HisPur™ Ni-NTA Magnetic Beads

Catalog Numbers 88831, 88832

Pub. No. MAN0011794 Rev. B.0

Product description

The Thermo Scientific™ HisPur™ Ni-NTA Magnetic Beads enable effective immobilized metal affinity chromatography (IMAC) purification of polyhistidine-tagged proteins from a soluble protein extract. The beads contain nickel-charged nitrilotriacetic acid (Ni-NTA) chelate immobilized onto a blocked magnetic surface. The HisPur™ Ni-NTA Magnetic Beads are compatible with native or denaturing conditions and can be used in manual applications with a magnetic stand, or automated applications with an instrument such as the Thermo Scientific KingFisher™ Flex System (see Table 1). HisPur™ Ni-NTA Magnetic Beads are 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.

Table 1 Properties of Thermo Scientific™ HisPur™ Ni-NTA Magnetic Beads

Property	Description
Composition	Nickel on nitrilotriacetic acid covalently coupled on a blocked magnetic bead surface
Magnetization	Superparamagnetic (no magnetic memory)
Mean diameter	1 µm (nominal)
Density	2 g/cm^3
Bead concentration	12.5 mg/mL in 20% ethanol
Binding capacity	≥ 40 µg green fluorescent protein (GFP)/mg of bead

Contents and storage

Table 2 HisPur™ Ni-NTA Magnetic Beads

Cat. No.	Amount	Storage
88831	2 mL[1]	4°C[2]
88832	10 mL[1]	

[1] Supplied at 12.5 mg/mL in 20% ethanol.
[2] Product is shipped with an ice pack.

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.

Item	Source
For buffer preparation:	
Sodium phosphate	MLS
Sodium chloride	MLS
Imidazole	MLS
2-(*N*-morpholine)-ethanesulfonic acid	MLS
Tween™-20 Surfact-Amps™ Detergent Solution	28320
Guanidine-HCl	24110
For manual purification of His-tagged proteins:	
1.5 mL microcentrifuge tubes	MLS
DynaMag™-2 Magnet	12321D
For automated purification of His-tagged proteins:	
KingFisher™ with 96 deep-well head	5400630
KingFisher™ 96 deep-well plate, v-bottom, polypropylene	95040450
KingFisher™ 96 tip comb for deep-well magnets	97002534

General guidelines

- Do not centrifuge, dry, or freeze the HisPur™ Ni-NTA Magnetic Beads. 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. 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, 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.
- 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.
- 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 HisPur™ Ni-NTA Magnetic Beads.
- Concentration of proteins in the eluted fractions can be determined by using the Thermo Scientific™ Pierce™ 660 nm Protein Assay Kit (Cat. No. 22662).
- When scaling up, use 2–3 volumes of Equilibration, Wash, and Elution Buffers per volume of bead slurry.
- Do not regenerate the HisPur™ Ni-NTA Magnetic Beads.

Prepare buffers

Note: The buffers listed below are recommendations. To decrease nonspecific binding and increase yield, the following 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 Buffer from 10 mM to 50 mM.
- Purify Protein L from cell lysate. This is optimal with 10 mM imidazole in the Equilibration Buffer and 25 mM imidazole in the Wash Buffer.
- Purify GFP and β-galactosidase from cell lysate. These are optimal with 30 mM imidazole in the Equilibration Buffer and 50 mM imidazole in the Wash Buffer.

Buffer	Components
Buffers for native conditions	
Equilibration Buffer, pH 8.0	• 100 mM sodium phosphate • 600 mM sodium chloride • 0.05% Tween™-20 Surfact-Amps™ Detergent Solution • 30 mM imidazole
Wash Buffer, pH 8.0	• 100 mM sodium phosphate • 600 mM sodium chloride • 0.05% Tween™-20 Surfact-Amps™ Detergent Solution • 50 mM imidazole
Elution Buffer, pH 8.0	• 100 mM sodium phosphate • 600 mM sodium chloride • 250 mM imidazole
Buffers for denaturing conditions	
Equilibration Buffer, pH 8.0	• 100 mM sodium phosphate • 600 mM sodium chloride • 6 M guanidine-HCl • 0.05% Tween™-20 Surfact-Amps™ Detergent Solution • 30 mM imidazole
Wash Buffer, pH 8.0	• 100 mM sodium phosphate • 600 mM sodium chloride • 6 M guanidine-HCl • 0.05% Tween™-20 Surfact-Amps™ Detergent Solution • 50 mM imidazole
Elution Buffer, pH 8.0	• 100 mM sodium phosphate • 600 mM sodium chloride • 6 M guanidine-HCl • 250 mM imidazole

