INSTRUCTIONS

HisPur™ Ni-NTA Superflow Agarose

25214 25215 25216 25217

2406.0

Number	Description
25214	**HisPur Ni-NTA Superflow Agarose,** 10mL settled resin
25215	**HisPur Ni-NTA Superflow Agarose,** 50mL settled resin
25216	**HisPur Ni-NTA Superflow Agarose,** 250mL settled resin
25217	**HisPur Ni-NTA Superflow Agarose,** 1000mL settled resin

Binding Capacity: ≥ 60mg/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

The Thermo Scientific HisPur Ni-NTA Superflow Agarose enables high-yield, high-purity purification of polyhistidine-tagged proteins. This immobilized metal affinity chromatography (IMAC) purification support consists of nitrilotriacetic acid (NTA) chelator-activated agarose beads that are subsequently charged with divalent nickel (Ni^{2+}) ions. HisPur Ni-NTA Superflow Agarose 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.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce

Table 1. Properties of Thermo Scientific HisPur Ni-NTA Superflow Agarose.

Support :	Superflow 6 Resin, 6% highly crosslinked agarose
Bead Size :	60-160µm
Recommended Linear Flow Rate:	≤ 150cm/hr (binding, wash, elution)
Maximum Linear Flow Rate†:	1200cm/hr
Metal Loading Capacity:	≥ 15µM Ni^{2+}/mL resin
	Typical Static Capacity: ~60mg 6xHis-GFP/mL resin
	Typical Dynamic Capacity*: ~20mg 6xHis-GFP/mL resin
Chemical Compatibility:	1M acetic acid, pH 2; 0.1M HCl, pH 1; 1% SDS, pH 7; 6M guanidine•HCl; 70% ethanol: ≥ 1 week at 37°C
	8M urea, 10mM DTT, 5mM TCEP: ≥ 2hr 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 (cm^2)

*Dynamic binding conditions (10% breakthrough):
Sample: 1mg/mL 6xHis-GFP (27kDa) pure protein in 20mM NaH_2PO_4, 300mM NaCl, 10mM 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. For some proteins, adjustments to the imidazole concentration may be required to decrease nonspecific binding and increase yield.
- To avoid sample loss, try to not 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 cell lysis procedures 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. Confirm cell disruption before proceeding to protein purification. Add EDTA-free protease inhibitors, such as Thermo Scientific Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (Product No. 78437), during lysis procedures to protect proteins from degradation.
- Over-expressed proteins can often be sequestered in inclusion bodies. The Ni-NTA 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 Ni-NTA Superflow Resin, but a denaturant must be added to buffers so the protein remains soluble throughout the procedure.
- For liquid chromatography 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 resin function and potentially strip the nickel from the resin.
- Reducing agents, such as 10mM DTT and 5mM TCEP, have been tested and do not affect function of the resin. Avoid using higher concentrations of these reducing agents.

Recommended Buffers

For native conditions:

- Equilibration Buffer: 20mM sodium phosphate, 300mM sodium chloride, 10mM imidazole; pH 7.4
- Wash Buffer: 20mM sodium phosphate, 300mM sodium chloride, 20-30mM imidazole; pH 7.4
- Elution Buffer: 20mM sodium phosphate, 300mM sodium chloride, 300mM imidazole; pH 7.4

For denaturing conditions:

- Equilibration Buffer: 20mM sodium phosphate, 300mM sodium chloride, 6M guanidine•HCl, 10mM imidazole; pH 7.4
- Wash Buffer: 20mM sodium phosphate, 300mM sodium chloride, 6M guanidine•HCl, 20-30mM imidazole; pH 7.4
- Elution Buffer: 20mM sodium phosphate, 300mM sodium chloride, 6M guanidine•HCl, 300mM imidazole; pH 7.4

For regeneration:

- Regeneration Buffer: 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 Purifying His-Tagged Proteins Using a Liquid Chromatography 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.

A. Additional Materials Required

- Suitable liquid chromatography (LC) system
- Empty column for resin packing (follow column manufacturer's protocol for packing)
- Recommended buffers (see above) and volumes (see below)

B. Procedure

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 that will be used during purification.
2. Equilibrate the column and all buffers to working temperature. Purifications can be performed at room temperature or 4°C. Ensure 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 the 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.

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 Related Thermo Scientific 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 (proceed to step 1), storage (proceed to step 9) or routine clean-in-place procedures (see Procedure for Resin Cleaning-in-Place Section).

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 Ni-NTA Superflow Resin allows for customization of your purification strategy. Purification conditions can be scaled as needed 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

- Suitable vessel or 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 Ni-NTA Superflow Resin to a container with 3-4 times the volume of the resin quantity used. Centrifuge for 2 minutes at 700 × *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 × *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 stays suspended. For best results, use an end-over-end rotary mixer.
6. Centrifuge for 2 minutes at 700 × *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 × *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 making sure resin stays suspended.
10. Spin tube for 2 minutes at 700 × *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). To avoid interference with the assay, dilute the samples at least 1:2 to decrease the imidazole concentration before performing the protein 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., Zeba™ Spin Desalting Columns or Slide-A-Lyzer™ Dialysis Cassettes (see the Related Thermo Scientific Products Section).
13. Regenerate the resin by resuspending the resin with 10 resin-bed volumes of Regeneration Buffer. Centrifuge tube for 2 minutes at 700 × *g*. Discard supernatant. Repeat once.
14. Resuspend resin with 10 resin-bed volumes of ultrapure water. Centrifuge tube for 2 minutes at 700 × *g*. Discard supernatant. Repeat once. The column is now ready for reuse (proceed to step 1), storage (proceed to step 15) or routine clean-in-place procedures (see Procedure for Resin Cleaning-in-Place Section).
15. For storage, resuspend resin with 1 column volume of 20% ethanol. Seal column and store at 4°C.

Procedure for Resin Cleaning-in-Place

The Ni-NTA 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 or decrease in performance 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., ThermoScientific Triton X-100 Surfact Amps Detergent Solution) 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 make sure 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 imidizole concentration in Wash Buffer
		Reduce amount of Wash Buffer used

Continued on next page

Continued from previous page

Poor protein purity	Insufficient washing	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

25228-31 **HisPur Cobalt Superflow Agarose**

25236-9 **Pierce™ Glutathione Superflow Agarose**

89896-8 **Pierce Centrifuge Columns**

87785 **Halt™ Protease Inhibitor Cocktail (100X), EDTA-free,** 1mL

88666 **Pierce Protease Inhibitor Tablets, EDTA-free,** 30 tablets

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

88282 **Pierce LAL Chromogenic Endotoxin Quantitation Kit**

90078 **B-PER™ Bacterial Protein Extraction Reagent with Enzymes,** 250mL

23238 **Coomassie Plus™ (Bradford) Assay Reagent,** 300mL

22660 **Pierce 660nm Protein Assay Reagent,** 750mL

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

24115 **8M Guanidine•HCl Solution,** 200mL

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**

Triton is a trademark of the Dow Chemical Company.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.