Pierce™ High-Capacity Ni-IMAC Magnetic Beads, EDTA Compatible

Catalog Numbers A50588, A50589, A50590, and A50591

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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 Magnetic Beads, EDTA Compatible are ferrimagnetic agarose beads coupled to a novel, proprietary ligand loaded with nickel ions. The beads enable efficient purification of recombinant polyhistidine-tagged proteins from a soluble protein extract or mammalian cell culture supernatant and are compatible with native or denaturing conditions. They can be used in manual applications with a magnetic stand or automated applications with an instrument such as the Thermo Fisher™ KingFisher™ Flex System. They have high binding affinity for His-tagged proteins and low-metal, ion-leaching characteristics, even in the presence of chemical additives including 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 magnetic beads.

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

Composition	Magnetite-embedded agarose beads coupled to a novel, proprietary ligand loaded with nickel ions
Magnetization	Ferrimagnetic with low remanence
Mean Diameter	30 µm
pH Tolerance	2–13
Reusability	Up to 5 times
Binding Capacity	≥80 mg green fluorescent protein (GFP)/mL of settled beads
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 Magnetic Beads, EDTA Compatible	A50588	1 mL of 25% slurry	Store at 4°C.
	A50589	5 mL of 25% slurry	
	A50590	25 mL of 25% slurry	
	A50591	100 mL of 25% slurry	

Important product information

- Do not centrifuge, dry, or freeze the Pierce™ High-Capacity Ni-IMAC Magnetic Beads, EDTA Compatible. Handling the beads in this way will cause the beads to aggregate and lose binding capacity.
- 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. 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.
- 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).
- When scaling up, use 2–3 volumes of Equilibration, Wash, and Elution Buffers per volume of settled beads.

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.

- Vary the imidazole concentration in the Elution Buffer from 250 mM to 500 mM.
- Vary the imidazole concentration in the Equilibration Buffer from 5 mM to 50 mM and in the Wash Buffers from 10 mM to 50 mM.
- Purification of GFP from bacterial cell lysate is optimal with 10 mM imidazole in the Equilibration Buffer and 20 mM imidazole in the Wash Buffer.

For native conditions, prepare the following buffers:

- Equilibration Buffer: 50mM 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: 50 mM monosodium phosphate, 10 mM Tris base, 8 M urea in water; pH 8.0
- Wash Buffer: 50 mM monosodium phosphate, 10 mM Tris base, 8 M urea in water; pH 6.3
- Elution Buffer: 50 mM monosodium phosphate, 10 mM Tris base, 8 M urea in water; pH 4.5

For magnetic bead 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 4.5

Manual purification of His-tagged proteins

Materials required but not provided

- 1.5 mL microcentrifuge tubes
- Sample containing His-tagged proteins
- Magnetic stand (e.g., Thermo Scientific™ MagnaBind™ Magnet for 6 × 1.5 mL microcentrifuge tubes, Cat. No. 21359)

Perform manual purification

Refer to “Materials required but not provided” on page 2 for composition of the recommended buffers when using Native or Denaturing conditions.

1. Place 40 µL (10 µL of settled beads) of Pierce™ High-Capacity Ni-IMAC Magnetic Beads, EDTA Compatible into a 1.5-mL microcentrifuge tube.
2. Add 500 µL of Equilibration Buffer and mix by vortexing. Place the tube on a magnetic microtube stand until the beads are separated and discard the supernatant.
3. Pipet 500 µL of the clarified sample onto the equilibrated magnetic beads and incubate the sample/magnetic bead mixture at 4°C for 30 mins on an end-over-end shaker.
4. Place the tube on the magnetic stand until the beads separate and remove the supernatant. Optimize separation by briefly centrifuging the sample to collect liquid from the lid before placing it on the magnetic separator.

5. Remove the tube from the magnet. Add 500 µL of Wash Buffer and mix by vortexing. Place the tube again on the magnetic stand and allow the beads to separate. Remove the supernatant.
6. Repeat step 5 twice.
7. Elute the His-tagged protein using 100 µL of Elution Buffer.

   Note: Depending on the protein expression rate and desired protein concentration, the elution volume can be adjusted from 25 µL to 500 µL.
8. Repeat step 7 three times. Collect each elution fraction in a separate tube and determine the protein concentration of each fraction.
9. Analyze all fractions by SDS-PAGE.

