INSTRUCTIONS

HisPur™ Cobalt Spin Columns

89967 89968 89969

1852.2

Number	Description
89967	**HisPur Cobalt Spin Columns, 0.2mL resin bed,** 25 each
89968	**HisPur Cobalt Spin Columns, 1.0mL resin bed,** 5 each
89969	**HisPur Cobalt Spin Columns, 3.0mL resin bed,** 5 each

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

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

Introduction

The Thermo Scientific HisPur Cobalt Spin Columns enable efficient purification of polyhistidine-tagged proteins from bacterial, mammalian and baculovirus-infected cells. His-tagged proteins are purified from either native or denatured total soluble protein extract using a cobalt-charged tetradentate chelator immobilized onto 6% crosslinked agarose. Many immobilized metal affinity chromatography (IMAC) resins use nickel (Ni^{+2}) as the metal source for purifying His-tagged proteins. Although Ni^{+2} chelate resins achieve high protein yields, purity is often suboptimal, resulting in the need for additional optimization of wash and elution steps. Cobalt achieves both high protein yield and purity with minimal optimization. Furthermore, the HisPur Cobalt Resin displays less metal leaching compared with Ni^{+2} resins.

Important Product Information

- 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 B-PER® Bacterial Protein Extraction Reagent (Product No. 78248), and mechanical methods, such as freeze/thaw cycles, sonication or French press.
- In some cases, overexpressed proteins are sequestered in inclusion bodies. Inclusion bodies of His-tagged proteins can be solubilized in 8M urea, 6M guanidine or Inclusion Body Solubilization Reagent (Product No. 78115) and purified with the cobalt resin, but a denaturant must be added to the buffers to ensure that the protein remains soluble throughout the procedure.
- One advantage of using cobalt is its low nonspecific binding. Although the buffer conditions described in these instructions work well for many samples, optimization may be required to further reduce nonspecific binding. To optimize conditions, adjust the imidazole concentration in the Equilibration/Wash Buffer or decrease the buffer's pH to protonate a greater proportion of histidine groups.
- IMAC relies on cobalt chelation to both the tetradentate chelator and the target histidine tag. 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 cobalt resin.

Additional Materials Required

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

For native conditions prepare the following buffers:

- 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

For denaturing conditions prepare the following buffers:

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

Procedure for Spin Purification of His-tagged Protein

The HisPur Cobalt Spin Columns also may be used for gravity-flow purifications. Purifications may be performed at room temperature or at 4°C.

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.

1. Equilibrate column(s) to working temperature.
2. Prepare sample by mixing the protein extract with Equilibration/Wash Buffer. Use an amount of Equilibration/Wash Buffer at least equal to the sample volume; the total volume should be greater than or equal to two resin-bed volumes.
3. Remove the bottom tab from the HisPur Cobalt Spin Column. Place column into a centrifuge tube.

   Note: Use 1.5mL, 15mL or 50mL centrifuge tubes for the 0.2mL, 1mL 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/Wash Buffer. Allow buffer to enter the resin bed.
6. Centrifuge column at 700 × *g* for 2 minutes to remove buffer.
7. Place the bottom plug on the column and add the prepared protein extract. Mix on an orbital shaker or end-over-end mixer for 30 minutes.
8. Remove the bottom plug. Centrifuge column at 700 × *g* for 2 minutes and collect the flow-through in a 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.
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.
11. 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., spin desalting columns or dialysis cassettes). Samples containing 6M guanidine•HCl must be dialyzed against a buffer containing 8M urea before SDS-PAGE analysis. Thermo Scientific Pierce SDS-PAGE Sample Prep Kit (Product No. 89888) may also be used to remove guanidine.

Procedure for Cobalt Resin Regeneration

The cobalt resin may be used up to three 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 20mM MES Buffer , 0.1M sodium chloride; pH 5.0.
2. Wash resin with 10 resin-bed volumes of ultrapure water.
3. Store resin as a 50% slurry in 20% ethanol.

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; alternatively, 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 the cell lysis protocol
	Fusion protein did not bind to the column	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
Poor protein purity	Insufficient column washing	Wash column additional times or modify imidazole concentration and pH of the Equilibration/Wash Buffer
Slow column flow	Column was overloaded	Apply less protein extract onto the column and make sure the extract is not too viscous or contaminated with cell debris

Additional Information

Please visit the website for additional information including the following items:

- Tech Tip #43: Protein stability and storage
- Tech Tip #40: Convert between times gravity (× *g*) and centrifuge rotor speed (RPM)
- Tech Tip #6: Extinction coefficients guide

Related Thermo Scientific Products

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

88282 **Pierce LAL Chromogenic Endotoxin Quantitation Kit**

16100 **Pierce Glutathione Agarose,** 10mL

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

89964 **HisPur Cobalt Resin,** 10mL settled resin

89965 **HisPur Cobalt Resin,** 100mL settled resin

89966 **HisPur Cobalt Resin,** 500mL settled resin

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

78410 **Halt™ Protease Inhibitor Cocktail, EDTA-Free,** 1mL

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

89835 **DNAse I,** 5000 units

23236 **Coomassie Plus (Bradford) Assay**

89890 **Zeba Spin Desalting Columns, 2mL,** 25 columns, for 200-700µL samples

89892 **Zeba Spin Desalting Columns, 5mL,** 25 columns, for 500-2000µL samples

89894 **Zeba Spin Desalting Columns, 10mL,** 25 columns, for 700-4000µL samples

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").

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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.