INSTRUCTIONS

HisPur™ Ni-NTA Spin Plates

88230 2280.1

Number	Description
88230	**HisPur Ni-NTA Spin Plates,** 2 filter plates
	Contents: Each well of the 96-well filter plates contains 50µL of resin bed (200µL of 25% resin slurry) and six wash/collection plates
	Resin: Crosslinked 6% agarose supplied in 20% ethanol
	Binding capacity: 1mg/well at > 90% purity of a 28kDa His-tagged protein from a bacterial source
	Metal ion capacity: ≥ 15µmol nickel/mL of resin
	Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific HisPur Ni-NTA Spin Plates are convenient, ready-to-use pre-dispensed filter plates for the efficient purification of polyhistidine-tagged proteins from bacterial, mammalian and baculovirus-infected cells. His-tagged proteins are purified from total soluble protein extract using nickel-charged nitrilotriacetic acid (NTA) chelate immobilized onto 6% crosslinked agarose. The HisPur Ni-NTA Spin Plates are compatible with centrifugation and vacuum manifold systems for manual or automated purification. The plates enable fast, consistent well-to-well and plate-to-plate reproducibility for small-scale high-throughput separations.

Important Product Information

- The HisPur Ni-NTA Spin Plates are compatible with variable-speed centrifuges with rotors and carriers capable of handling stacked plates. Use a centrifuge speed of 1000 × *g*.
- If the purification is performed using centrifugation, the spin plate must be balanced throughout the procedure with a duplicate plate or a balance plate (Product No. 45205) filled with an appropriate volume of water.
- Purification may be performed with standard vacuum manifold systems, depending upon sample properties and pre-treatment. Suggested flow rates are 4-8 inches Hg (2-4 psi), which is equivalent to 1-2 drops/second. Avoid overdrying resin when using a vacuum system as this can result in channeling or cracking of the resin bed.
- Each well of the spin plate has a typical binding capacity of up to 1mg of His-tagged protein. Depending upon protein expression, the maximum total protein (lysate) loading amount is 4mg. Typical yields are 10-25% of the total protein loaded into the well. For optimal results, do not exceed the capacity of the resin.
- Optimization of the lysis procedure is critical for maximizing protein yield. Some methods for protein extraction include using detergent-based reagents, such as Thermo Scientific B-PER Bacterial Protein Extraction Reagent in Phosphate Buffer (Product No. 78266), and mechanical methods, such as freeze/thaw cycles, sonication or French press.
- Although the buffer conditions described in these instructions work well for a majority of proteins, optimization may be required to further reduce nonspecific binding. (See Troubleshooting Section)
- The HisPur Ni-NTA Resin relies on nickel chelation to both the NTA chelator and the target histidine tag. Avoid using any additives that contain metal 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

- Plate or orbital shaker (optional)
- Variable-speed centrifuge with rotor and carriers capable of handling stacked plates (4.4cm height) at 1000 × *g* or a vacuum manifold system
- Equilibration/Binding Buffer: 50mM sodium phosphate, 300mM sodium chloride, 0-10mM imidazole; pH 7.4
- Wash Buffer: 50mM sodium phosphate, 300mM sodium chloride, 5-10mM imidazole; pH 7.4
- Elution Buffer: 50mM sodium phosphate, 300mM sodium chloride, 150mM imidazole; pH 7.4. A high-purity low-absorbance imidazole (Fisher BP-305-50) is ideal when baseline interference by imidazole is problematic for monitoring protein concentration. Alternatively, adjust the elution buffer to pH 5.0 and omit the imidazole.
- EDTA-free protease inhibitors such as Thermo Scientific Halt Protease Inhibitor Single Use Cocktail, EDTA-Free (Product No 78425)
- Balance Plate (Product No. 45205)
- Optional: Additional plates for collecting and storing extra fractions
- Optional: Thermo Scientific Zeba 96-well Spin Desalting Plates (Product No. 89808) for buffer exchange
- Optional: Sealing tape for 96-well plates (Product No. 15036)

Procedure for Purification of His-tagged Protein Using Centrifugation

1. Equilibrate plate(s) to working temperature. Perform purifications at room temperature or at 4°C.
2. Prepare samples by diluting bacterial protein extract 1:1 with Equilibration/Binding Buffer so the total volume is ≤ 200µL/well.

