INSTRUCTIONS

HisPur™ Ni-NTA Spin Columns

88224 88225 88226

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Number	Description
88224	**HisPur Ni-NTA Spin Columns, 0.2mL resin bed,** 25 columns
88225	**HisPur Ni-NTA Spin Columns, 1mL resin bed,** 5 columns
88226	**HisPur Ni-NTA Spin Columns, 3mL resin bed,** 5 columns

Binding Capacity: ≤ 60mg of a 28kDa 6xHis-tagged protein from a bacterial source per milliliter of settled resin

Resin: Crosslinked 6% agarose

Supplied: 50% slurry in 20% ethanol

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

Introduction

The Thermo Scientific™ HisPur™ Ni-NTA Spin Columns enable effective immobilized metal affinity chromatography (IMAC) purification of polyhistidine-tagged proteins from a soluble protein extract. These spin columns contain nickel-charged nitrilotriacetic acid (NTA) chelate immobilized onto 6% crosslinked agarose resin. The Ni-NTA resin is compatible with native or denaturing conditions and can be used in multiple formats, including conventional gravity-flow chromatography, spin column and FPLC. Ni-NTA resins are commonly chosen for His-tagged-protein purification because of the four metal-binding sites on the chelate, which allow for high-binding capacity and low-metal ion leaching.

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 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 and Phosphatase Inhibitor Cocktail, EDTA-free (Product No. 78441), to protect proteins from degradation.
- Sometimes overexpressed proteins are sequestered in inclusion bodies. Inclusion bodies of 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 resin, but a denaturant must be added to buffers so the protein remains soluble throughout the procedure.
- These instructions are effective for many types of samples; however, optimization might be required to further reduce nonspecific binding. To optimize conditions, adjust the recommended imidazole concentration in the Equilibration, Wash and Elution Buffer.
- 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 nickel resin.
- When using the Thermo Scientific™ Coomassie Plus™ (Bradford) Assay (Product No. 23238) or Thermo Scientific Pierce 660nm Protein Assay (Product No. 22660) to monitor protein concentration in the elution fractions, dilute the samples at least 1:2 before performing the protein assay.

Additional Materials Required

Note: The buffers listed below are recommended. To decrease nonspecific binding and increase yield, adjustments to the imidazole concentration might be required for specific proteins.

For native conditions prepare the following buffers:

- Equilibration Buffer: 20mM sodium phosphate, 300mM sodium chloride (PBS) with 10mM imidazole; pH 7.4
- Wash Buffer: PBS with 25mM imidazole; pH 7.4
- Elution Buffer: PBS with 250mM imidazole; pH 7.4

For denaturing conditions prepare the following buffers:

- Equilibration Buffer: PBS with 6M guanidine•HCl and 10mM imidazole; pH 7.4
- Wash Buffer: PBS with 6M guanidine•HCl and 25mM imidazole; pH 7.4
- Elution Buffer: PBS with 6M guanidine•HCl and 250mM imidazole; pH 7.4

For resin regeneration prepare the following buffer:

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

Procedure for Spin Purification of His-Tagged Proteins

Note: The total volume of the 0.2, 1 and 3mL columns are 1, 8 and 22mL, respectively. If a sample volume is greater than the column, perform multiple applications and centrifugations until the entire sample has been processed. Be careful not to exceed the resin's binding capacity. The HisPur Ni-NTA Spin Columns also may be used for gravity-flow purifications.

1. Equilibrate column(s) to working temperature. Perform purifications at room temperature or at 4°C.
2. Prepare sample by mixing the protein extract with an equal volume of Equilibration Buffer. Use the Equilibration Buffer to adjust the total volume to be ≥ 2 resin-bed volumes.
3. Remove the bottom tab from the HisPur Ni-NTA Spin Column by gently twisting. Place column into a centrifuge tube.

   Note: Use 2.0, 15 or 50mL centrifuge tubes for the 0.2, 1 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 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 in the column and add the prepared protein extract. Mix on an orbital shaker or end-over-end mixer for 30 minutes at room temperature or 4°C.
8. Remove the bottom plug. Centrifuge the 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 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 Reagent (Product No. 23238). The eluted protein can be directly analyzed by SDS-PAGE.

    Note: To remove 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 Ni-NTA Resin Regeneration

The Ni-NTA resin may be used at least five 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 MES Buffer.
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 expression conditions
	His-tagged protein forms 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., 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 and/or 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

Visit the website for additional information relating to this product including the following:

- 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

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

88227 **HisPur Ni-NTA Purification Kit,** 0.2mL, 25 each

88270 **Pierce High Capacity Endotoxin Removal Resin**

88282 **Pierce LAL Chromogenic Endotoxin Quantitation Kit**

89967 **HisPur Cobalt Spin Columns,** 0.2mL, 25 each

89968 **HisPur Cobalt Spin Columns,** 1mL, 5 each

89969 **HisPur Cobalt Spin Columns,** 3mL, 5 each

24110 **Guanidine•HCl,** 500g

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

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

78441 **Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X),** 1mL

23238	**Coomassie Plus (Bradford) Assay Reagent,** 300mL
22660	**Pierce 660nm Protein Assay Reagent,** 750mL
78115	**Inclusion Body Solubilization Reagent,** 100mL
89890	**Zeba Spin Desalting Columns, 7K MWCO, 2mL,** 25 columns, for 200-700µL samples
89892	**Zeba Spin Desalting Columns, 7K MWCO, 5mL,** 25 columns, for 500-2000µL samples
89894	**Zeba Spin Desalting Columns, 7K MWCO, 10mL,** 25 columns, for 1500-4000µL samples
87769	**Zeba Spin Desalting Columns, 40K MWCO, 2mL,** 25 columns, for 200-900µL samples
87771	**Zeba Spin Desalting Columns, 40K MWCO, 5mL,** 25 columns, for 300-2000µL samples
87773	**Zeba Spin Desalting Columns, 40K MWCO, 10mL,** 25 columns, for 1000-4000µL samples
87730	**Slide-A-Lyzer G2 Dialysis Cassettes, 10K MWCO, 3mL,** 10 cassettes
87731	**Slide-A-Lyzer G2 Dialysis Cassettes, 10K MWCO, 15mL,** 8 cassettes

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