HisPur™ Ni-NTA Resin

Catalog Numbers  88221, 88222, 88223

Pub. No.  MAN0011700  Rev.  B.0

Product description

The Thermo Scientific™ HisPur™ Ni-NTA Resin enables effective immobilized metal affinity chromatography (IMAC) purification of polyhistidine-tagged proteins from a soluble protein extract. This resin is composed of nickel-charged nitrilotriacetic acid (NTA) chelate immobilized onto 6% crosslinked agarose. 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.

HisPur™ Ni-NTA Resin features and formats:

- Binding capacity: ≤ 60 mg of a 28-kDa 6xHis-tagged protein from a bacterial source per milliliter of settled resin
- Resin: Crosslinked 6% agarose
- Supplied: 50% slurry in 20% ethanol

Contents and storage

Table 1  HisPur™ Ni-NTA Resin

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Amount</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>88221</td>
<td>10 mL</td>
<td></td>
</tr>
<tr>
<td>88222</td>
<td>100 mL</td>
<td></td>
</tr>
<tr>
<td>88223</td>
<td>500 mL</td>
<td>4°C[1]</td>
</tr>
</tbody>
</table>

[1] The product is shipped at ambient temperature.

Required materials not supplied

Unless otherwise indicated, all materials are available through thermofisher.com. "MLS" indicates that the material is available from fisherscientific.com or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium phosphate</td>
<td>MLS</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>MLS</td>
</tr>
<tr>
<td>Imidazole</td>
<td>MLS</td>
</tr>
<tr>
<td>2-(N-morpholine)-ethanesulfonic acid</td>
<td>MLS</td>
</tr>
<tr>
<td>Guanidine-HCl</td>
<td>24110</td>
</tr>
</tbody>
</table>

General Guidelines

- Immunoglobulins are known to have multiple histidines in their Fc region that can bind to IMAC supports. High background and false positives can result if sufficient blocking is not performed to prevent binding of immunoglobulins in the absence of the His-tagged protein. Albumins, such as bovine serum albumin (BSA), also have multiple histidines that can bind to IMAC supports, but with a lower affinity than immunoglobulins or His-tagged proteins, which can displace the albumin.

For Research Use Only. Not for use in diagnostic procedures.
Protein yield and purity are dependent upon the expression level, conformation, and solubility characteristics of the recombinant fusion protein. 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™ with Enzymes Bacterial Protein Extraction Kit (Cat. 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 (100X) (Cat. No. 78441), to protect proteins from degradation.

Overexpressed proteins can be sequestered in inclusion bodies. Inclusion bodies of His-tagged proteins can be solubilized in 8 M urea, 6 M guanidine, or Thermo Scientific™ Inclusion Body Solubilization Reagent (Cat. No. 78115), and purified with the Ni-NTA resin. 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 may 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™ Pierce™ Coomassie Plus (Bradford) Assay Reagent (Cat. No. 23238) or Thermo Scientific™ Pierce™ 660 nm Protein Assay Reagent (Cat. No. 22660) to monitor protein concentration in the elution fractions, dilute the samples at least 1:2 before performing the protein assay.

Prepare buffers

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

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Buffers for native conditions</strong></td>
<td></td>
</tr>
</tbody>
</table>
| Equilibration Buffer, pH 7.4 | 20 mM sodium phosphate  
300 mM sodium chloride  
10 mM imidazole           |
| Wash Buffer, pH 7.4      | 20 mM sodium phosphate  
300 mM sodium chloride  
25 mM imidazole            |
| Elution Buffer, pH 7.4   | 20 mM sodium phosphate  
300 mM sodium chloride  
250 mM imidazole                      |
| **Buffers for denaturing conditions** |                                           |
| Equilibration Buffer, pH 7.4 | 20 mM sodium phosphate  
300 mM sodium chloride  
6 M guanidine-HCl  
10 mM imidazole           |
| Wash Buffer, pH 7.4      | 20 mM sodium phosphate  
300 mM sodium chloride  
6 M guanidine-HCl  
25 mM imidazole            |
| Elution Buffer, pH 7.4   | 20 mM sodium phosphate  
300 mM sodium chloride  
6 M guanidine-HCl  
250 mM imidazole                      |
| **Buffer for resin degeneration** |                                    |
| MES Buffer, pH 5.0       | 20 mM 2-((N-morpholine)-ethanesulfonic acid   
0.1 M sodium chloride            |
Purification of His-tagged proteins by batch method

The HisPur™ Ni-NTA Resin allows for purification strategy customization. Purification conditions can be scaled as needed. The procedure may be performed at room temperature or at 4°C.

