HisPur™ Ni-NTA Resin

Catalog Numbers 88221, 88222, 88223

Pub. No. MAN0011700 Rev. B.0

Product description

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.

HisPur™ Ni-NTA Resin features and formats:

- Binding capacity: ≤ 60 mg of a 28-kDa 6xHis-tagged protein from a bacterial source per milliliter of settled resin
- Resin: Crosslinked 6% agarose
- Supplied: 50% slurry in 20% ethanol

Contents and storage

Table 1 HisPur™ Ni-NTA Resin

Cat. No.	Amount	Storage
88221	10 mL	4°C[1]
88222	100 mL	
88223	500 mL	

[1] The product is shipped at ambient temperature.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Sodium phosphate	MLS
Sodium chloride	MLS
Imidazole	MLS
2-(*N*-morpholine)-ethanesulfonic acid	MLS
Guanidine-HCl	24110

General Guidelines

- Immunoglobulins are known to have multiple histidines in their Fc region that can bind to IMAC supports. High background and false positives can result if sufficient blocking is not performed to prevent binding of immunoglobulins in the absence of the His-tagged protein. Albumins, such as bovine serum albumin (BSA), also have multiple histidines that can bind to IMAC supports, but with a lower affinity than immunoglobulins or His-tagged proteins, which can displace the albumin.

- Protein yield and purity are dependent upon the expression level, conformation, and solubility characteristics of the recombinant fusion protein. 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™ with Enzymes Bacterial Protein Extraction Kit (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 (100X) (Cat. No. 78441), to protect proteins from degradation.
- Overexpressed proteins can be 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. 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 may 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™ Pierce™ Coomassie Plus (Bradford) Assay Reagent (Cat. No. 23238) or Thermo Scientific™ Pierce™ 660 nm Protein Assay Reagent (Cat. No. 22660) to monitor protein concentration in the elution fractions, dilute the samples at least 1:2 before performing the protein assay.

Prepare buffers

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

Buffer	Components
Buffers for native conditions	
Equilibration Buffer, pH 7.4	• 20 mM sodium phosphate • 300 mM sodium chloride • 10 mM imidazole
Wash Buffer, pH 7.4	• 20 mM sodium phosphate • 300 mM sodium chloride • 25 mM imidazole
Elution Buffer, pH 7.4	• 20 mM sodium phosphate • 300 mM sodium chloride • 250 mM imidazole
Buffers for denaturing conditions	
Equilibration Buffer, pH 7.4	• 20 mM sodium phosphate • 300 mM sodium chloride • 6 M guanidine-HCl • 10 mM imidazole
Wash Buffer, pH 7.4	• 20 mM sodium phosphate • 300 mM sodium chloride • 6 M guanidine-HCl • 25 mM imidazole
Elution Buffer, pH 7.4	• 20 mM sodium phosphate • 300 mM sodium chloride • 6 M guanidine-HCl • 250 mM imidazole
Buffer for resin degeneration	
MES Buffer, pH 5.0	• 20 mM 2-(*N*-morpholine)-ethanesulfonic acid • 0.1 M sodium chloride

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 Ni-NTA resin and Equilibration Buffer

1.1. Add an appropriate amount of Ni-NTA resin to a tube.

1.2. Centrifuge the tube for 2 minutes at 700 × *g*, then carefully remove and discard the supernatant.

1.3. Add two resin-bed volumes of Equilibration Buffer, then mix until the resin is fully suspended.

1.4. Centrifuge the tube for 2 minutes at 700 × *g*, then carefully remove and discard the buffer.

2 Prepare sample, add prepared protein extract, then centrifuge

2.1. 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.

2.2. Add the prepared protein extract to the tube, then mix on an end-over-end rotator for 30 minutes.

2.3. Centrifuge the tube for 2 minutes at 700 × *g*.

Optional: Save the supernatant for downstream analysis.

3 Wash resin, then elute His-tagged proteins

3.1. Wash the resin with two resin-bed volumes of Wash Buffer.

3.2. Centrifuge the tube for 2 minutes at 700 × *g*.

Optional: Save the supernatant for downstream analysis.

3.3. Repeat steps 3.1 and 3.2.

Monitor the supernatant by measuring its absorbance at 280 nm until baseline is reached.

3.4. Elute bound His-tagged proteins using one resin-bed volume of Elution Buffer.

3.5. Centrifuge tube for 2 minutes at 700 × *g*. Carefully remove and save the supernatant.

3.6. Repeat steps 3.4 and 3.5 twice, saving each supernatant fraction in a separate tube.

3.7. Monitor protein elution by measuring the absorbance of the fractions at 280 nm, or by Coomassie Plus (Bradford) Assay Reagent (Cat. No. 23238) or Pierce™ 660 nm Protein Assay Reagent (Cat. No. 22660).

The eluted protein can be directly analyzed by SDS-PAGE.

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, prepare sample, then equilibrate the column

1.1. Pack the column with an appropriate amount of Ni-NTA resin.

Allow storage buffer to drain from resin by gravity flow.

1.2. Prepare sample by mixing protein extract with an equal volume of Equilibration Buffer.

1.3. Equilibrate the column with two resin-bed volumes of Equilibration Buffer.

Allow buffer to drain from the column.

2	Add prepared protein, wash resin, then elute His-tagged proteins	**2.1.** Add the prepared protein extract to the resin, then collect the flow-through in a tube. *Optional*: Re-apply the flow-through once to maximize binding. **2.2.** Wash resin with two resin-bed volumes of Wash Buffer, then collect the flow-through. Repeat this step using a new collection tube until the absorbance of the flow-through fraction at 280 nm approaches baseline. **2.3.** 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. **2.4.** Monitor protein elution by measuring the absorbance of the fractions at 280 nm or by Coomassie Plus (Bradford) Assay Reagent (Cat. No. 23238) or Pierce™ 660 nm Protein Assay Reagent (Cat. 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 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.

Regenerate Ni-NTA resin

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

Observation	Possible cause	Recommended action
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.
		Solubilize inclusion bodies and perform the purification with a compatible denaturant (e.g., Inclusion Body Solubilization Reagent, Cat. 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 was overloaded.	Apply less protein extract onto the column and make sure the extract is not too viscous or highly particulate.

Supplemental information

User tips

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

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

Related Thermo Scientific products

Item	Amount	Source
HisPur™ Ni-NTA Spin Columns, 0.2 mL resin bed	25 columns	88224
HisPur™ Ni-NTA Spin Columns, 1 mL resin bed	5 columns	88225
HisPur™ Ni-NTA Spin Columns, 3 mL resin bed	5 columns	88226
Pierce™ High Capacity Endotoxin Removal Resin	10 mL	88270
Pierce™ LAL Chromogenic Endotoxin Quantitation Kit	50 tests	88282
HisPur™ Cobalt Spin Columns, 0.2 mL	25 columns	89967
HisPur™ Cobalt Spin Columns, 1 mL	5 columns	89968
HisPur™ Cobalt Spin Columns, 3 mL	5 columns	89969
B-PER™ with Enzymes Bacterial Protein Extraction Kit	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 500–2,000 µL samples	89892
Zeba™ Spin Desalting Columns, 7K MWCO, 10 mL	25 columns, for 1,500–4,000 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 300–2,000 µL samples	87771
Zeba™ Spin Desalting Columns, 40K MWCO, 10 mL	25 columns, for 1,000–4,000 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

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  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0011700

Revision	Date	Description
B.0	17 November 2021	The buffer components for native conditions and denaturing conditions were updated.
A.0	17 October 2015	Initial release with new publication number format.