INSTRUCTIONS

HisPur™ Cobalt Resin

89964 89965 89966

1851.2

Number	Description
89964	**HisPur Cobalt Resin,** 10mL settled resin
89965	**HisPur Cobalt Resin,** 100mL settled resin
89966	**HisPurCobalt Resin,** 500mL settled resin
	Binding Capacity: ≥ 10mg at > 90% purity of a 28kDa His-tagged protein from a bacterial source per milliliter of settled resin
	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 Resin enables 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. The cobalt resin is compatible using native or denaturing conditions and can be used in multiple formats including conventional gravity flow chromatography, spin column and FPLC.

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, 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 Thermo Scientific B-PER Bacterial Protein Extraction Reagent (Product No. 78248), and mechanical methods, such as freeze/thaw cycles, sonication or French press.
- Sometimes 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 buffers so 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 Purification of His-Tagged Proteins by Batch Method

The HisPur Cobalt Resin allows customization of a purification strategy. Purification conditions detailed within these instructions can be scaled as desired. The procedure may be performed at room temperature or at 4°C.

1. Add an appropriate amount of cobalt resin to a tube. Centrifuge tube for 2 minutes at 700 × *g* and carefully remove and discard the supernatant.
2. Add two resin-bed volumes of Equilibration/Wash Buffer and mix until the resin is fully suspended.
3. Centrifuge tube for 2 minutes at 700 × *g* and carefully remove and discard buffer.
4. Prepare sample by mixing the protein extract with an equal volume of Equilibration/Wash Buffer.
5. Add the prepared protein extract to the tube and mix on an end-over-end rotator for 30 minutes.
6. Centrifuge the tube for 2 minutes at 700 × *g*. If desired, save supernatant for downstream analysis.
7. Wash the resin with two resin-bed volumes of Equilibration/Wash Buffer. Centrifuge the tube for 2 minutes at 700 × *g*. If desired, save supernatant for downstream analysis.
8. Repeat wash step and monitor supernatant by measuring its absorbance at 280nm until baseline is reached.
9. Elute bound His-tagged proteins using one resin-bed volume of Elution Buffer. Centrifuge tube for 2 minutes at 700 × *g*. Carefully remove and save the supernatant. Repeat this step twice, saving each supernatant fraction in a separate tube.
10. 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 (e.g., Thermo Scientific Zeba Spin Desalting Columns) or dialysis (e.g., Thermo Scientific Slide-A-Lyzer Dialysis Cassettes). Samples containing 6M guanidine•HCl must be dialyzed against a buffer containing 8 M urea before SDS-PAGE analysis. The Thermo Scientific Pierce SDS-PAGE Sample Prep Kit (Product No. 89888) may also be used to remove guanidine.

Procedure for Purification of His-tagged Proteins using a Gravity-flow Column

The HisPur Cobalt Resin allows customization of a purification strategy. Purification conditions detailed within these instructions can be scaled as desired. Perform the procedure at room temperature or at 4°C.

1. Pack column with an appropriate amount of cobalt resin. Allow storage buffer to drain from resin by gravity flow.
2. Prepare sample by mixing the protein extract with an equal volume of Equilibration/Wash Buffer.
3. Equilibrate column with two resin-bed volumes of Equilibration/Wash Buffer. Allow buffer to drain from resin, flow rate should be 0.5-1mL/minute.
4. Add the prepared protein extract onto the resin. Collect the flow-through in a tube. If desired, re-apply the flow-through once to maximize binding.
5. Wash resin with two resin-bed volumes of Equilibration/Wash Buffer and collect the flow-through. Repeat this step using a new collection tube until the absorbance of the flow-through fraction at 280nm approaches baseline.

6. Elute His-tagged proteins from the resin with two resin-bed volumes of Elution Buffer. Repeat this step twice, collecting each fraction in a separate tube.
7. 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 (e.g., Zeba™ Spin Desalting Columns) or dialysis (e.g., Slide-A-Lyzer® Dialysis Cassettes). Samples containing 6M guanidine•HCl must be dialyzed against buffer containing 8M urea before SDS-PAGE analysis. The 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 forms 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 cell lysis protocol
	Fusion protein does 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 washing	Wash resin additional times or modify imidazole concentration and pH of the Equilibration/Wash Buffer
Slow column flow	Column is overloaded	Apply less protein extract onto the column and make sure the extract is not too viscous or highly particulate

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
- Tech Tip #7: Remove air bubbles from columns to restore flow rate
- Tech Tip #13: Pack beaded affinity resin into affinity columns

Related Thermo Scientific Products

16100	**Pierce Glutathione Agarose,** 10mL
88270	**Pierce High Capacity Endotoxin Removal Resin, 10mL**
88282	**Pierce LAL Chromogenic Endotoxin Quantitation Kit**
88221	**HisPur Ni-NTA Resin,** 10mL
89967	**HisPur Cobalt Spin Columns, 0.2mL,** 25 each
89968	**HisPur Cobalt Spin Columns, 1.0mL,** 5 each
89969	**HisPur Cobalt Spin Columns, 3.0mL,** 5 each
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, 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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Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.