Pierce™ High-Capacity Ni-IMAC Resin, EDTA Compatible

Catalog Numbers A50584

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Pierce™ High-Capacity Ni-IMAC Resin, EDTA Compatible enables efficient purification of recombinant polyhistidine-tagged proteins from a soluble protein extract or mammalian cell culture supernatant. This resin consists of 6% crosslinked agarose beads coupled to a novel proprietary ligand loaded with nickel ions. The novel high-affinity chelator of the resin enables high binding capacity of His-tagged proteins in the presence of chemical additives such as chelators (EDTA), strong reducing agents (DTT), or components of cell culture supernatants that typically strip off Ni ions and reduce the functionality of most IMAC resins. The resin is compatible with native or denaturing conditions and can be used in multiple formats, including conventional gravity-flow chromatography, spin column, and FPLC.

Table 1 Characteristics of Pierce™ High-Capacity Ni-IMAC Resin, EDTA Compatible.

Binding Capacity	Magnetite-embedded agarose beads coupled to a novel, proprietary ligand loaded with nickel ions
Resin	Ferrimagnetic with low remanence
pH Tolerance	2–13
Supplied	50% slurry in 20% ethanol
Reusable	Up to 10 times
Chelator Stability	Stable in buffer containing 20 mM EDTA and 20 mM DTT

Contents and storage

Product	Cat. No.	Amount	Storage
Pierce™ High-Capacity Ni-IMAC Resin, EDTA Compatible	A50584	20 mL of 50% slurry	Store at 4°C.
	A50585	100 mL of 50% slurry	
	A50586	500 mL of 50% slurry	
	A50587	1,000 mL of 50% slurry	

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 attemtping 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 including freeze/thaw cycles, sonication or, French press. Add protease inhibitors Thermo Scientific™ Halt™ Protease and Phosphatase Inhibitor Cocktail (100X) (Cat. No. 78444) 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-IMAC 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 may be required to further reduce nonspecific binding. To optimize conditions, adjust the recommended imidazole concentration in the Equilibration, Wash, and Elution Buffers.
- Concentration of proteins in the eluted fractions can be determined by using the Thermo Scientific™ Pierce™ Detergent Compatible Bradford Assay Kit (Cat. No. 23246) or Pierce™ 660nm Protein Assay Kit (Cat. No. 22662).
- For liquid chromatography applications, use highly pure buffer components and ultrapure water. Use low-absorbance imidazole (Fisher Scientific, Cat. No. BP 305-50) to avoid UV interference. Degas or filter buffers through a 0.45 µm filter before use.

Materials required but not provided

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

For native conditions, prepare the following buffers:

- Equilibration Buffer: 50 mM monosodium phosphate, 300 mM sodium chloride, 10 mM imidazole in water; pH 8.0
- Wash Buffer: 50 mM monosodium phosphate, 300 mM sodium chloride, 20 mM imidazole in water; pH 8.0
- Elution Buffer: 50 mM monosodium phosphate, 300 mM sodium chloride, 500 mM imidazole in water; pH 8.0

For denaturing conditions, prepare the following buffers:

- Equilibration Buffer: 100 mM monosodium phosphate, 10 mM Tris base, 8 M urea in water; pH 8.0
- Wash Buffer: 100 mM monosodium phosphate, 10 mM Tris base, 8 M urea in water; pH 6.3
- Elution Buffer: 100 mM monosodium phosphate, 10 mM Tris base, 8 M urea in water; pH 4.5

For resin regeneration, prepare the following buffers:

- 0.1 M NaOH, pH 13
- Neutralization Buffer: 150 mM sodium chloride; 200 mM Na_2HPO_4, pH 7.0
- Storage Buffer: 20% ethanol; 10 mM sodium acetate, pH 6.5

Purify His-tagged proteins by Batch Method (Native and Denaturing conditions)

The Pierce™ High-Capacity Ni-IMAC Resin, EDTA Compatible allows for purification strategy customization. Purification conditions can be scaled as needed. The procedure may be performed at room temperature or at 4°C. Refer to “Materials required but not provided” on page 2 for corresponding buffer formulations under Native and Denaturing Conditions.

1. Resuspend the resin by inverting the bottle until the suspension is homogeneous. Transfer 1 mL of the 50% suspension (corresponding to 500 µL bed volume) to a 15 mL conical centrifuge tube. Allow the resin to settle by gravity and remove the supernatant. Alternatively, resin equilibration can be performed directly in a disposable gravity flow column (see **thermofisher.com** for Pierce™ Disposable Columns).

2. Add 2.5 mL of Equilibration Buffer and gently resuspend the slurry to equilibrate the resin. Allow the resin to settle by gravity and remove 2 mL of supernatant.

