Pierce™ Ni-NTA Magnetic Agarose Beads

Catalog Numbers 78605 and 78606

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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 Thermo Scientific™ Pierce™ Ni-NTA Magnetic Agarose Beads (Table 1) provide a fast, convenient method for purification of polyhistidine-tagged recombinant proteins. The beads are incubated with cell lysate containing His-tagged protein and then magnetically separated from the supernatant manually or through automation using an instrument such as the Thermo Scientific™ KingFisher™ Flex Magnetic Particle Processor. Nonspecifically bound protein can be washed away before dissociating bound His-tagged protein with elution buffer. Automated instruments are especially useful for higher throughput purification and screening of purification conditions.

The Pierce™ Ni-NTA Magnetic Agarose Beads consist of highly crosslinked agarose beads embedded with magnetite and a covalently attached tetradentate nitrilotriacetic acid (NTA) chelator charged with divalent nickel ions. The density of the ligand on the magnetic agarose bead results in binding capacity similar to or better than traditional agarose resins with the added feature of magnetic handling. Magnetic agarose beads are a valuable tool for small-scale (~1 mg) purification of multiple His-tagged proteins and for scouting expression and purification conditions to be used in larger scale purifications with agarose chromatography supports.

Table 1 Characteristics of the Thermo Scientific™ Pierce™ Ni-NTA Magnetic Agarose Beads.

Composition	Magnetite-embedded 6% agarose coupled to an NTA-chelating ligand loaded with nickel ions
Magnetization	Ferrimagnetic with low remanence
Mean diameter	10-40 µm
Bead concentration	25% slurry in 20% ethanol
Binding capacity	>75 mg His-tagged green fluorescent protein (GFP)/mL settled beads

Contents

Contents	Cat. No. 78605	Cat. No. 78606	Storage
Pierce™ Ni-NTA Magnetic Agarose Beads	4 mL, supplied at 25% v/v suspension in 20% ethanol	20 mL, supplied at 25% v/v suspension in 20% ethanol	Store at 4°C.

Additional information

- Do not centrifuge, dry or freeze the beads. Handling the beads in this way will cause the beads to aggregate and lose binding capacity.
- Cell lysates can be prepared from a variety of methods, including Thermo Scientific™ Cell Lysis Reagents (e.g., B-PER™ Complete Bacterial Protein Extraction Reagent , Product No. 89821, 89822; Y-PER™ PLUS Dialyzable Yeast Protein Extraction Reagent, Product No. 78999; M-PER™ Mammalian Protein Extraction Reagent, Product No. 78501), sonication and French press.
- To minimize protein degradation, include protease inhibitors (e.g., Halt™ Protease Inhibitor Cocktail, EDTA-free (100X), Product No. 78425) in cell lysate preparations.
- 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.
- Concentration of proteins in the eluted fractions can be determined by using the Thermo Scientific™ Pierce™ Detergent Compatible Bradford Assay Kit (Product No. 23246).

Manual purification of His-tagged proteins

Materials required but not supplied for manual purification

- 1.5 mL low protein binding microcentrifuge tubes (Product No. 90410)
- Sample containing His-tagged protein

  Note: Samples can be concentrated using Thermo Scientific™ Pierce™ Protein Concentrators (thermofisher.com/concentrators).
- Magnetic stand (e.g., Thermo Scientific™ DynaMag™–2 magnet; Product No. 12321D)
- Nutating mixer (e.g., Thermo Scientific™ HulaMixer™ Sample Mixer; Product No. 15920D)
- **Equilibration Buffer: 50 mM sodium phosphate, 0.3 M NaCl , 10 mM imidazole, 0.05% Tween™-20 Detergent, pH 8.0**
- **Wash Buffer: 50 mM sodium phosphate, 0.3 M NaCl, 15 mM imidazole, 0.05% Tween™-20 Detergent, pH 8.0**
- **Elution Buffer: 50 mM sodium phosphate, 0.3 M NaCl, 0.3 M imidazole pH 8.0**

Perform manual purification of His-tagged proteins

The protocols listed below are designed for the purification of 0.5-1 mg of recombinant His-tagged protein. Adjust volumes accordingly for smaller or larger scale purifications. Required bead volume can vary with the amount of protein in a sample and the magnetic separator used.

Table 2

Binding capacity	Settled bead volume
1.9 mg	25 µL
7.5 mg	100 µL
18.8 mg	250 µL
37.5 mg	500 µL
75 mg	1000 µL

1. Place 100 µL bead slurry (25µL settled beads) into a 1.5 mL microcentrifuge tube.
2. Add 400 µL of Equilibration Buffer to the beads and vortex for 10 seconds to mix.
3. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.
4. Add 500 µL of Equilibration Buffer to the tube. Vortex the beads for 10 seconds and collect the beads with a magnetic stand. Remove and discard the supernatant.
5. Prepare 1 mL final volume of sample by diluting 500 µL protein extract with an equal volume of Equilibration/Wash Buffer.

   Note: Reserve a small volume of load for downstream analysis by SDS-PAGE.
6. Add 500 µL of prepared protein extract to the washed beads, vortex for 10 seconds and then mix on an end-over-end rotator for 30-60 minutes.
7. Collect the beads by placing the tube on a magnetic stand. If desired, save the supernatant (flow-through) for downstream analysis.
8. Add 500 µL of Wash Buffer to the tube and mix well. Collect the beads with a magnetic stand, then remove and discard the supernatant or reserve in separate tube for downstream analysis if necessary.
9. Repeat wash step.
10. Add 250 µL of Elution Buffer to the tube and then mix on an end-over-end rotator for 10 minutes.
11. Collect beads on a magnetic stand. Carefully remove and save the supernatant containing the His-tagged protein.
12. Repeat the elution steps (steps 10-11) using 250 µL of Elution Buffer.
13. Monitor the elution for protein content by measuring absorbance at 280 nm or by using the Pierce™ Detergent Compatible Bradford Assay Kit (Product No. 23246) or Pierce™ 660nm Protein Assay Reagent (Product No. 22660). Eluted protein can also be directly analyzed by SDS-PAGE. If needed, elution fractions can be pooled after purity and concentration have been assessed.

