INSTRUCTIONS

HisPur™ Cobalt Chromatography Cartridges

90093 90094

2061.2

Number	Description
90093	**HisPur Cobalt Chromatography Cartridge,** 5 × 1mL
90094	**HisPur Cobalt Chromatography Cartridge,** 2 × 5mL

Each product contains an accessory pack (1 female Luer-Lok® Adapter, 1 connector fitting, 1 column plug and 1 or 2 bottom caps).

Binding capacity: ≥ 10mg of 6xHis-tagged protein/ml of resin

Metal ion capacity: ≥ 12µmol cobalt/mL of resin

Note: The Pierce HisPur 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 Cobalt Chromatography Cartridges are convenient, ready-to-use pre-packed devices for efficient purification of polyhistidine-tagged proteins from bacterial, mammalian and baculovirus-infected cells. His-tagged proteins are purified from total soluble protein extract using a cobalt-charged tetradentate chelator immobilized onto 6% crosslinked agarose. Many immobilized metal affinity chromatography (IMAC) resins contain nickel (Ni^{2+}) for purifying His-tagged proteins. Although Ni^{2+} chelate resins achieve high protein yields, purity is often suboptimal, requiring additional clean-up steps. Cobalt achieves both high protein yield and purity with minimal optimization. The HisPur Cobalt Resin binds fewer nonspecific proteins, displays less metal leaching and enables less stringent elution conditions compared to Ni^{2+} resins.

Pierce Cartridges are compatible with the major automated liquid-chromatography systems or manual syringe processing (see Table 1 for cartridge general properties). 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 Pierce HisPur Cobalt Chromatography Cartridges.

Support	Crosslinked 6% beaded agarose
Ligand	Cobalt Charged Tetradentate Chelator
Metal ion capacity	≥12µmol cobalt/mL of resin
Binding Capacity	≥ 10mg 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 (1mL column); 5mL/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

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce

Important Product Information

- Typical binding capacity is ≥10mg protein/mL resin. Depending upon protein expression, the maximum total protein (lysate) loading amount recommended is 40mg and 200mg for the 1mL and 5mL cartridges, respectively. Typical yields are 10-25% of the total protein loaded onto the column. For optimal results, do not exceed the capacity of the resin.
- Avoid using chelator-containing protease inhibitors or other additives. EDTA and strong reducing agents, such as DTT and β-mercaptoethanol, disrupt the function of the cobalt resin.
- Optimization of the lysis procedure is critical for maximizing yield. Some methods include using commercially available detergent-based reagents, such as Thermo Scientific B-PER Bacterial Protein Extraction Reagent in Phosphate Buffer (Product No. 78266), and mechanical methods, such as freeze/thaw cycles, sonication or French press.
- For liquid-chromatography applications, use highly pure, low-absorbance imidazole (Fisher, Product No. BP 305-50). Also, use highly pure buffer components and water. For best results, degas or filter buffers through a 0.45µm filter.
- The indicated buffers (see Additional Materials Required Section) are effective for most proteins. If needed, perform an imidazole gradient to establish optimal concentrations for binding and washing. To minimize nonspecific binding, use 5-10mM of imidazole in the Equilibration/Binding and Wash Buffer but do not exceed 10mM. If the target protein does not bind to the column, omit imidazole from the Equilibration/Binding Buffer (See Troubleshooting).
- For elution, a stepwise imidazole gradient is preferable because linear gradients result in broader peaks. Alternatively, elute using a pH gradient or adjust the elution buffer to pH 5.0 and omit the imidazole (pH Elution Buffer: 50mm sodium phosphate, 300mM sodium chloride; pH 5.0).
- Pierce Cartridges can be used singly or connected in series (2-3 columns) to increase capacity. Back pressure is greater when cartridges are used in a series than when used as single columns.
- To monitor protein as it elutes from the cartridge, measure the UV absorbance at 280nm.

Additional Materials Required

Note: For best results, process all buffers through a 0.45µm filter before use in LC applications.

