

HisPur™ Cobalt Superflow Agarose

25228 25229 25230 25231

2407.0

Number	Description
25228	HisPur Cobalt Superflow Agarose , 10mL settled resin
25229	HisPur Cobalt Superflow Agarose , 50mL settled resin
25230	HisPur Cobalt Superflow Agarose , 250mL settled resin
25231	HisPur Cobalt Superflow Agarose , 1000mL settled resin
	Binding Capacity: ≥ 30 mg/mL of settled resin for a 27kDa 6xHis-tagged protein from a bacterial source
	Resin: Highly crosslinked 6% Superflow agarose
	Supplied: 50% slurry in a 20% ethanol solution

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

Table of Contents

Introduction	1
Important Product Information	2
Recommended Buffers	3
Procedure for Purification of His-tagged Proteins Using an LC System	3
Procedure for Purification of His-tagged Proteins by Batch Method	4
Procedure for Resin Cleaning-in-Place	5
Troubleshooting	6
Related Thermo Scientific Products	6

Introduction

The Thermo Scientific HisPur Cobalt Superflow Agarose enables high-yield, high-purity purification of polyhistidine-tagged proteins. This immobilized metal affinity chromatography (IMAC) purification support consists of a cobalt-charged tetradentate chelator immobilized onto Superflow 6% agarose, which exhibits minimal metal leaching and is compatible with a wide range of chemicals and pH values. The highly crosslinked Superflow Support has a high dynamic binding capacity across a wide range of flow rates and is stable to multiple reuses making it ideal for large-scale FPLC purifications.

The HisPur Cobalt Superflow Agarose protocol uses gentle wash and elution conditions to typically produce > 90% pure target protein after purification. Protein purities achieved with the Cobalt Superflow Resin are generally higher than those achieved with nickel IMAC resins, resulting in the Cobalt Superflow Resin being a valuable tool for users interested in one-step purifications.

Table 1. Properties of Thermo Scientific HisPur Cobalt Superflow Agarose.

Support: Superflow 6 Resin, 6% highly crosslinked agarose
Bead Size: 60-160µm
Recommended Linear Flow ≤ 150cm/hr (for binding, washing and elution steps)
Maximum Linear Flow Rate[†]: 1200cm/hr
Metal Loading: ≥ 11µMol Co²⁺/mL resin
Binding Capacity: Typical Static Capacity: ~30mg 6xHis-GFP/mL resin
Typical Dynamic Capacity*: ~20mg 6xHis-GFP/mL resin
Chemical Compatibility: 1M acetic acid, pH 2; 1% SDS, pH 7; 6M guanidine HCl; 70% ethanol: ≥ 1 week at 37°C
8M urea, 10mM DTT, 5mM TCEP: ≥ 2 hours at 22°C
pH Limits: pH 3-9 (≥1 week at 4°C)
pH 2-3 or 9-12 (≥ 2 hours at 22°C)
Storage Solution: 20% ethanol
Reuse: Up to 25 times

[†]Maximum linear flow-rate conditions:

Column dimensions (w × h): 13mm × 38mm (5mL resin)

Ultrapure water at room temperature

Linear flow rate = volumetric flow rate (mL/min) × 60 (min/hr)/cross sectional area (cm²)

*Dynamic binding conditions (10% breakthrough):

Sample: 1mg/mL 6xHis-GFP (27kDa) pure protein in 20mM NaH₂PO₄, 300mM NaCl, 5mM imidazole

Column dimensions (w × h): 5mm × 50mm (1mL resin)