Manual purification of His-tagged proteins

Note: To ensure homogeneity, mix the beads thoroughly before use by repeated inversion, gentle vortexing, or slow mixing using a rotating platform.

1 Combine magnetic beads and Equilibration Buffer, vortex, then place on magnetic stand

1.1. Place 40 µL (0.5 mg) of HisPur™ Ni-NTA Magnetic Beads into a 1.5 mL microcentrifuge tube.

Note: This procedure can be scaled up to accommodate higher volumes of beads (see "General guidelines" on page 2).

1.2. Add 160 µL of Equilibration Buffer to the beads, then vortex for 10 seconds to mix.

1.3. Place the tube into a magnetic stand to collect the beads against the side of the tube.

Remove and discard the supernatant.

2 Add Equilibration Buffer, prepare the sample, then place on magnetic stand

2.1. Add 400 µL of Equilibration Buffer to the tube, then vortex the beads for 10 seconds.

2.2. Collect the beads by placing the tube on a magnetic stand.

Remove and discard the supernatant.

2.3. Prepare sample by diluting the protein extract with an equal volume of Equilibration Buffer.

2.4. Add 400 µL of prepared protein extract to the tube, vortex the beads for 10 seconds, then mix on an end-over-end rotator for 30 minutes.

2.5. Collect the beads by placing the tube on a magnetic stand.

Optional: Save the supernatant (flow-through) for downstream analysis.

3 Wash twice, add Elution Buffer, then place on magnetic stand

3.1. Complete a wash:

a. Add 400 µL of Wash Buffer to the tube, then vortex for 10 seconds to mix.

b. Collect the beads by placing the tube on a magnetic stand, then remove and discard the supernatant.

3.2. Repeat step 3.1 once.

3.3. Add 25 µL of Elution Buffer to the tube, then vortex for 15 seconds.

If needed, centrifuge the tube for 1 minute at 700 × *g* to ensure all of the beads are submerged in the Elution Buffer.

3.4. Incubate the beads for 15 minutes on a rotating platform. Alternatively, vortex the tube for 15 seconds every 5 minutes.

3.5. Collect the beads by placing the tube on a magnetic stand.

Carefully remove and save the supernatant containing the His-tagged protein.

3.6. Repeat steps 3.3 through 3.5 once using 25 µL of Elution Buffer.

3.7. Incubate the beads for 10 minutes.

Optional: Combine the two eluates.

3.8. Monitor the elution for protein content using the Pierce 660 nm Protein Assay Kit (Cat. No. 22662).

Eluted protein can also 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.

Automated purification of His-tagged proteins

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

1 Prepare, then set up the plate

1.1. Download the **His_Tag_Protein_Purification** protocol from the Thermo Fisher Scientific website (**http://www.thermoscientific.com/bindit-protocols**) into the BindIt™ Software on an external computer.

1.2. Transfer the protocol to the KingFisher™ Flex System from an external computer.

See the BindIt™ Software User Manual (Pub. No. N07974) for detailed instructions on importing protocols.

1.3. Set up plates according to the following table:

Plate number	Plate name	Content	Volume	Time/Speed
1	Beads	Beads	40 µL	15 seconds
		Equilibration Buffer	160 µL	
2	Bead Equilibration	Equilibration Buffer	400 µL	30 seconds/Medium
3	Bind	Protein in Equilibration Buffer	400 µL	30 minutes/Slow
4	Wash 1	Wash Buffer	400 µL	15 seconds/Slow
5	Wash 2	Wash Buffer	400 µL	15 seconds/Slow
6	Elution 1	Elution Buffer	100 µL	15 minutes/Medium
7	Elution 2	Elution Buffer	100 µL	10 minutes/Medium
8	Tip Plate	KingFisher™ 96 tip comb for deep-well magnets	—	10 seconds/Fast

2 Perform automated purification of His-tagged proteins

2.1. Select the protocol using the arrow keys on the instrument keypad, then press **Start**.

See the *Thermo Scientific™ KingFisher™ Flex User Manual* (Pub. No. MAN0019870) for detailed information.

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

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

2.4. After sample processing, remove the plates as instructed by the instrument's display. Press **Start** after each plate. Press **Stop** after removing all of the 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 96 deep-well plate. See the *Thermo Scientific™ KingFisher™ Flex User Manual* (Pub. No. MAN0019870) for detailed instructions.
- A minimum volume of 100 µL 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).
	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.
Poor proteins purity	Insufficient washing.	Wash beads a minimum of two additional times.
		Adjust imidazole concentration of the Equilibration and/or Wash Buffer.
Beads aggregate during the binding step	Detergent was 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%).

Supplemental information

User tips

Visit the website for additional information relating to this product, including the following:

- Tech Tip #43—Protein stability and storage
- Visit **www.thermoscientific.com/kingfisher** for information on the KingFisher products.
- In the U.S.A., purchase KingFisher supplies from Fisher Scientific. Outside the U.S.A., contact your local Thermo Fisher Scientific office to purchase KingFisher supplies.

Related Thermo Scientific products

Item	Amount	Source
Pierce™ NHS-Activated Magnetic Beads	1 mL, 5 mL	88826, 88827
Pierce™ Direct Magnetic IP/Co-IP Kit	40 reactions	88828
Pierce™ Protein A/G Magnetic Beads	1 mL, 5 mL	88802, 88803
Pierce™ Classic Magnetic IP/Co-IP Kit	40 reactions	88804
Pierce™ Crosslink Magnetic IP/Co-IP Kit	40 reactions	88805
Pierce™ Streptavidin Magnetic Beads	1 mL, 5 mL	88816, 88817
Pierce™ Glutathione Magnetic Agarose Beads	1 mL, 5 mL	78601, 78602
Pierce™ Anti-HA Magnetic Beads	1 mL, 5 mL	88836, 88837
Pierce™ HA-Tag Magnetic IP/Co-IP Kit	40 reactions	88838
Pierce™ 660 nm Protein Assay Reagent	750 mL	22660
Pierce™ 660 nm Protein Assay Kit	450 mL	22662
HisPur™ Ni-NTA Resin	10 mL, 100 mL, 500 mL	88221, 88222, 88223
HisPur™ Ni-NTA Spin Columns, 0.2 mL	25 columns	88224
HisPur™ Ni-NTA Spin Columns, 1 mL	5 columns	88225
HisPur™ Ni-NTA Spin Columns, 3 mL	5 columns	88226
HisPur™ Ni-NTA Spin Purification Kit, 0.2 mL	25 columns	88227
HisPur™ Ni-NTA Spin Purification Kit, 1 mL	5 columns	88228

Item	Amount	Source
HisPur™ Ni-NTA Spin Purification Kit, 3 mL	5 columns	88229
HisPur™ Cobalt Resin	10 mL, 100 mL, 500 mL	89964, 89965, 89966
HisPur™ Cobalt Spin Columns, 0.2 mL	25 columns	89967
HisPur™ Cobalt Spin Columns, 1 mL	5 columns	89968
HisPur™ Cobalt Spin Columns, 3 mL	5 columns	89969
HisPur™ Cobalt Purification Kit, 0.2 mL	25 columns	90090
HisPur™ Cobalt Purification Kit, 1 mL	5 columns	90091
HisPur™ Cobalt Purification Kit, 3 mL	5 columns	90092
Tween™-20 Surfact-Amps™ Detergent Solution	6 × 10 mL	28320

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  - 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

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

Revision history: Pub. No. MAN0011794

Revision	Date	Description
B.0	17 November 2021	The buffer components for native conditions and denaturing conditions were updated.
A.0	17 October 2015	Initial release with new publication number format.