INSTRUCTIONS

HisPur™ Cobalt Purification Kit

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Rev. B
Doc. Part No. 2162060

90090 90091 90092

Number	Description
90090	**HisPur Cobalt Purification Kit,** 0.2mL **Kit Contents:** **HisPur Cobalt Spin Columns,** 25 each, pre-filled with 0.2mL resin bed **Equilibration/Wash Buffer,** 100mL **Elution Buffer,** 50mL **Collection Tubes,** 80 each
90091	**HisPur Cobalt Purification Kit,** 1mL **Kit Contents:** **HisPur Cobalt Spin Columns,** 5 each, pre-filled with 1mL resin bed **Equilibration/Wash Buffer,** 100mL **Elution Buffer,** 50mL
90092	**HisPur Cobalt Purification Kit,** 3mL **Kit Contents:** **HisPur Cobalt Spin Columns,** 5 each, pre-filled with 3mL resin bed **Equilibration/Wash Buffer,** 225mL **Elution Buffer,** 75mL

Binding Capacity: ≥ 10mg at > 90% purity of a 28kDa His-tagged protein from a bacterial source per milliliter of resin bed

Resin: Crosslinked 6% agarose in a 20% ethanol solution

Equilibration/Wash Buffer: 50mM sodium phosphate, 300mM sodium chloride, 10mM imidazole; pH 7.4.

Elution Buffer: 50mM sodium phosphate, 300mM sodium chloride, 150mM imidazole; pH 7.4

Note: Buffers contain 0.01% sodium azide as an antimicrobial.

Storage: Upon receipt store kit at 4°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific™ HisPur™ Cobalt Purification Kit contains HisPur Cobalt Spin Columns and pre-formulated Equilibration/Wash and Elution Buffers for the efficient purification of recombinant 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 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.

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.
- Typical binding capacity is ≥10mg protein/mL resin. Depending upon expression of the protein, the maximum total protein (lysate) loading amount recommended is 8mg for the 0.2mL spin column, 40mg for the 1mL spin column and 120mg for the 3mL spin column. 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 protein yield. Some methods for protein extraction 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.

Additional Materials Required

- Regeneration MES Buffer: 20mM 2-(*N*-morpholine)-ethanesulfonic acid, 0.1M sodium chloride; pH 5.0
- EDTA-free protease inhibitors such as Thermo Scientific™ Halt™ Protease Inhibitor Single Use Cocktail, EDTA-Free (Product No. 78425)

Procedure for Spin Purification of His-tagged Protein

Note: The total volume of the 0.2mL, 1mL and 3mL column devices are 1.0mL, 8mL and 22mL, respectively. If a sample volume is greater than the column device, perform multiple applications and centrifugations until the entire sample has been processed. Be careful not to exceed the working capacity of the resin. The HisPur Cobalt Spin Columns also can be used for gravity-flow purifications.

1. Equilibrate column(s) to working temperature. Perform purifications at room temperature or at 4°C.
2. Remove the bottom closure (SAVE closure for later use) from the HisPur Cobalt Spin Column. Place column into a centrifuge tube and loosen top cap.

   Note: Use 1.5mL, 15mL or 50mL centrifuge tubes for the 0.2mL, 1mL and 3mL spin columns, respectively.
3. Centrifuge column at 700 × *g* for 2 minutes to remove storage buffer.
4. Equilibrate column by adding two resin-bed volumes of Equilibration/Wash Buffer. Invert column to mix.
5. Centrifuge column at 700 × *g* for 2 minutes to remove buffer.
6. Prepare sample by mixing 1 part protein extract with 1 part Equilibration/Wash Buffer.
7. 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. Add the prepared protein extract to the tube and cap top of column. Invert column to mix.

   Note: For maximal binding, incubate for 30 minutes at 4°C on an end-over-end rocking platform.
8. Centrifuge column at 700 × *g* for 2 minutes and collect the flowthrough in a new centrifuge tube.
9. Wash resin with two resin-bed volumes of Equilibration/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. 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.
10. Monitor protein elution by measuring the absorbance of the fractions at 280nm or by Thermo Scientific™ Coomassie Plus™ (Bradford) Protein Assay (Product No. 23236). The eluted protein can be directly analyzed by SDS-PAGE. To remove excess imidazole for downstream applications, use gel filtration.

Procedure for Column Regeneration

The cobalt spin column 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 column to one specific fusion protein.

1. Wash column with 10 column volumes of Regeneration MES Buffer.
2. Wash column with 10 column volumes of ultrapure water. For storage, apply 20% ethanol to the column. 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, then screw on the top cap.
3. Before reuse, re-equilibrate the column with Equilibration/Binding Buffer until the pH returns to the buffer value.

Troubleshooting

Problem	Possible Cause	Solution
Low protein yield	Poor expression of soluble protein.	Optimize bacterial expression conditions.
	His-tagged protein formed 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 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 cobalt column.	Optimize the equilibration/wash buffer by decreasing the concentration of imidazole (i.e., 50mM sodium phosphate, 300mM sodium chloride, 0-10mM imidazole; pH 7.4).
Poor protein purity	Insufficient column washing.	Wash column additional times or modify the imidazole concentration; alternatively, adjust the Equilibration/Wash Buffer to pH 7.0 to decrease nonspecific protein binding.
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

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 Thermo Scientific 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 Procedure for Spin Purification of His-tagged Protein.)

If using 8M urea, you may proceed directly to SDS-PAGE without sample clean-up. If using 6M guanidine, perform one of the following steps before SDS-PAGE: 1) Dilute sample 1:6 in ultrapure water; 2) Dialyze against a compatible buffer; 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/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

Related Thermo Scientific Products

88270	**Pierce™ High Capacity Endotoxin Removal Resin,** 10mL
88282	**Pierce LAL Chromogenic Endotoxin Quantitation Kit**
16100	**Pierce Glutathione Agarose,** 10mL
78425	**Halt™ Protease Inhibitor Single Use Cocktail, EDTA Free,** 24 × 100µL microtubes, each 100µL microtube contains sufficient cocktail to treat 10mL of lysate
88221	**HisPur™ Ni-NTA Resin,** 10mL
89967	**HisPur Cobalt Spin Columns, 0.2mL resin bed,** 25/pkg
89968	**HisPur Cobalt Spin Columns, 1.0mL resin bed,** 5/pkg
89969	**HisPur Cobalt Spin Columns, 3.0mL resin bed,** 5/pkg
89964	**HisPur Cobalt Resin,** 10mL settled resin
78266	**B-PER™ Bacterial Protein Extraction Reagent (in Phosphate Buffer),** 500mL
78248	**B-PER Bacterial Protein Extraction Reagent,** 500mL
78260	**B-PER II Bacterial Protein Extraction Reagent,** 250mL
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

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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.

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