Flow rate: 1mL/min

Important Product Information

- Protein yield and purity are dependent upon the expression level, conformation and solubility characteristics of the recombinant fusion protein, as well as the buffer conditions and flow rates used. 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. Decreasing the flow rate during the sample load will increase binding capacity.
- To avoid sample loss, try not to exceed the maximum resin binding capacity for the target protein for the purification conditions used. Volumes will vary based on the protein and expression efficiency and will have to be determined and optimized for each over-expressed protein. Typically over-expressed proteins represent 1-30% of the final sample protein concentration. Adjust resin or sample volume as appropriate.
- 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 with Enzymes (Product No. 90078), and mechanical methods, such as freeze/thaw cycles, sonication or French press. Add EDTA-free protease inhibitors, such as Thermo Scientific Halt Protease Inhibitor Cocktail, EDTA-free (Product No. 78437), to protect proteins from degradation.
- Overexpressed proteins can be sequestered in inclusion bodies. The Cobalt Superflow Resin is compatible with purification under native or denaturing conditions. Inclusion bodies containing His-tagged proteins can be solubilized in 8M urea, 6M guanidine or Thermo Scientific Inclusion Body Solubilization Reagent (Product No. 78115) and purified with the Cobalt Superflow Resin, but a denaturant must be added to buffers so the protein remains soluble throughout the procedure.
- For liquid chromatography (LC) applications, use highly pure buffer components and ultrapure water. Use low-absorbance imidazole (Fisher Scientific, Product No. BP 305-50) to avoid UV interference. Degas or filter buffers through a 0.45µm filter before use.
- Avoid using chelators such as EDTA, which will disrupt the function of the cobalt resin and potentially strip cobalt from the resin.
- Reducing agents, such as 10mM DTT and 5mM TCEP, have been tested and do not affect function of the resin; however, avoid using higher concentrations of these reducing agents.

Recommended Buffers

Note: For some specific proteins or expression systems, adjustments to the imidazole concentration may be required to decrease nonspecific binding or increase yield.

For native conditions:

- Equilibration Buffer: 20mM sodium phosphate, 300mM sodium chloride, 5mM imidazole; pH 7.4
- Wash Buffer: 20mM sodium phosphate, 300mM sodium chloride, 10-15mM imidazole; pH 7.4
- Elution Buffer: 20mM sodium phosphate, 300mM sodium chloride, 150mM imidazole; pH 7.4

For denaturing conditions:

- Equilibration Buffer: 20mM sodium phosphate, 300mM sodium chloride, 6M guanidine•HCl, 5mM imidazole; pH 7.4
- Wash Buffer: 20mM sodium phosphate, 300mM sodium chloride, 6M guanidine•HCl, 10-15mM imidazole; pH 7.4
- Elution Buffer: 20mM sodium phosphate, 300mM sodium chloride, 6M guanidine•HCl, 150mM imidazole; pH 7.4

For regeneration:

- MES Buffer: 20mM 2-(*N*-morpholine)-ethanesulfonic acid, 0.1M sodium chloride; pH 5.0
- Ultrapure water
- 20% ethanol in ultrapure water

For clean-in-place:

- 6M guanidine•HCl with 1% non-ionic detergent
- Ultrapure water

Procedure for Purification of His-tagged Proteins Using an LC System

Note: Monitor and collect all fractions during a purification to avoid accidental loss of target protein. User can adjust sample collection based on their needs and comfort level with the purification methods used. Maximum flow rates will be dependent on application and equipment used. The procedure may be performed at room temperature or 4°C.

Additional Materials Required

- Suitable LC system
 - Empty column for resin packing (follow column manufacturer's protocol for packing)
 - Recommended buffers (see Recommended Buffers Section) and volumes (see below)
1. Pack an appropriate-sized column with resin according to column manufacturer's protocol. Ensure the packing flow rate is at least 20% faster than the flow rate to be used during purification.
 2. Equilibrate the column and all buffers to working temperature. Perform purifications at room temperature or at 4°C. Ensure that all solutions are degassed.
 3. Prepare the LC system by washing pumps and filling tubing with buffer. To avoid introducing air into the system, allow a few drops of buffer to flow from the tubing into the column top. Connect column to the tubing.
 4. Equilibrate the column with 5-10 column volumes of the Equilibration Buffer at a flow rate of 300cm/hr or less (150cm/hr recommended).

