HisPur™ Ni-NTA Chromatography Cartridge

Catalog Numbers 90098, 90099

Pub. No. MAN0011701 Rev. B.0

Product description

The Thermo Scientific™ HisPur™ Ni-NTA Chromatography Cartridges are convenient, ready-to-use pre-packed devices for the immobilized metal affinity chromatography (IMAC) purification of polyhistidine-tagged proteins from a soluble protein extract. The 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. 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 Chromatography Cartridges are compatible with the major automated liquid-chromatography systems or manual syringe processing (see Table 1). The cartridges attach directly to AKTA™ or FPLC Systems without additional connectors. The included accessory pack readily adapts cartridges for use with Luer-Lok Syringe Fittings or 1/16” tubing. These cartridges enable fast, easy and reproducible chromatographic separations and can be regenerated for multiple uses.

Table 1 Properties of Thermo Scientific™ HisPur™ Ni-NTA Chromatography Cartridges

Property	Description
Support	Crosslinked 6% agarose
Ligand	Nickel-charged nitrilotriacetic acid (NTA) chelator
Metal ion capacity	≥ 15 µmol nickel/mL of resin
Binding capacity	≤ 60 mg of 6×His-tagged protein/mL of resin
Cartridge dimensions	0.7 × 2.7 cm (1 mL column); 1.3 × 3.8 cm (5 mL column)
Particle size	45–165 µm
Void volume	0.32 mL (1 mL column); 1.5 mL (5 mL column)
Recommended flow rate	1–2 mL/min (1 mL column); 10 mL/min (5 mL column)
Maximum recommended flow rate	4 mL/min (1 mL column); 10 mL/min (5 mL column)
pH limits	2–14 (2 hours); 3–10 (24 hours)
Maximum operating pressure	0.3 MPa, 43.5 psi or 3 bar
Cartridge material	Polypropylene
Frit	Polypropylene, 10 µm
Storage solution	20% ethanol
Accessory pack	Luer-Lok Adapter to 10–32 male Ginger-tight 10–32 connector fitting for 1/16" OD tubing Cap 1/16 male

Contents and storage

Table 2 HisPur™ Ni-NTA Chromatography Cartridges

Cat. No.	Amount	Storage
90098	5 × 1 mL	4–8°C[1]
90099	2 × 5 mL	

[1] The product is shipped at ambient temperature. Do not freeze.

Note: Each product contains an accessory pack (1 female Luer-Lok™ Adapter, 1 connector fitting, 1 column plug, and bottom caps).

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
Liquid chromatography system (LC procedure) with 1/16" tubing or syringes	MLS
Connectors and fittings to attach to the Bio-Rad BioLogic™ System	MLS
Halt Protease Inhibitor Cocktail (100X), EDTA-free, 1 mL[1]	87785
Sodium phosphate	MLS
Sodium chloride	MLS
Imidazole	MLS
2-(*N*-morpholine)-ethanesulfonic acid	MLS
Guanidine-HCl	24110

[1] Or any EDTA-free protease inhibitors

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. 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™ 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, but a denaturant must be added to buffers so the protein remains soluble throughout the procedure.
- 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.
- For liquid-chromatography applications, use highly pure, low-absorbance imidazole. Also, use highly pure buffer components and water. For best results, filter buffers through a 0.45 µm filter and degas before use.

Prepare buffers

Note: The Thermo Scientific™ Phosphate Buffered Saline (20X) (Cat. No. 28348) diluted to 10X may be used to prepare the recommended buffers listed below. To decrease nonspecific binding and increase yield, optimization of the imidazole concentration may be required for specific proteins.

Buffer	Components
Buffers for native conditions	
Equilibration/Binding 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 • 20–40 mM imidazole[1]
Elution Buffer, pH 7.4	• 20 mM sodium phosphate • 300 mM sodium chloride • 300 mM imidazole
Buffers for denaturing conditions	
Equilibration/Binding 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 • 20–40 mM imidazole
Elution Buffer, pH 7.4	• 20 mM sodium phosphate • 300 mM sodium chloride • 6 M guanidine-HCl • 300 mM imidazole
Buffer for resin degeneration	
MES Buffer, pH 5.0	• 20 mM 2-(*N*-morpholine)-ethanesulfonic acid • 0.1 M sodium chloride

[1] The optimum imidazole wash concentration is protein specific; 20-40 mM is appropriate for many proteins.

Purify His-tagged proteins using a liquid chromatography system

Note: For syringe application, 30 drops per minute is equivalent to a flow rate of 1 mL per minute.

1 Equilibrate the cartridge, prepare the LC system, then equilibrate the cartridge again

1.1. Equilibrate the cartridge and all buffers to working temperature. Perform purifications at room temperature or at 4°C. Ensure that all solutions are degassed.