1. Add Ni-NTA resin and Equilibration Buffer
   1.1. Add an appropriate amount of Ni-NTA resin to a tube.
   1.2. Centrifuge the tube for 2 minutes at 700 × g, then carefully remove and discard the supernatant.
   1.3. Add two resin-bed volumes of Equilibration Buffer, then mix until the resin is fully suspended.
   1.4. Centrifuge the tube for 2 minutes at 700 × g, then carefully remove and discard the buffer.

2. Prepare sample, add prepared protein extract, then centrifuge
   2.1. Prepare sample by mixing the protein extract with an equal volume of Equilibration Buffer.
   The total volume should equal at least two volumes of the resin bed.
   2.2. Add the prepared protein extract to the tube, then mix on an end-over-end rotator for 30 minutes.
   2.3. Centrifuge the tube for 2 minutes at 700 × g.
   Optional: Save the supernatant for downstream analysis.

3. Wash resin, then elute His-tagged proteins
   3.1. Wash the resin with two resin-bed volumes of Wash Buffer.
   3.2. Centrifuge the tube for 2 minutes at 700 × g.
   Optional: Save the supernatant for downstream analysis.
   3.3. Repeat steps 3.1 and 3.2.
   Optional: Save the supernatant for downstream analysis.
   3.4. Elute bound His-tagged proteins using one resin-bed volume of Elution Buffer.
   3.5. Centrifuge tube for 2 minutes at 700 × g. Carefully remove and save the supernatant.
   3.6. Repeat steps 3.4 and 3.5 twice, saving each supernatant fraction in a separate tube.
   3.7. Monitor protein elution by measuring the absorbance of the fractions at 280 nm, or by Coomassie Plus (Bradford) Assay Reagent (Cat. No. 23238) or Pierce™ 660 nm Protein Assay Reagent (Cat. No. 22660).
   The eluted protein can be directly analyzed by SDS-PAGE.

Purification of His-tagged proteins using a gravity-flow column

The HisPur™ Ni-NTA Resin allows for purification strategy customization. Purification conditions can be scaled as desired. Perform the procedure at room temperature or at 4°C.

1. Pack column, prepare sample, then equilibrate the column
   1.1. Pack the column with an appropriate amount of Ni-NTA resin.
   Allow storage buffer to drain from resin by gravity flow.
   1.2. Prepare sample by mixing protein extract with an equal volume of Equilibration Buffer.
   1.3. Equilibrate the column with two resin-bed volumes of Equilibration Buffer.
   Allow buffer to drain from the column.
Add prepared protein, wash resin, then elute His-tagged proteins

1. Add the prepared protein extract to the resin, then collect the flow-through in a tube. 
   Optional: Re-apply the flow-through once to maximize binding.

2. Wash resin with two resin-bed volumes of Wash Buffer, then collect the flow-through. 
   Repeat this step using a new collection tube until the absorbance of the flow-through fraction at 280 nm approaches baseline.

3. Elute His-tagged proteins from the resin with two resin-bed volumes of Elution Buffer. 
   Repeat this step twice, collecting each fraction in a separate tube.

4. Monitor protein elution by measuring the absorbance of the fractions at 280 nm or by Coomassie Plus (Bradford) Assay Reagent (Cat. No. 23238) or Pierce™ 660 nm Protein Assay Reagent (Cat. No. 22660). 
   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 6 M 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 (Cat. No. 89888) may also be used to remove guanidine.

Regenerate Ni-NTA resin

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

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low protein yield</td>
<td>Poor expression of soluble protein.</td>
<td>Optimize expression conditions.</td>
</tr>
<tr>
<td></td>
<td>His-tagged protein formed inclusion bodies.</td>
<td>Alter growth conditions to minimize inclusion body formation and maximize soluble protein yield.</td>
</tr>
<tr>
<td></td>
<td>Insufficient cell lysis and extraction.</td>
<td>Optimize cell lysis protocol.</td>
</tr>
<tr>
<td></td>
<td>Fusion protein did not bind to the column.</td>
<td>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.</td>
</tr>
<tr>
<td>Poor protein purity</td>
<td>Insufficient washing.</td>
<td>Wash resin additional times or modify imidazole concentration and pH of the Equilibration and/or Wash Buffer.</td>
</tr>
<tr>
<td>Slow column flow</td>
<td>Column was overloaded.</td>
<td>Apply less protein extract onto the column and make sure the extract is not too viscous or highly particulate.</td>
</tr>
</tbody>
</table>

