thermo scientific | USER GUIDE

HisPur™ Cobalt Spin Columns

Catalog Numbers 89967, 89968, and 89969

Doc. Part No. 2161852 Pub. No. MAN0011582 Rev. B

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

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.

Contents and storage

Item	Catalog Number	Storage
HisPur™ Cobalt Spin Columns, 0.2 mL resin bed	89967	Store at 4°C
HisPur™ Cobalt Spin Columns, 1.0 mL resin bed, 5 each	89968	
HisPur™ Cobalt Spin Columns, 3.0 mL resin bed, 5 each	89969	
Binding Capacity: ≥10 mg at >90% purity of a 28 kDa His-tagged protein from a bacterial source per milliliter of resin bed Resin: Crosslinked 6% agarose in a 20% ethanol solution		

Required materials not supplied

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

For native conditions prepare the following buffers:

- Equilibration/Wash Buffer: 50 mM sodium phosphate, 300 mM sodium chloride, 10 mM imidazole; pH 7.4
- Elution Buffer: 50 mM sodium phosphate, 300 mM sodium chloride, 150 mM imidazole; pH 7.4

For denaturing conditions prepare the following buffers:

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

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 (Cat. 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 8 M urea, 6 M guanidine or Inclusion Body Solubilization Reagent (Cat. 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.

Perform 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 0.2 mL, 1 mL and 3 mL column devices are 1.0 mL, 8 mL and 22 mL, 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 cap (Save for later use) from the HisPur™ Cobalt Spin Column. Place column into a centrifuge tube.

   Note: Use 1.5 mL, 15 mL or 50 mL centrifuge tubes for the 0.2 mL, 1 mL and 3 mL spin columns, respectively.
4. Centrifuge column at 700 x *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 x *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 x *g* for 2 minutes and collect the flowthrough in a centrifuge tube.
9. Wash resin with two resin-bed volumes of Equilibration/Wash Buffer. Centrifuge at 700 x *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 x *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 280 nm or by Coomassie Plus™ (Bradford)™ Assay (Cat. No. 23236). The eluted protein can be directly analyzed by SDS-PAGE. To remove excess imidazole for downstream applications, use gel filtration or dialysis (for example, spin desalting columns or dialysis cassettes). Samples containing 6M guanidine-HCl must be dialyzed against a buffer containing 8 M urea before SDS-PAGE analysis. Thermo Scientific™ Pierce™ SDS-PAGE Sample Prep Kit (Cat. 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 20 mM MES Buffer, 0.1 M 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. To cap the bottom, invert the snap-off column closure from step 3 and apply to the column bottom with gentle pressure.

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 (for example, Inclusion Body Solubilization Reagent, Cat. 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.

Related products

Product	Catalog Number
Pierce™ High Capacity Endotoxin Removal Resin, 10 mL	88270
Pierce™ LAL Chromogenic Endotoxin Quantitation Kit	88282
Pierce™ Glutathione Agarose, 10 mL	16100
HisPur™ Ni-NTA Resin, 10 mL	88221
HisPur™ Cobalt Resin, 10 mL settled resin	89964
HisPur™ Cobalt Resin, 100 mL settled resin	89965
HisPur™ Cobalt Resin, 500 mL settled resin	89966
B-PER™ Bacterial Protein Extraction Reagent, 500 mL	78248
B-PER™ II Bacterial Protein Extraction Reagent, 250 mL	78260
I-PER™ Insect Cell Protein Extraction Reagent, 250 mL	89802

Product	Catalog Number
Halt™ Protease Inhibitor Cocktail, EDTA-Free, 1 mL	78410
Inclusion Body Solubilization Reagent, 100 mL	78115
DNAse I, 5,000 units	89835
Coomassie Plus™ (Bradford)™ Assay	23236
Zeba™ Spin Desalting Columns, 2 mL, 25 columns, for 200–700 µL samples	89890
Zeba™ Spin Desalting Columns, 5 mL, 25 columns, for 500–2000 µL samples	89892
Zeba™ Spin Desalting Columns, 10 mL, 25 columns, for 700–4000 µL samples	89894
Slide-A-Lyzer™ Dialysis Cassettes Kit, 10K MWCO, 0.1–0.5 mL	66385
Slide-A-Lyzer™ Dialysis Cassettes Kit, 10K MWCO, 0.5–3 mL	66382
Slide-A-Lyzer™ Dialysis Cassettes Kit, 10K MWCO, 3–12 mL	66807

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0011582 B

Revision	Date	Description
B	13 May 2024	Removing bottom caps to correspond with product change.
A.0	17 October 2015	New document for HisPur™ Cobalt Spin Columns.

The information in this guide is subject to change without notice.

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