INSTRUCTIONS

HisPur™ Ni-NTA Chromatography Cartridge

90098 90099

2205.1

Number	Description
90098	**HisPur Ni-NTA Chromatography Cartridge,** 5 × 1mL
90099	**HisPur Ni-NTA Chromatography Cartridge,** 2 × 5mL

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

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

Note: The HisPur Ni-NTA Chromatography Cartridge is supplied in 20% ethanol.

Storage: Upon receipt store at 4-8°C. Product is shipped at ambient temperature. Do not freeze.

Introduction

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.

Thermo Scientific Cartridges are compatible with the major automated liquid-chromatography systems or manual syringe processing (see Table 1). The cartridges attach directly to ÄKTA™ 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 the Thermo Scientific HisPur Ni-NTA Chromatography Cartridges.

Support	Crosslinked 6% agarose
Ligand	Nickel-charged nitrilotriacetic acid (NTA) chelator
Metal Ion Capacity	≥ 15µmol nickel/mL of resin
Binding Capacity	≤ 60mg of 6xHis-tagged protein/ml of resin
Cartridge Dimensions	0.7 × 2.7cm (1mL column); 1.3 × 3.8cm (5mL column)
Particle Size	45-165µm
Void Volume	0.32mL (1mL column); 1.5mL (5mL column)
Recommended Flow Rate	1-2mL/min (1mL column); 1-5mL/min (5mL column)
Maximum Recommended Flow Rate	4mL/min (1 mL column); 10 mL/min (5mL column)
pH Limits	2-14 (2 hours); 3-10 (24 hours)
Maximum Operating Pressure	0.3MPa, 43.5psi or 3 bar
Cartridge Material	Polypropylene
Frit	Polyethylene, 10µm
Storage Solution	20% Ethanol
Accessory Pack	Luer-Lok Adapter to 10-32 male
	Finger-tight 10-32 connector fitting for 1/16" OD tubing
	Plug for 10-32 coned port
	Cap 1/16 male

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Important Product Information

- Protein yield and purity are dependent upon the expression level, conformation and solubility characteristics of the recombinant fusion protein. Typical yields are 10-25% of the total protein loaded onto the column. For optimal results, do not exceed the capacity of the resin.
- 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.
- 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.
- 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 0.45µm filter and degas before use.

Additional Materials Required

Note: The Thermo Scientific 20X PBS Buffer (Product 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 might be required for specific proteins.

- Suitable liquid chromatography system (LC procedure) with 1/16” tubing or syringes
- EDTA-free protease inhibitors such as Halt Protease Inhibitor Cocktail (100X), EDTA-free, 1mL (Product No. 87785)
- Additional connectors and fittings are required to attach to the Bio-Rad BioLogic™ System.

For native conditions prepare the following buffers:

- Equilibration/Binding Buffer: 20mM sodium phosphate, 300mM sodium chloride (PBS) with 10mM imidazole; pH 7.4
- Wash Buffer: PBS with 20-40mM imidazole; pH 7.4 (The optimum imidazole wash concentration is protein specific; 20-40mM is appropriate for many proteins)
- Elution Buffer: PBS with 300mM imidazole; pH 7.4

For denaturing conditions prepare the following buffers:

- Equilibration/Binding Buffer: PBS with 6M guanidine•HCl and 10mM imidazole; pH 7.4
- Wash Buffer: PBS with 6M guanidine•HCl and 20-40mM imidazole; pH 7.4
- Elution Buffer: PBS with 6M guanidine•HCl and 300mM imidazole; pH 7.4

For cartridge regeneration prepare the following buffer:

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

Procedure for Purifying His-tagged Proteins Using a Liquid Chromatography System

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

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.
2. Prepare the LC system by filling tubing with buffer. Remove 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 tubing into cartridge top then connect cartridge top to the tubing; allow a few drops to emerge from the cartridge before connecting to the LC inlet port.
3. Equilibrate the cartridge with 5-10 column volumes of Equilibration/Binding Buffer at a flow rate of 1-2mL/minute for the 1mL cartridge or 1-5mL/minute for the 5mL cartridge.
4. 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 (0.45µm) the sample immediately before use. Apply a volume that does not exceed column capacity.
5. Apply the clarified sample to the cartridge. For maximum binding, apply at a flow rate of 0.5-1mL/minute for the 1mL cartridge and 1-2mL/minute for the 5mL cartridge. Collect fractions.
6. Wash the resin with 10-15 column volumes of Wash Buffer or until the absorbance approaches baseline.
7. Elute with approximately 5-10 column volumes of Elution Buffer and collect fractions. Elute using a one step or linear gradient. A shallow gradient (≥ 20 column volumes) might separate proteins with similar binding properties.
8. Monitor protein elution by measuring the absorbance of the fractions at 280nm or by Coomassie Plus (Bradford) Assay (Product 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 the Related Thermo Scientific Products Section).
9. For storage, wash the cartridge with five column volumes of water and store in 20% ethanol. Attach supplied bottom cap followed by the top plug. Store the cartridge at 4°C.

Procedure for Cartridge Regeneration

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

Problem	Possible Cause	Solution
Low protein yield	Poor expression of soluble protein	Optimize bacterial expression conditions
	His-tagged protein forms inclusion bodies	Alter bacterial 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, Product No. 78115)
	Insufficient cell lysis and extraction	Optimize cell lysis protocol
	His tag is absent	Verify the sequence or perform an ELISA or Western blot using an antibody against the His tag
	His tag is inaccessible using native conditions	See the Important Product Information Section for denaturing conditions
	Flow rate is too fast	Decrease the flow rate during sample application

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Problem	Possible Cause	Solution
Poor protein purity	Insufficient column washing	Wash cartridge additional times or increase the imidazole concentration of the wash buffer
Slow column flow	Cartridge is overloaded	Decrease the flow rate, apply less sample or clarify sample by filtration or centrifugation
High back pressure (exceeds 0.3MPa)	Cell debris clogging the cartridge	Clarify sample by filtration (0.45µm) or centrifugation and apply less sample

Related Thermo Scientific Products

89971 **Accessory Pack** (1 female Luer-Lok Adapter, 1 connector fitting, 1 column plug and 1 bottom cap)

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

88282 **Pierce LAL Chromogenic Endotoxin Quantitation Kit**

28348 **20X PBS Buffer,** 500mL

90093 **HisPur Cobalt Chromatography Cartridge,** 5 × 1mL

90094 **HisPur Cobalt Chromatography Cartridge,** 2 × 5mL

89935 **Pierce Desalting Chromatography Cartridge,** 5 × 5mL

87785 **Halt Protease Inhibitor Cocktail (100X), EDTA Free,** 1mL

78441 **Halt Protease and Phosphatase Inhibitor Cocktail (100X), EDTA-Free,** 1mL

90078 **B-PER Bacterial Protein Extraction Reagent with Enzymes,** 250mL

78266 **B-PER Bacterial Protein Extraction Reagent (in Phosphate Buffer),** 500mL

78248 **B-PER Bacterial Protein Extraction Reagent,** 500mL

78115 **Inclusion Body Solubilization Reagent,** 100mL

89835 **DNAse I,** 5000 units

24110 **Guanidine•HCl,** 500g

23238 **Coomassie Plus (Bradford) Assay Reagent,** 300mL

89894 **Zeba Spin Desalting Columns, 7K MWCO, 10mL,** 25 columns

87730 **Slide-A-Lyzer G2 Dialysis Cassettes, 10K MWCO, 3mL,** 10 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").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.