- Suitable liquid chromatography system (LC procedure only) with 1/16” tubing or syringes
- Equilibration/Binding Buffer: 50mM sodium phosphate, 300mM sodium chloride, 0-10mM imidazole; pH 7.4
- Wash Buffer: 50mM sodium phosphate, 300mM sodium chloride, 5-10mM imidazole; pH 7.4
- Elution Buffer: 50mM sodium phosphate, 300mM sodium chloride, 150mM imidazole; pH 7.4. A high-purity low-absorbance imidazole (Fisher BP-305-50) is ideal for LC applications.
- Regeneration MES Buffer: 20mM 2-(*N*-morpholine)-ethanesulfonic acid, 0.1M sodium chloride; pH 5.0
- EDTA-free protease inhibitors such as Halt Protease Inhibitor Single Use Cocktail, EDTA-Free (Product No 78425)
- Additional connectors and fittings are required to attach to the Bio-Rad BioLogic™ System.

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

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 the Equilibration/Binding Buffer at a flow rate of 1-4mL/minute for the 1mL cartridge or 2-5mL/minute for the 5mL cartridge.

4. For maximum binding, prepare sample by mixing protein extract 1:1 with Equilibration/Binding Buffer (to adjust the sample to the ionic strength and pH of the Equilibration/Binding Buffer) before applying to the cartridge. Alternatively, buffer-exchange the sample against the Equilibration/Binding Buffer. If the sample contains insoluble matter, centrifuge or filter (0.45µm filter) it before use. Apply any volume that does not exceed column capacity.
5. Apply the diluted sample to the cartridge. For maximum binding, apply at a flow rate of 0.5-1mL/minute. 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 0.5-1mL fractions.
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 (e.g., Thermo Scientific Zeba Spin Desalting Columns or Slide-A-Lyzer Dialysis Cassettes).
9. The cartridge can be regenerated with Regeneration MES Buffer and reused multiple times without significant loss of binding capacity. See procedure for regeneration of cartridge.
10. 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 Regeneration of HisPur Chromatography Cartridge

The cobalt 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 Regeneration MES Buffer.
2. Wash cartridge with 10 column volumes of ultra pure 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.

Procedure for Purification of His-tagged Protein Using a Syringe

Note: The void volumes are 0.320mL for the 1mL cartridge and 1.5mL for the 5mL cartridge.

1. Equilibrate the cartridge and all solutions to working temperature. Perform purifications at room temperature or at 4°C.
2. Fill a syringe with 5-10 column volumes of Equilibration/Binding Buffer.
3. Attach the syringe to the Luer-Lok Adapter included in the accessory pack. Remove top plug from cartridge and carefully snap off the end-tab. To avoid introducing air into the cartridge, allow a few drops to emerge from the Luer-Lok Adapter and then connect to the cartridge top. Securely tighten the connection.
4. Equilibrate the cartridge with 5-10 column volumes of Equilibration/Binding Buffer at a flow rate of ~1mL/minute for the 1mL cartridge or ~2-5mL/minute for the 5mL cartridge. Remove syringe from the Luer-Lok Adapter.
5. For maximum binding, adjust the sample to the ionic strength and pH of the Equilibration/Binding Buffer by diluting it at least 1:1 before applying to the cartridge. Alternatively, buffer-exchange the sample against the Equilibration/Binding Buffer. If the sample contains insoluble matter, centrifuge or filter (0.45µm filter) it before use. Any volume may be applied provided the column capacity is not exceeded.
6. Fill a syringe with the diluted sample and connect it to the Luer-Lok Adapter. Depress the syringe plunger to pass the sample through the cartridge. For maximum binding, apply at a flow rate of 0.5-1mL/minute. Collect fractions.
7. Change the syringe and wash the resin with 6-10 column volumes of Wash Buffer.
8. Change syringe and elute with approximately five column volumes of Elution Buffer and collect 0.5-1mL fractions.
9. Analyze the purified fractions directly by SDS-PAGE, or dialyze or desalt the sample into a buffer that is compatible.
10. The cartridge may be regenerated with Regeneration MES Buffer and reused multiple times without significant loss of binding capacity. See procedure for regeneration of cartridge.