5. Apply any sample volume that does not exceed column-binding capacity for target protein at a flow rate of 300cm/hr or less (150cm/hr recommended).
Note: Binding capacity is flow rate- and protein-dependent. Decreasing the flow rate during the sample load will increase binding capacity. Higher flow rates will decrease production time but may result in losing a small portion of the target protein in the flow-through fraction.
Note: For maximum binding, prepare sample by mixing protein extract 1:1 with Equilibration Buffer (to adjust the sample to the ionic strength and pH of the Equilibration Buffer) before applying to the column.
Note: If the sample contains insoluble matter, centrifuge or filter (0.45µm filter) before use.
6. Wash the resin at a flow rate of 300cm/hr or less (150cm/hr recommended) with 10-15 column volumes of Wash Buffer or until the absorbance approaches baseline.
Note: Due to the gentle binding characteristics of cobalt IMAC resin, excessive washing can elute target protein from the column.
7. Elute at a flow rate of 300cm/hr or less (150cm/hr recommended) with approximately 5-10 column volumes of Elution Buffer and collect fractions.
Note: Monitor protein elution by measuring the absorbance of the fractions at 280nm. 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; see the Thermo Scientific Related Products Section).
8. Regenerate column by washing with 10 column volumes of Regeneration Buffer, followed by 10 column volumes of ultrapure water at a flow rate of 300cm/hr or less (150cm/hr recommended). The column is now ready for reuse (return to step 1), storage (proceed to step 9) or routine clean-in-place procedures (see the Procedure for Resin Cleaning-In-Place).
9. For storage, equilibrate the column with 5 column volumes of 20% ethanol. Seal column and store at 4°C.

Procedure for Purification of His-tagged Proteins by Batch Method

Note: The procedure may be performed at room temperature or 4°C. The Cobalt Superflow Resin allows for customization of your purification strategy. Purification conditions can be scaled as needed and performed in several formats. A batch method based on centrifugation is included below. Alternatively, methods based on vacuum filtration or gravity flow can be used to collect flow-through, wash and elution fractions.

A. Additional Materials Required

- Sample-handling containers, such as centrifugation bottle or spin filters/columns
- Recommended buffers (see Recommended Buffers Section) and volumes (see below)
- End-over-end rotary mixer or equivalent mixing apparatus

B. Procedure

1. Add the required amount of Cobalt Superflow Resin to a container with 3-4X greater volume. Centrifuge for 2 minutes at $700 \times g$ and carefully remove and discard the supernatant.
2. Add two resin-bed volumes of Equilibration Buffer and mix until the resin is fully suspended.
3. Centrifuge for 2 minutes at $700 \times g$ and carefully remove and discard buffer.
4. Prepare sample by mixing the protein extract with Equilibration Buffer to a volume greater than or equal to the resin bed volume.
5. Add the prepared protein extract to the tube and mix slowly for 30 minutes ensuring the resin remains suspended. For best results, use an end-over-end rotary mixer.
6. Centrifuge for 2 minutes at $700 \times g$ and carefully remove supernatant. If desired, save supernatant for downstream analysis.