   Note: If sample volume per well is > 200µL, perform multiple applications and centrifugation steps until the entire sample has been processed. Be careful not to exceed the binding capacity of the resin.
3. Remove the bottom seal from the plate(s) and place the plate on top of a wash/collection plate. Remove the top seal.
4. Place the plate assembly into a centrifuge with a 96-well plate-carrier and centrifuge for 1 minute at 1000 × *g* to remove the storage solution. Discard the solution.
5. Add 250µL of Equilibration/Binding Buffer to each well, centrifuge for 1 minute at 1000 × *g* and discard the buffer. Repeat this two more times.
6. Place the purification plate on top of the wash/collection plate. Apply up to 200µL of prepared sample (from Step 2) to each well. Incubate for 15 minutes with moderate agitation (optional) on a plate or orbital shaker.
7. Centrifuge the plate assembly for 1 minute at 1000 × *g*. Make sure the applied sample has drained from all wells. Discard the flow-through or use additional collection plates to collect and store the flow-through.
8. Place the purification plate on a wash/collection plate, and wash resin by adding 250µL/well of Wash Buffer. Centrifuge at 1000 × *g* for 1 minute. Repeat this step two more times, discarding the wash each time. Depending upon the sample, additional wash steps might be required.
9. Place the purification plate on a new collection plate. Add 250µL of Elution Buffer to each well and incubate with gentle agitation (optional) for 1 minute.
10. Centrifuge the plate assembly at 1000 × *g* for 1 minute to collect the eluate. Repeat the elution step once for maximum protein concentration or twice for maximum yield. Vary the elution volumes as needed for the desired protein concentration (i.e., 100µL). Use additional plates for collecting and storing extra fractions.
11. Seal collection plate top with sealing tape and store protein at 4ºC.
12. Monitor protein elution by measuring the absorbance of the protein in each well at 280nm or by the Coomassie Plus (Bradford) Assay (Product No. 23238) or Pierce 660nm Protein Assay (Product No. 22660). The eluted protein can be directly analyzed by SDS-PAGE. Use gel filtration to remove excess imidazole for downstream applications (e.g., Zeba™ 96-well Spin Desalting Plates).

Procedure for Purification of His-tagged Protein Using a Vacuum Manifold

1. Adjust the vacuum to obtain a flow rate of 4-8 inches Hg (2-4 psi), which is equivalent to 1-2 drops/second.
2. Equilibrate plate(s) to working temperature. Purification may be performed at room temperature or at 4°C.
3. Prepare samples by diluting bacterial protein extract 1:1 with Equilibration/Binding Buffer so the total volume is ≤ 200µL/well.

   Note: If the sample volume per well is > 200µL, perform multiple applications and vacuum steps until the entire sample has been processed. Be careful not to exceed the binding capacity of the resin.
4. Remove the bottom seal from the plate(s) and place the plate upright. Remove the top seal and mount the plate onto the top of a vacuum manifold. Apply vacuum to remove the storage solution. Let the solution drain or collect in a wash/collection plate. Discard the solution.
5. Equilibrate the plate by adding 250µL of Equilibration/Binding Buffer to each well. Apply vacuum and let the buffer drain or collect in a wash/collection plate and discard. Repeat this step two more times.
6. Align the purification plate on top of the wash/collection plate in the vacuum manifold.
7. Apply up to 200µL of prepared sample (from Step 3) to each well. Incubate for 15 minutes with (optional) moderate agitation.

   Note: Detergent lysis solutions may foam and result in cross-contamination. Decreasing the pressure (Step 9) can help to prevent this from occurring.
8. Apply the vacuum. Make sure the sample has been drained from all wells. Discard the flow-through or use extra collection plates to collect and store the flow-through.
9. Place the purification plate and wash/collection plate on the vacuum manifold and wash the resin by adding 250µL/well of Wash Buffer. Apply the vacuum. Repeat this step two more times, discarding the wash each time. Depending upon the sample, additional wash steps might be required.
10. Place the purification plate and a new collection plate on the vacuum manifold. Add 250µL of Elution Buffer to each well and incubate with gentle agitation (optional) for 1 minute.
11. Apply vacuum to collect the eluate. Repeat the elution step once to collect the maximum protein concentration or twice to collect the maximum yield. If needed, use additional plates for collecting and storing extra fractions.
12. Seal collection plate top with Sealing Tape (Product No. 15036) and store protein at 4ºC.
13. Monitor protein elution by measuring the absorbance of the protein in each well at 280nm or by the Coomassie Plus (Bradford) Assay (Product No. 23238) or Pierce 660nm Protein Assay (Product No. 22660). The eluted protein can be directly analyzed by SDS-PAGE. Use gel filtration to remove excess imidazole for downstream applications (e.g., Zeba 96-well Spin Desalting Plates).