3. Add 10 mL of clarified sample to the equilibrated resin and incubate at 4°C for 1 hour on an end-over-end shaker. Alternatively, batch binding can be performed directly in a gravity flow column with closed bottom and top outlets.

4. Transfer the binding suspension to a disposable gravity flow column with a capped bottom outlet. Use Equilibration Buffer to rinse the centrifuge tube and remove resin adhered to the wall.

5. Remove the bottom cap of the column and collect the flowthrough.

6. Wash the column with 5 mL of Wash Buffer. Repeat the washing step at least 3 times.

7. Elute the His-tagged protein 5 times using 0.5 mL of Elution Buffer. Collect each eluate in a separate tube and determine the protein concentration of each fraction. *(Optional)* To increase protein yields, incubate the resin for 15 mins in Elution Buffer before collecting the eluate.

   Note: Monitor protein elution by measuring the absorbance of the fractions at 280 nm. 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 “Related products” on page 4).

8. Analyze all fractions by SDS-PAGE.

   Note: Do not boil membrane proteins. Instead, incubate samples at 46°C for 30 mins in preparation for SDS-PAGE analysis.

Automated purification of His-tagged proteins

Materials required but not provided

- Suitable liquid chromatography (LC) system
- Empty column for resin packing (follow column manufacturer's protocol for packing)
- Recommended buffers and volumes (see “Materials required but not provided” on page 2)

Protocol

1. Pack an appropriate-sized column with resin according to the 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 300 cm/hr or less (150 cm/hr recommended).
5. Apply any sample volume that does not exceed the column binding capacity for target protein at a flow rate of 300 cm/hr or less (150 cm/hr recommended).
   - 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 flowthrough fraction.
   - For maximum binding, prepare the 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.
   - If the sample contains insoluble matter, centrifuge or filter (0.45µm filter) before use.
6. Wash the resin at a flow rate of 300 cm/hr or less (150 cm/hr recommended) with 10-15 column volumes of Wash Buffer or until the absorbance approaches baseline.
7. Elute at a flow rate of 300 cm/hr or less (150 cm/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 280 nm. 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 “Related products” on page 4).
8. For regeneration and storage, refer to “Regenerate the resin” on page 3.

Regenerate the resin

Pierce™ High-Capacity Ni-IMAC Resin, EDTA Compatible may be used up to 10 times without affecting protein yield or purity (depending on sample type). 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 column volumes (CV) of ultrapure water.
2. Wash resin with 10 column volumes (CV) of 0.1 M NaOH.
3. Wash resin with 10 column volumes (CV) of ultrapure water.
4. Wash resin with 10 column volumes (CV) of Neutralization Buffer (150 mM sodium chloride; 200 mM Na_2HPO_4, pH 7.0).
5. Wash resin with 10 column volumes (CV) of ultrapure water.
6. Store resin as a 50% slurry in 20% ethanol and 10 mM sodium acetate, pH 6.5.

Troubleshooting

Observation	Possible cause	Recommended action
Low protein yield.	Poor expression of soluble protein.	Optimize expression conditions.
	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., Inclusion Body Solubilization Reagent, Cat. No. 78115).
	Insufficient cell lysis and extraction.	Optimize the 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. Decrease imidazole concentration in the Equilibration and/or Wash Buffer.
Poor protein purity.	Insufficient washing.	Wash resin additional times or modify imidazole concentration and pH of the Equilibration or Wash Buffer.
Slow column flow.	Column was overloaded.	Apply less protein extract onto the column and make sure the extract is not too viscous or highly particulate.

Related products

Product	Catalog Number
Pierce™ High-Capacity Ni-IMAC Magnetic Beads, EDTA Compatible	A50588-91
B-PER™ Bacterial Protein Extraction Reagent with Enzymes	90078
Expi293™ Expression System Kit	A14635
ExpiCHO™ Expression System Kit	A29133
ExpiSf™ Expression System Starter Kit	A38841
Pierce™ Detergent Compatible Bradford Assay Kit	23246
Pierce™ 660nm Protein Assay Kit	22662
Pierce™ DTT (Dithiothreitol), No-Weigh™ Format	A39255
UltraPure™ 0.5M EDTA, pH 8.0	15575020
Pierce™ Protease and Phosphatase Inhibitor Tablets	Multiple products available at thermofisher.com
Halt™ Protease Inhibitor Cocktail	Multiple products available at thermofisher.com
Pierce™ Concentrators	Multiple products available at thermofisher.com
Novex™ WedgeWell™ Tris-Glycine Mini Gels	Multiple products available at thermofisher.com
Zeba™ Spin Desalting Columns	Multiple products available at thermofisher.com
Slide-A-Lyzer™ Dialysis Cassettes	Multiple products available at thermofisher.com

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**.

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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.

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Revision	Date	Description
A.0	15 February 2021	New manual.