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

Regenerate the magnetic beads

1. After completion of purification, add 500 µL of deionized water to 10 µL of settled magnetic beads, then place the tube on the magnetic stand and allow beads to separate. Remove the supernatant.
2. Repeat step 1.
3. Add 500 µL of NaOH to the magnetic beads and incubate for 10 mins. Place tube on the magnetic stand and allow beads to separate. Remove the supernatant.
4. Add 500 µL of deionized water to the magnetic beads, then place the tube on the magnetic stand and allow beads to separate. Remove the supernatant.
5. Add 500 µL of Neutralization Buffer (150 mM sodium chloride; 200 mM Na_2HPO_4, pH 7.0) to magnetic beads, then place the tube on the magnetic stand and allow the beads to separate. Remove the supernatant.
6. Add 500 µL of deionized water to the magnetic beads, then place the tube on the magnetic stand and allow beads to separate. Remove the supernatant.
7. Repeat step 6 and proceed to purification.
8. For long-term storage, add 500 µL of Storage Buffer and store at 4°C.

Automated purification of His-tagged proteins

Materials required but not provided

- KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head (Cat. No. 5400630) or KingFisher™ Duo Prime Purification System (Cat. No. 5400110)
- Thermo Scientific™ Microtiter Deep Well 96 Plate, V-bottom, polypropylene (100–1,000 µL, Cat. No. 95040450)
- KingFisher™ 96 tip comb for deep-well magnets (Cat. No. 97002534)

Instrument preparation and plate setup

Note: The following protocol is designed for use with the KingFisher™ Flex instrument. The protocol can be modified according to your needs using the BindIt™ Software provided with the instrument.

1. Download the "Ni-IMAC" protocol from the Thermo Fisher Scientific website at **https://www.thermofisher.com/us/en/home/life-science/dna-rna-purification-analysis/automated-purification-extraction/automated-protocols-software.html?open=protein** into the BindIt™ Software on an external computer.

2. Transfer the protocol to the KingFisher™ Flex instrument from an external computer. See the BindIt™ Software User Manual for detailed instructions on importing protocols.

3. Set up plates according to Table 2.

Table 2 Pipetting instructions for the His-tagged Protein Purification protocol using the Thermo Scientific™ Microtiter Deep Well 96 Plates.

Plate #	Plate Name	Content	Volume	Time/Speed
1	Beads	Beads	40 µL	15 secs
		Equilibration Buffer	500 µL	
2	Bead Equilibration	Equilibration Buffer	500 µL	30 secs/Medium
3	Bind	Protein in Equilibration Buffer	500 µL	30 mins/Medium
4	Wash 1	Wash Buffer	500 µL	30 secs/Medium
5	Wash 2	Wash Buffer	500 µL	30 secs/Medium
6	Wash 3	Wash Buffer	500 µL	30 secs/Medium
7	Elution 1	Elution Buffer	200 µL	10 mins/Medium
8	Tip Plate	KingFisher™ 96 tip comb for DW magnets	—	10 secs/Fast

Perform automated purification

1. Select the protocol using the arrow keys on the instrument keypad and press **Start**. See the KingFisher™ Flex instrument user guide for detailed information.

2. Slide open the door of the instrument's protective cover.

3. Load plates into the instrument according to the protocol requests, placing each plate in the same orientation. Confirm each action by pressing **Start**.

4. After sample processing, remove the plates as instructed by the instrument's display. Press **Start** after each plate. Press **Stop** after removal of plates.

Note:

- If fewer than 96 wells are used, fill the same wells in each plate. For example, if using wells A1 through A12, use these same wells in all plates.
- Combine the Tip Comb with a Deep Well 96 Plate. See the instrument user manual for detailed instructions.
- A minimum volume of 100 mL is required for efficient elution of bound protein

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., Thermo Scientific™ Inclusion Body Solubilization Reagent, Cat. No. 78115) using optimized denaturing conditions. See "Materials required but not provided" on page 2 for recommended denaturing buffers.

Observation	Possible cause	Recommended action
Low protein yield. *(continued)*	Insufficient cell lysis and extraction.	Optimize the cell lysis protocol.
	Fusion protein did not bind to the magnetic beads.	Verify the sequence.
		Perform an ELISA or western blot using an antibody against the His-tagged protein to ensure the His-tagged protein is present. Decrease imidazole concentration in the Equilibration and/or Wash Buffer.
Poor protein purity.	Insufficient washing.	Wash beads a minimum of two additional times.
		Adjust imidazole concentration of the Equilibration and/or Wash Buffer.

Related products

Product	Catalog Number
Pierce™ High-Capacity Ni-IMAC Resin, EDTA Compatible	A50584-7
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
MagnaBind™ Magnet for 6 × 1.5mL microcentrifuge tubes	21359
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.

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