Troubleshooting

Problem	Possible Cause	Solution
Low 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
		Solubilize inclusion bodies in 8M urea, 6M guanidine or other solubilization reagent (Product No. 78115) and purify using the Ni-NTA plate (see the Additional Information Section)
	Insufficient cell lysis and extraction	Optimize cell lysis protocol
	Fusion protein does not bind to the resin	Omit imidazole from the Equilibration/Binding Buffer or adjust the pH to 8.0 – note that amino acids (other than histidine) can adsorb at high pH, which affects resin capacity and purity of the target protein
		Verify the sequence or perform an ELISA or Western blot using an antibody against the His tag to make sure the tag is present
Poor protein purity	Insufficient column washing	Wash column additional times
	Nonspecific binding	Adjust the imidazole concentration of the Equilibration/ Binding Buffer to 10mM (do not exceed 10mM), or decrease buffer pH to 7.0
Slow flow rate	Well is overloaded	Apply less protein extract to the column and make sure the extract is not too viscous or contaminated with cell debris

Additional Information

A. Fusion Proteins Expressed in Inclusion Bodies

Over-expressed proteins are sometimes sequestered in inclusion bodies. Inclusion bodies can be solubilized in 8M urea, 6M guanidine or the Inclusion Body Solubilization Reagent (Product No. 78115) and purified using the Ni-NTA resin; however, a denaturant must be added to the buffers to ensure the protein remains soluble throughout the procedure. (**Follow the Procedure for Spin Purification of His-tagged Protein.)**

Note: Purified samples containing 6M guanidine•HCl must be dialyzed against a buffer containing 8M urea before SDS-PAGE analysis. Alternatively, use the Thermo Scientific Pierce SDS-PAGE Sample Prep Kit (Product No. 89888) for removing guanidine.

For denaturing conditions prepare the following buffers:

- Equilibration/Wash Buffer: 50mM sodium phosphate, 300mM sodium chloride, 6M guanidine•HCl, 0-10mM imidazole; pH 7.4
- Elution Buffer: 50mM sodium phosphate, 300mM sodium chloride, 6M guanidine•HCl, 150mM imidazole; pH 7.4

B. Tech Tips Available from our Website

- Tech Tip #43: Protein stability and storage
- Tech Tip #6: Extinction coefficients guide
- Tech Tip #40: Convert between times gravity (× g) and centrifuge rotor speed (RPM)

Related Thermo Scientific Products

78425	**Halt™ Protease Inhibitor Single Use Cocktail, EDTA-Free,** 24 × 100µL microtubes
88221-23	**HisPur Ni-NTA Resin,** 10mL, 100mL or 500mL of settled resin
88224-26	**HisPur Ni-NTA Spin Columns,** 0.2mL, 1mL or 3mL resin bed
88227-29	**HisPur Ni-NTA Purification Kit,** 0.2mL, 1mL or 3mL resin bed
88270	**Pierce High Capacity Endotoxin Removal Resin,** 10mL
88282	**Pierce LAL Chromogenic Endotoxin Quantitation Kit**
78266	**B-PER® Bacterial Protein Extraction Reagent (in Phosphate Buffer),** 500mL
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
78115	**Inclusion Body Solubilization Reagent,** 100mL
89835	**DNAse I,** 5000 units
23236	**Coomassie Plus (Bradford) Assay Kit**
45205	**Balance Plate**
89808	**Zeba 96-well Spin Desalting Plates,** 4 plates, for sample volumes of 20-100µL
89806	**Protein Stabilizing Cocktail, 4X Concentrated Solution,** 10mL
16111	**Pierce Glutathione Spin Plates,** 2/pkg
90095	**HisPur Cobalt Spin Plates,** 2/pkg
90098-99	**HisPur Ni-NTA Chromatography Cartridges,** 1mL or 5mL

General Reference

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**:248-54.

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