1.2. Prepare the LC system by filling tubing with buffer. Remove the top plug from cartridge and carefully snap off the end-tab (do not twist).

To avoid introducing air into the system, let a few drops of buffer flow from the tubing into the cartridge top, then connect the cartridge top to the tubing. Allow a few drops to emerge from the cartridge before connecting to the LC inlet port.

1.3. Equilibrate the cartridge with 5–10 column volumes of Equilibration/Binding Buffer at a flow rate of 1–2 mL/minute for the 1 mL cartridge, or 1–5 mL/minute for the 5 mL cartridge.

2	Mix the sample, apply to the cartridge, then wash the resin	**2.1.** Mix the sample 1:1 with Equilibration/Binding Buffer to adjust the ionic strength and pH. Alternatively, buffer-exchange the sample against the Equilibration/Binding Buffer. If the sample contains insoluble matter, centrifuge or filter (0.45 µm) the sample immediately before use. Apply a volume that does not exceed column capacity. **2.2.** Apply the clarified sample to the cartridge. For maximum binding, apply at a flow rate of 0.5–1 mL/minute for the 1 mL cartridge, or 1–2 mL/minute for the 5 mL cartridge. Collect the fractions. **2.3.** Wash the resin with 10-15 column volumes of Wash Buffer, or until the absorbance approaches baseline.
3	Elute, then store the cartridge	**3.1.** Elute with approximately 5–10 column volumes of Elution Buffer, then collect then fractions. Elute using a one step or linear gradient. A shallow gradient (≥ 20 column volumes) may separate proteins with similar binding properties. **3.2.** Monitor protein elution by measuring the absorbance of the fractions at 280 nm or by Pierce™ Coomassie Plus (Bradford) Assay Kit (Cat. No. 23236). The eluted protein can be directly analyzed by SDS-PAGE. To remove excess imidazole for downstream applications, use gel filtration or dialysis (see "Related Thermo Scientific products" on page 5). **3.3.** To store, wash the cartridge with five column volumes of water, then store in 20% ethanol. Attach supplied bottom cap, followed by the top plug. Store the cartridge at 4°C.

Regenerate Ni-NTA resin

The Ni-NTA cartridge can be used multiple times without affecting protein yield or purity. After each use and before storing, perform the procedure as described below to remove residual imidazole and any nonspecifically adsorbed protein. To prevent cross contamination of samples, designate a given cartridge to one specific fusion protein.

1. Wash cartridge with 10 column volumes of MES Buffer.
2. Wash cartridge with 10 column volumes of ultrapure water.
3. Before reuse, re-equilibrate with Equilibration/Binding Buffer until the pH returns to the buffer value.
4. Store the cartridge 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.
	His tag was absent.	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.
	His tag was inaccessible using native conditions.	See "General guidelines" on page 2 for denaturing conditions.
	Flow rate was too fast.	Decrease the flow rate during sample application.
Poor protein purity	Insufficient column washing.	Wash the cartridge additional times or increase the imidazole concentration of the wash buffer.
Slow column flow	Cartridge was overloaded.	Decrease the flow rate, apply less sample or clarify sample by filtration or centrifugation.
High back pressure (exceeds 0.3 MPa)	Cell debris clogged the cartridge.	Clarify sample by filtration (0.45 µm) or centrifugation and apply less sample.

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
Pierce™ Chromatography Cartridge Accessory Pack	1 pack	89971
Pierce™ High Capacity Endotoxin Removal Resin	10 mL	88270
Pierce™ LAL Chromogenic Endotoxin Quantitation Kit	50 tests	88282
Pierce™ 20X Phosphate Buffered Saline	500 mL	28348
HisPur™ Cobalt Chromatography Cartridges, 1 mL	5 × 1 mL	90093
HisPur™ Cobalt Chromatography Cartridges, 5 mL	2 × 5 mL	90094
Halt™ Protease Inhibitor Cocktail (100X), EDTA-free	1 mL	87785
Halt™ Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X)	1 mL	78441
B-PER™ with Enzymes Bacterial Protein Extraction Kit	250 mL	90078
B-PER™ Reagent (in Phosphate Buffer)	500 mL	78226
B-PER™ Bacterial Protein Extraction Reagent	500 mL	78248
Inclusion Body Solubilization Reagent	100 mL	78115
Coomassie Plus (Bradford) Assay Reagent	300 mL	23238
Zeba™ Spin Desalting Columns, 7K MWCO, 10 mL	25 columns, for 1,500–4,000 µL samples	89894
Slide-A-Lyzer™ G2 Dialysis Cassettes, 10K MWCO, 3 mL	10 cassettes	87730

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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. MAN0011701

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.