11. For storage, wash cartridge with five column volumes of water containing 20% ethanol. Attach supplied bottom cap followed by the top plug. Store the cartridge at 4°C.

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., Thermo Scientific 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 Additional Information Section for denaturing conditions
	His-tagged protein has a low affinity to the cobalt column	Decrease the concentration of imidazole or adjust the pH of the equilibration/binding and wash buffers (see the Important Product Information Section)
	Flow rate is too fast	Decrease the flow rate during sample application
Poor protein purity	Insufficient column washing	Wash cartridge additional times or increase the imidazole concentration to 5-10mM; alternatively, adjust the equilibration/binding and wash buffer to pH 7.0 to decrease nonspecific protein binding
Slow column flow	Column is overloaded	Apply less protein extract into the cartridge 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) and purified using the cobalt cartridge; however, a denaturant must be added to the buffers to ensure the protein remains soluble throughout the procedure. (**Follow the Procedure for Purification of His-tagged Protein using a Liquid Chromatography System or a Syringe.)**

Note: Purified samples containing 6M guanidine•HCl must be dialyzed against a buffer containing 8M urea before SDS-PAGE analysis. Alternatively, use the Thermo Scientific Pierce SDS-PAGE Sample Prep Kit (Product No. 89888) for removing guanidine.

For denaturing conditions prepare the following buffers:

- Equilibration/Binding Buffer: 50mM sodium phosphate, 300mM sodium chloride, 6M guanidine•HCl, 0-10mM imidazole; pH 7.4
- Wash Buffer: 50mM sodium phosphate, 300mM sodium chloride, 6M guanidine•HCl, 0-10mM imidazole; pH 7.4
- Elution Buffer: 50mM sodium phosphate, 300mM sodium chloride, 6M guanidine•HCl, 150mM imidazole; pH 7.4

B. Tech Tips Available from our Website

- Tech Tip #43: Protein stability and storage
- Tech Tip #6: Extinction coefficients guide

Related Thermo Scientific Products

189971	**Accessory Pack** (1 female Luer-Lok Adapter, 1 connector fitting, 1 column plug and 1 bottom cap)
6109	**Pierce® Glutathione Chromatography Cartridges, 1mL,** 5/pkg
16110	**Pierce Glutathione Chromatography Cartridges, 5mL,** 2/pkg
88270	**Pierce High Capacity Endotoxin Removal Resin,** 10mL
88282	**Pierce LAL Chromogenic Endotoxin Quantitation Kit**
89935	**Pierce Chromatography Cartridge Desalting,** 5 × 5mL
90098	**HisPur Cobalt Chromatography Cartridges, 1mL,** 5/pkg
90099	**HisPur Cobalt Chromatography Cartridges, 5mL,** 2/pkg
78425	**Halt™ Protease Inhibitor Single Use Cocktail, EDTA Free,** 24 × 100µL microtubes, each 100µL microtube contains sufficient cocktail to treat 10mL of lysate
78266	**B-PER® Bacterial Protein Extraction Reagent (in Phosphate Buffer),** 500mL
89802	**I-PER® Insect Cell Protein Extraction Reagent,** 250mL
78115	**Inclusion Body Solubilization Reagent,** 100mL
89835	**DNAse I,** 5000 units
23236	**Coomassie Plus (Bradford) Assay Kit**
89890	**Zeba™ Spin Desalting Columns, 7K MWCO, 2mL,** 25/pkg
89892	**Zeba Spin Desalting Columns, 7K MWCO, 5mL,** 25/pkg
89894	**Zeba Spin Desalting Columns, 7K MWCO, 10mL,** 25/pkg
66385	**Slide-A-Lyzer® Dialysis Cassettes Kit, 10K MWCO,** 0.1-0.5mL
66382	**Slide-A-Lyzer Dialysis Cassettes Kit, 10K MWCO,** 0.5-3mL
66807	**Slide-A-Lyzer Dialysis Cassettes Kit, 10K MWCO,** 3-12mL

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.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce