INSTRUCTIONS

HisPur™ Ni-NTA Purification Kit

Pub. No. MAN0011702
Rev. B
Doc. Part No. 2162206

88227 88228 88229

Number	Description
88227	**HisPur Ni-NTA Purification Kit, 0.2mL**
	Kit Contents:
	HisPur Ni-NTA Spin Columns, 0.2mL resin bed, 25 each
	Phosphate-Buffered Saline (10X), 30mL, 200mM sodium phosphate, 3M sodium chloride, pH 7.4
	Imidazole (2M), 8mL, pH 7.4
	Collection Tubes, 80 each
	Column Plugs, 25 each
88228	**HisPur Ni-NTA Purification Kit, 1mL**
	Kit Contents:
	HisPur Ni-NTA Spin Columns, 1mL resin bed, 5 each
	Phosphate-Buffered Saline (10X), 30mL, 200mM sodium phosphate, 3M sodium chloride, pH 7.4
	Imidazole (2M), 8mL, pH 7.4
88229	**HisPur Ni-NTA Purification Kit, 3mL**
	Kit Contents:
	HisPur Ni-NTA Spin Columns, 3mL resin bed, 5 each
	Phosphate-Buffered Saline (10X), 30mL, 200mM sodium phosphate, 3M sodium chloride, pH 7.4
	Imidazole (2M), 25mL, pH 7.4
	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 Purification Kit enables effective immobilized metal affinity chromatography (IMAC) purification of polyhistidine-tagged proteins from a soluble protein extract. The Ni-NTA 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

- 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. 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.
- 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.

Additional Materials Required

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

Material Preparation

Depending on the specific protein, buffers might require some optimization. Use the table below to make buffers with different imidazole concentrations. Adjust the total volume depending on the resin volume used. For most proteins, the following imidazole concentrations are recommended:

- Equilibration Buffer: 10mM imidazole
- Wash Buffer: 25mM imidazole
- Elution Buffer: 250mM imidazole

Imidazole Final Conc. (mM)	10X PBS* (mL)	2M Imidazole (µL)	Water (mL)
10	1	50	8.95
25	1	125	8.875
40	1	200	8.8
60	1	300	8.7
75	1	375	8.625
150	1	750	8.25
200	1	1,000	8
250	1	1,250	7.75
500	1	2,500	6.5

*Phosphate-buffered saline

Procedure for Spin Purification of His-Tagged Proteins

Note: The total volume of the 0.2, 1 and 3mL columns are 1, 8 and 22mL, 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 at 4°C.
2. Prepare sample by mixing protein extract with Equilibration Buffer so the total volume equals two resin-bed volumes.
3. Remove the bottom closure (SAVE for later use) from the HisPur Ni-NTA Spin Column by gently twisting. Place column into a centrifuge tube.

   Note: Use 1.5, 15 or 50mL centrifuge tubes for the 0.2, 1 and 3mL spin columns, respectively.
4. Centrifuge column at 700 × *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 × *g* for 2 minutes to remove buffer.
7. Add the prepared protein extract to the column and allow it to enter the resin bed.

   Note: For maximal binding, the sample can be incubated for 30 minutes at room temperature or 4°C on an end-over-end rocking platform.
8. Centrifuge column at 700 × *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 × *g* for 2 minutes and collect fraction in a centrifuge tube. Repeat this step two more times collecting each fraction in a separate centrifuge tube.

   Note: If desired, perform additional washes. Monitor washes by measuring their absorbance at 280nm.
10. Elute His-tagged proteins from the resin by adding one resin-bed volume of Elution Buffer. Centrifuge at 700 × *g* for 2 minutes. Repeat this step two more times, collecting each fraction in a separate tube.

    Note: If performing gravity-flow add two resin-bed volumes of Elution Buffer to achieve proper flow characteristics.
11. Monitor protein elution by measuring the absorbance of the fractions at 280nm or by Coomassie Plus (Bradford) Assay Reagent (Product No. 23238). 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).

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. Reseal the column by inverting the original snap-off closure, and with a slight twisting motion, press it firmly to the bottom tip of the column. Reseal the top cap, then store vertically.

Troubleshooting

Problem	Possible Cause	Solution
Low protein yield	Poor expression of soluble	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., Thermo Scientific Inclusion Body Solubilization Reagent, Product No. 78115).
	Insufficient cell lysis and	Optimize cell lysis protocol.
	His tag was absent.	Verify the sequence or perform an ELISA or Western blot using an antibody against the His tag.
	His tag was inaccessible using native conditions.	See the Additional Information Section for denaturing conditions.
	His-tagged protein had a low affinity to the column.	Optimize the Equilibration or Wash buffer by decreasing the concentration of imidazole (see table in the Material Preparation Section).
Poor protein purity	Insufficient column washing.	Wash column additional times or modify the imidazole concentration (see table in Material Preparation Section).
Slow column flow	Column was overloaded.	Apply less protein extract to the column and make sure the extract is not too viscous or contaminated with cell debris.

Additional Information

A. Fusion Proteins Expressed in Inclusion Bodies

Over-expressed proteins are sometimes sequestered in inclusion bodies. Inclusion bodies can be solubilized in 8M urea, 6M guanidine or the Inclusion Body Solubilization Reagent (Product No. 78115); however, a denaturant must be added to the buffers to ensure the protein remains soluble throughout the procedure. (Follow the spin purification procedure.)

If using 8 M urea, you may proceed directly to SDS-PAGE without sample clean-up. If using 6 M guanidine, perform one of the following steps before SDS-PAGE: 1) Dilute sample 1:6 in ultrapure water; 2) Perform buffer exchange with dialysis or desalting; 3) Perform TCA precipitation; 4) Use the Thermo Scientific Pierce SDS-PAGE Sample Prep Kit (Product No. 89888).

For denaturing conditions prepare the following buffers:

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

Related Thermo Scientific Products

88270 **Pierce™ High Capacity Endotoxin Removal Resin,** 10mL

88282 **Pierce™ LAL Chromogenic Endotoxin Quantitation Kit**

88221 **HisPur Ni-NTA Resin,** 10mL

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 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 200-700µL samples
89892	**Zeba Spin Desalting Columns, 7K MWCO, 5mL,** 25 columns, for 500-2,000µL samples
89894	**Zeba Spin Desalting Columns, 7K MWCO, 10mL,** 25 columns, for 1,500-4,000µL samples
87769	**Zeba Spin Desalting Columns, 40K MWCO, 2mL,** 25 columns, for 200-900µL samples
87771	**Zeba Spin Desalting Columns, 40K MWCO, 5mL,** 25 columns, for 300-2,000µL samples
87773	**Zeba Spin Desalting Columns, 40K MWCO, 10mL,** 25 columns, for 1,000-4,000µL 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

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. The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0011702 B

Revision	Date	Description
B	31 July 2024	Correcting spin column usage.
A	17 October 2015	New document for HisPur™ Ni-NTA Purification Kit.