    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. Alternatively, the Pierce™ SDS-PAGE Sample Prep Kit (Product No. 89888) may be used to remove guanidine.

Automated purification of His-tagged proteins

Materials required but not supplied for automated purification

- KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head (Product No. 5400630) or KingFisher™ Duo Prime Magnetic Particle Processor (Product No. 5400110)
- KingFisher™ Deepwell 96 Plate, V-bottom, polypropylene (100-1000 µL; Product No. 95040450)
- 96 Deep-Well Tip Combs for KingFisher™ Flex Magnetic Particle Processor (Product No. 97002534)
- **Equilibration, Wash, and Elution Buffers (same as above)**

Prepare instrument and set up plates

Note: The following protocol is designed for use with the KingFisher™ Flex Magnetic Particle Processor. The protocol can be modified according to customer needs using the Thermo Scientific™ BindIt™ Software provided with the instrument.

1. Download the appropriate BindIt™ Software protocol from the product page (Product No. 78605, 78606) on the Thermo Fisher Scientific website 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 3 Plate set-up.

Plate #	Plate name	Content	Volume
1	Beads	Beads	100 µL
		Equilibration Buffer	400 µL
2	Bead equilibration	Equilibration Buffer	500 µL
3	Bind	Protein in Equilibration Buffer	500 µL
4	Wash 1	Wash Buffer	500 µL
5	Wash 2	Wash Buffer	500 µL
6	Elution 1	Elution Buffer	250 µL
7	Elution 2	Elution Buffer	250 µL
8	Tip plate	KingFisher™ 96 tip comb for DW magnets	—

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.
- To ensure bead homogeneity, mix the vial thoroughly by repeated inversion, gentle vortexing or rotating platform before adding the beads to plate 1.
- Combine the Tip Comb with a Deep Well 96 Plate. See the instrument user manual for detailed instructions.
- A minimum volume of 100 µL is required for efficient elution of bound protein.

Execute the His-tag purification protocol on the Kingfisher Flex instrument

1. Select the protocol using the arrow keys on the instrument keypad and press **Start**. See the KingFisher™ Flex Magnetic Particle Processor Instrument user manual 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. Stop after removing all of the plates.

Frequently asked questions for the Kingfisher instruments

Question	Answer
Which plates are compatible with the KingFisher™ Flex Magnetic Particle Processor?	The KingFisher™ Flex Magnetic Particle Processor is compatible with the KingFisher™ Flex 24 Deep-Well Plates, KingFisher™ Deepwell 96 Plate, V-bottom, polypropylene, KingFisher™ 96 and 96 PCR plates.
Is it possible to concentrate samples during the run?	Both deep-well plates and KingFisher™ 96 plates can be used during the same run. Therefore, it is possible to start the processing using larger volumes (in a deep-well plate) and elute the purified sample to a smaller volume (in a KingFisher™ 96 plate).
Is it possible to heat samples during the run?	The heating block is located inside the instrument and can be used automatically during the sample process. All plates compatible with the KingFisher™ Flex Magnetic Particle Processor can be heated using specially designed, interchangeable heating blocks.
Why do the beads stick to the plastic tips and wells? Why does the eluted protein stick to the wells?	Eluted proteins and proteins conjugated to beads can nonspecifically bind to plastics. Adding detergent to Binding/Wash Buffer prevents the protein conjugated to the bead from sticking (0.05%-0.1% Tween™-20 Detergent). Also include a small amount of detergent in the elution buffer or silanize the elution plate.
Are the reagent volumes in each well critical?	For best results, keep the specified volumes within defined limits to avoid spillover.

Related products

Product	Cat. no.
HisPur™ Ni-NTA Resin	88221
HisPur™ Cobalt Resin	89964
HisPur™ Ni-NTA Superflow Agarose	25214
HisPur™ Cobalt Superflow Agarose	25228
HisPur™ Ni-NTA Chromatography Cartridges	90098
Halt™ Protease Inhibitor Cocktail, EDTA-free (100X)	78425
Slide-A-Lyzer™ Dialysis Cassettes Kit	66382
Zeba™ Spin Desalting Columns, 7K MWCO	89892
Coomassie Plus (Bradford) Assay	23236
B-PER™ Complete Bacterial Protein Extraction Reagent	89821
B-PER™ with Enzymes Bacterial Protein Extraction Kit	90078
Zeba™ 96-well Spin Desalting Plates	89808
Slide-A-Lyzer™ Dialysis Cassettes	See thermofisher.com for product lines.
Pierce™ Protease and Phosphatase Inhibitor Tablets	
Pierce™ Concentrators	
Novex™ WedgeWell™ Tris-Glycine Mini Gels	

Troubleshooting

Observation	Possible cause	Recommended action
Beads aggregate during binding step.	Detergent missing or insufficient in the Equilibration Buffer.	Vortex beads periodically during the binding step (e.g., every 10 minutes).
		Increase Equilibration Buffer detergent concentration (e.g., increase detergent concentration from 0.05% to 0.1%).
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, Product No. 78115).
	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.

Limited product warranty

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Revision history: Pub. No. MAN0015957

Revision	Date	Description
A.0	15 August 2016	New manual

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