7. Wash the resin with two resin-bed volumes of Wash Buffer. Centrifuge for 2 minutes at $700 \times g$ and carefully remove supernatant. If desired, save supernatant for downstream analysis.
8. Repeat wash step three times.
9. Elute bound His-tagged proteins by resuspending resin bed in one resin-bed volume of Elution Buffer. Mix slowly for 10 minutes ensuring that resin remains suspended.
10. Centrifuge for 2 minutes at $700 \times g$. Carefully remove and save the supernatant.
11. Repeat elution steps 9-10 two to four times, saving each supernatant fraction in a separate tube.
12. Monitor protein elution by measuring the absorbance of the fractions at 280nm or by Thermo Scientific Coomassie Plus (Bradford) Assay Reagent (Product No. 23238) or Pierce 660nm Protein Assay (Product No. 22660). Dilute the samples at least 1:2 to decrease the imidazole concentration before performing the protein assay to avoid interference with the assay. The eluted protein can be directly analyzed by SDS-PAGE. To remove excess imidazole for other downstream applications, use gel filtration or dialysis (e.g., Thermo Scientific Zeba Spin Desalting Columns or Slide-A-Lyzer Dialysis Cassettes).
13. Regenerate the resin by resuspending resin with 10 resin-bed volumes of Regeneration Buffer. Centrifuge tube for 2 minutes at $700 \times g$ and discard the supernatant. Repeat once.
14. Resuspend resin with 10 resin-bed volumes of ultrapure water. Centrifuge tube for 2 minutes at $700 \times g$. Discard supernatant. Repeat once. The column is now ready for reuse (return to step 1), storage (proceed to step 15) or routine clean-in-place procedures (see the Procedure for Resin Cleaning-in-Place).
15. For storage, re-suspend resin with 1 column volume of 20% ethanol. Seal column and store at 4°C .

Procedure for Resin Cleaning-in-Place

Note: The Cobalt Superflow Resin can be used multiple times without affecting protein yield or purity. To prevent cross contamination of samples, designate a given column to one specific fusion protein. If an increase in backpressure is observed, the following cleaning procedures can be followed.

1. To remove precipitated or denatured proteins and hydrophobic substances, wash resin with 2 volumes of 6M guanidine•HCl plus 1% nonionic detergent (e.g., Thermo Scientific Triton X-100 Surfact-Amps Detergent Solution, Product No. 28314) with 10 minutes of contact time, followed by 5 volumes of ultrapure water at a flow rate of less than 300cm/hr (150cm/hr recommended).
2. Store resin in 20% ethanol at 4°C .

Troubleshooting

Problem	Possible Cause	Solution	
Low protein yield	Poor expression of soluble protein	Optimize expression conditions (e.g., lower temperature during induction, vary induction time, optimize codon usage for expression system)	
	His-tagged protein formed inclusion bodies	Alter 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., Thermo Scientific Inclusion Body Solubilization Reagent, Product No. 78115)	
	Insufficient cell lysis and extraction	Optimize cell lysis protocol	
	Fusion protein did not bind to the column		Verify the sequence
			Perform an ELISA or Western blot using an antibody against the His tag to confirm the His tag is present
	Flow rate was too fast	Decrease flow rate during binding to allow for greater residence time and increased binding of fusion protein	
	Column washing was too extensive		Reduce imidazole concentration in wash buffer
Reduce amount of wash buffer used			
Poor protein purity	Insufficiently washed	Increase duration of wash	
		Modify imidazole concentration and pH of the Equilibration or Wash Buffer	
	Column was dirty	Follow clean-in-place procedure to remove nonspecifically bound proteins	
Slow column flow	Column was overloaded	Apply less protein extract onto the column	
	Extract was too viscous or highly particulate	Dilute lysate with Equilibration Buffer to decrease viscosity	
		Centrifuge lysate at higher speed to remove particulate	

Related Thermo Scientific Products

25214-7	HisPur Ni-NTA Superflow Agarose
25236-9	Pierce™ Glutathione Superflow Agarose
89896-8	Pierce Centrifuge Columns
87785	Halt™ Protease Inhibitor Cocktail (100X), EDTA-free
88661	Pierce Protease Inhibitor Tablets, EDTA-free
88270	Pierce High Capacity Endotoxin Removal Resin
90078	B-PER™ Bacterial Protein Extraction Reagent with Enzymes
88282	Pierce LAL Chromogenic Endotoxin Quantitation Kit
23238	Coomassie Plus™ (Bradford) Assay Reagent
22660	Pierce 660nm Protein Assay Reagent
78115	Inclusion Body Solubilization Reagent
89891-4	Zeba Spin Desalting Columns, 7K MWCO
87730-8	Slide-A-Lyzer™ G2 Dialysis Cassettes, 10K MWCO
28313-4	Triton™ X-100 Surfact Amps™ Detergent Solution

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