-NTA Resin</b> , 100mL settled resin
88223	<b>HisPur Ni-NTA Resin</b> , 500mL settled resin

Binding Capacity: ≤ 60mg of a 28kDa 6xHis-tagged protein from a bacterial source per milliliter of settled resin

Resin: Crosslinked 6% agarose

Supplied: 50% slurry in 20% ethanol

**Storage:** Upon receipt store at 4°C. Product shipped at ambient temperature.

## Introduction

The Thermo Scientific HisPur Ni-NTA Resin enables effective immobilized metal affinity chromatography (IMAC) purification of polyhistidine-tagged proteins from a soluble protein extract. This resin is composed of nickel-charged nitrilotriacetic acid (NTA) chelate immobilized onto 6% crosslinked agarose. 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 FPLC. Ni-NTA resins are commonly 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.

## 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 (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.
- Sometimes overexpressed proteins are sequestered in inclusion bodies. Inclusion bodies of His-tagged proteins can be solubilized in 8M urea, 6M guanidine or Thermo Scientific Inclusion Body Solubilization Reagent (Product 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 Buffer.
- 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 (Product No. 23238) or Thermo Scientific Pierce 660nm Protein Assay (Product No. 22660) to monitor protein concentration in the elution fractions, dilute the samples at least 1:2 before performing the protein assay.

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## Additional Materials Required

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

### For denaturing conditions prepare the following buffers:

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

### For resin regeneration prepare the following buffer:

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

## Procedure for Purification of His-Tagged Proteins by Batch Method

The HisPur Ni-NTA Resin allows for purification strategy customization. Purification conditions can be scaled as needed. The procedure may be performed at room temperature or at 4°C.

1. Add an appropriate amount of Ni-NTA resin to a tube. Centrifuge tube for 2 minutes at  $700 \times g$  and carefully remove and discard the supernatant.
2. Add two resin-bed volumes of Equilibration Buffer and mix until the resin is fully suspended.
3. Centrifuge tube for 2 minutes at  $700 \times g$  and carefully remove and discard buffer.
4. Prepare sample by mixing the protein extract with an equal volume of Equilibration Buffer. The total volume should equal at least two volumes of the resin bed.
5. Add the prepared protein extract to the tube and mix on an end-over-end rotator for 30 minutes.
6. Centrifuge the tube for 2 minutes at  $700 \times g$ . If desired, save supernatant for downstream analysis.
7. Wash the resin with two resin-bed volumes of Wash Buffer. Centrifuge the tube for 2 minutes at  $700 \times g$ . If desired, save supernatant for downstream analysis.
8. Repeat wash step and monitor supernatant by measuring its absorbance at 280nm until baseline is reached.
9. Elute bound His-tagged proteins using one resin-bed volume of Elution Buffer. Centrifuge tube for 2 minutes at  $700 \times g$ . Carefully remove and save the supernatant. Repeat this step twice, saving each supernatant fraction in a separate tube.
10. Monitor protein elution by measuring the absorbance of the fractions at 280nm or by Coomassie Plus (Bradford) Assay Reagent (Product No. 23238) or Pierce® 660nm Protein Assay (Product No. 22660). The eluted protein can 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. The Thermo Scientific Pierce SDS-PAGE Sample Prep Kit (Product No. 89888) may also be used to remove guanidine.

## Procedure for Purification of His-tagged Proteins using a Gravity-flow Column

The HisPur Ni-NTA Resin allows for purification strategy customization. Purification conditions can be scaled as desired. Perform the procedure at room temperature or at 4°C.

1. Pack column with an appropriate amount of Ni-NTA resin. Allow storage buffer to drain from resin by gravity flow.
2. Prepare sample by mixing protein extract with an equal volume of Equilibration Buffer.
3. Equilibrate column with two resin-bed volumes of Equilibration Buffer. Allow buffer to drain from the column.
4. Add the prepared protein extract to the resin. Collect the flow-through in a tube. If desired, re-apply the flow-through once to maximize binding.
5. Wash resin with two resin-bed volumes of Wash Buffer and collect the flow-through. Repeat this step using a new collection tube until the absorbance of the flow-through fraction at 280nm approaches baseline.
6. Elute His-tagged proteins from the resin with two resin-bed volumes of Elution Buffer. Repeat this step twice, collecting each fraction in a separate tube.
7. Monitor protein elution by measuring the absorbance of the fractions at 280nm or by Coomassie Plus (Bradford) Assay Reagent (Product No. 23238) or Pierce 660nm Protein Assay (Product No. 22660). The eluted protein can be directly analyzed by SDS-PAGE.

**Note:** To remove imidazole for downstream applications use gel filtration (e.g., Zeba™ Spin Desalting Columns) or dialysis (e.g., 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 Pierce SDS-PAGE Sample Prep Kit (Product 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.

## Troubleshooting

Problem	Possible Cause	Solution
Low protein yield	Poor expression of soluble protein	Optimize expression conditions
	His-tagged protein forms 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., Inclusion Body Solubilization Reagent, Product No. 78115)
	Insufficient cell lysis and extraction	Optimize cell lysis protocol
	Fusion protein does 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 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

## Additional Information

Visit the website for additional information relating to this product including the following:

- Tech Tip #43: Protein stability and storage
- Tech Tip #40: Convert between times gravity ( $\times g$ ) and centrifuge rotor speed (RPM)
- Tech Tip #6: Extinction coefficients guide

## Related Thermo Scientific Products

88224	HisPur Ni-NTA Spin Columns, 0.2mL, 25 each
88225	HisPur Ni-NTA Spin Columns, 1mL, 5 each
88226	HisPur Ni-NTA Spin Columns, 3mL, 5 each
88270	Pierce High Capacity Endotoxin Removal Resin, 10mL
88282	Pierce LAL Chromogenic Endotoxin Quantitation Kit
89967	HisPur Cobalt Spin Columns, 0.2mL, 25 each
89968	HisPur Cobalt Spin Columns, 1mL, 5 each
89969	HisPur Cobalt Spin Columns, 3mL, 5 each
24110	Guanidine•HCl, 500g
90078	B-PER <sup>®</sup> Bacterial Protein Extraction Reagent with Enzymes, 250mL
87785	Halt Protease Inhibitor Cocktail (100X), EDTA-free, 1mL
78441	Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X), 1mL
23238	Coomassie Plus (Bradford) Assay Reagent, 300mL
22660	Pierce 660nm Protein Assay Reagent, 750mL
78115	Inclusion Body Solubilization Reagent, 100mL
89890	Zeba Spin Desalting Columns, 7K MWCO, 2mL, 25 columns, for 200-700 $\mu$ L samples
89892	Zeba Spin Desalting Columns, 7K MWCO, 5mL, 25 columns, for 500-2000 $\mu$ L samples
89894	Zeba Spin Desalting Columns, 7K MWCO, 10mL, 25 columns, for 1500-4000 $\mu$ L samples
87769	Zeba Spin Desalting Columns, 40K MWCO, 2mL, 25 columns, for 200-900 $\mu$ L samples
87771	Zeba Spin Desalting Columns, 40K MWCO, 5mL, 25 columns, for 300-2000 $\mu$ L samples
87773	Zeba Spin Desalting Columns, 40K MWCO, 10mL, 25 columns, for 1000-4000 $\mu$ L samples
87730	Slide-A-Lyzer G2 Dialysis Cassettes, 10K MWCO, 3mL, 10 cassettes
87731	Slide-A-Lyzer G2 Dialysis Cassettes, 10K MWCO, 15mL, 8 cassettes

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

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Current product instructions are available at [www.thermoscientific.com/pierce](http://www.thermoscientific.com/pierce). For a faxed copy, call 800-874-3723 or contact your local distributor.

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