Supplemental information

User tips

Visit the website for additional information relating to this product, including the following:
- Tech Tip #6—Extinction coefficients guide
- Tech Tip #40—Convert between times gravity (x g) and centrifuge rotor speed (RPM)
- Tech Tip #43—Protein stability and storage
<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>HisPur™ Ni-NTA Spin Columns, 0.2 mL resin bed</td>
<td>25 columns</td>
<td>88224</td>
</tr>
<tr>
<td>HisPur™ Ni-NTA Spin Columns, 1 mL resin bed</td>
<td>5 columns</td>
<td>88225</td>
</tr>
<tr>
<td>HisPur™ Ni-NTA Spin Columns, 3 mL resin bed</td>
<td>5 columns</td>
<td>88226</td>
</tr>
<tr>
<td>Pierce™ High Capacity Endotoxin Removal Resin</td>
<td>10 mL</td>
<td>88270</td>
</tr>
<tr>
<td>Pierce™ LAL Chromogenic Endotoxin Quantiﬁcation Kit</td>
<td>50 tests</td>
<td>88282</td>
</tr>
<tr>
<td>HisPur™ Cobalt Spin Columns, 0.2 mL</td>
<td>25 columns</td>
<td>89967</td>
</tr>
<tr>
<td>HisPur™ Cobalt Spin Columns, 1 mL</td>
<td>5 columns</td>
<td>89968</td>
</tr>
<tr>
<td>HisPur™ Cobalt Spin Columns, 3 mL</td>
<td>5 columns</td>
<td>89969</td>
</tr>
<tr>
<td>B-PER™ with Enzymes Bacterial Protein Extraction Kit</td>
<td>250 mL</td>
<td>90078</td>
</tr>
<tr>
<td>Halt™ Protease Inhibitor Cocktail (100X), EDTA-free</td>
<td>1 mL</td>
<td>87785</td>
</tr>
<tr>
<td>Halt™ Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X)</td>
<td>1 mL</td>
<td>78441</td>
</tr>
<tr>
<td>Coomassie Plus (Bradford) Assay Reagent</td>
<td>300 mL</td>
<td>23238</td>
</tr>
<tr>
<td>Pierce™ 660 nm Protein Assay Reagent</td>
<td>750 mL</td>
<td>22660</td>
</tr>
<tr>
<td>Inclusion Body Solubilization Reagent</td>
<td>100 mL</td>
<td>78115</td>
</tr>
<tr>
<td>Zeba® Spin Desalting Columns, 7K MWCO, 2 mL</td>
<td>25 columns, for 200–700 μL samples</td>
<td>89890</td>
</tr>
<tr>
<td>Zeba® Spin Desalting Columns, 7K MWCO, 5 mL</td>
<td>25 columns, for 500–2,000 μL samples</td>
<td>89892</td>
</tr>
<tr>
<td>Zeba® Spin Desalting Columns, 7K MWCO, 10 mL</td>
<td>25 columns, for 1,500–4,000 μL samples</td>
<td>89894</td>
</tr>
<tr>
<td>Zeba® Spin Desalting Columns, 40K MWCO, 2 mL</td>
<td>25 columns, for 200–900 μL samples</td>
<td>87769</td>
</tr>
<tr>
<td>Zeba® Spin Desalting Columns, 40K MWCO, 5 mL</td>
<td>25 columns, for 300–2,000 μL samples</td>
<td>87771</td>
</tr>
<tr>
<td>Zeba® Spin Desalting Columns, 40K MWCO, 10 mL</td>
<td>25 columns, for 1,000–4,000 μL samples</td>
<td>87773</td>
</tr>
<tr>
<td>Slide-A-Lyzer™ G2 Dialysis Cassettes, 10K MWCO, 3 mL</td>
<td>10 cassettes</td>
<td>87730</td>
</tr>
<tr>
<td>Slide-A-Lyzer™ G2 Dialysis Cassettes, 10K MWCO, 15 mL</td>
<td>8 cassettes</td>
<td>87731</td>
</tr>
</tbody>
</table>
Customer and technical support

Visit thermofisher.com/support for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA

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

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

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0011700

<table>
<thead>
<tr>
<th>Revision</th>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.0</td>
<td>17 November 2021</td>
<td>The buffer components for native conditions and denaturing conditions were updated.</td>
</tr>
<tr>
<td>A.0</td>
<td>17 October 2015</td>
<td>Initial release with new publication number format.</td>
</tr>
</tbody>
</table>

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

©2021 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.