Secreted His-tagged Protein Purification Kit

Introduction

A polyhistidine tag called 6xHis-tag, His-tagged, and His-tag is a versatile tool for purifying the highly purified recombinant protein from various expression systems, including bacterial, yeast, plant cell, and mammalian cell systems. The tag comprises six or more consecutive histidine amino acid residues positioned at either N or C terminus of a recombinant protein. Due to its small size, His-tag has several distinctive features, including less immunogenicity, hydrophilic nature, and versatility under native and denaturing conditions. Additionally, it is unnecessary to cleave the tag from the recombinant protein since it rarely perturbs the structure and function of its fusion protein. The purification principle of the His-tag depends on immobilized metal ion affinity chromatography (IMAC).

Immobilized metal ion affinity chromatography (IMAC) is a rapid affinity purification chromatography where the his-tagged protein are separated based on their affinity for Ni^{2+} or Co^{2+}that have been immobilized by a chelator to a solid matrix such as beaded agarose or column. At pH 7-8, his tagged protein will bind to Ni^{2+} or Co^{2+}. The binding reaction with the tagged protein is affected by various independent variables such as pH, temperature, salt type, concentration, immobilized metal and ligand density, and protein size. The bound protein is eluted by a decreasing pH gradient, increasing imidazole concentration, or adding an EDTA chelator in a buffer. This technique is an ideal tool for capturing and purification of his-tagged recombinant protein in a quick, inexpensive, and straightforward manner.

BcMag™ Secreted His-tagged protein purification kit is based on magnetic beads coupled with a unique, proprietary ligand loaded with nickel ions. The ligands are extraordinarily firmly bonded and have a high affinity for His-tagged proteins, and exhibit low metal, ion-leaching properties even in the presence of chemical additives such as chelators (EDTA), strong reducing agents (DTT), or components of cell culture supernatants, which typically strip off Ni ions and reduce the functionality of most IMAC magnetic beads. The His-tagged protein purification resins allow the efficient purification of recombinant polyhistidine-tagged proteins directly from a soluble intracellular protein extract, HeLa, CHO mammalian cells, or Sf9 insect cells culture supernatant. They can be used manually with a magnetic stand or automatically with an instrument. It avoids extensive and time-consuming sample pretreatment processes, such as buffer exchange by dialysis in conjunction with concentration operations.

Magnetic resins have significant advantages over traditional chromatography such as columns, agarose, or non-magnetic resin. The magnetic bead-based format enables rapid high-yield processing of 96 samples in about 20 minutes, achieving more than 85% purities. When using column-based technologies, processing multiple samples in academic research labs may necessitate a significant quantity of hand pipetting. This pipetting can discourage differences in the yield of target biomolecules between experiments and people. Staff and students may require extensive training and practice to produce constant protein yields. It is due to the numerous benefits of magnetic resins, such as their ease of use, rapid experimental protocols, suitability, and convenience for high-throughput automated and miniaturized processing.

The workflow with magnetic resin is straightforward (Fig.1).

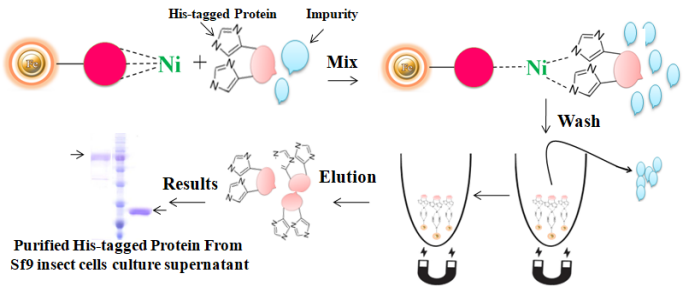

Fig.1 Workflow of Secreted His-tagged protein purification from the culture medium

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Mix the resins with the sample and incubate them with continuous rotation for a sufficient time. During mixing, the resins remain suspended in the sample solution, allowing the target molecules to interact with the immobilized ligand. After incubation, the beads are collected and separated from the sample using a magnet rack. Then the ultrapure His-tagged recombinant proteins are eluted by imidazole. During mixing, the beads remain suspended in the sample solution, allowing the target molecules to interact with the immobilized ligand. After incubation, the beads are collected and separated from the sample using a magnet rack. Then the ultrapure His-tagged recombinant proteins are eluted by imidazole.

Feature and benefits

- Magnetic beads exhibit less nonspecific binding than porous supports.
- Stable covalent bond with minimal ligand leakage
- The beads resist up to 20 mM EDTA and 20 mM reducing reagents without nickel leaching.
- Compatible with purification of overexpressed, secreted proteins in cell culture media.
- High protein purity
- Cost-effective: Eliminates columns, filters, repeat pipetting, and organic reagents.
- High-throughput: Compatible with many different automated liquid handling systems.

Applications

- Investigating protein structure and function
- Preparing recombinant proteins for X-ray crystallography
- Ideal for the study of protein interactions with protein or DNA
- Immunization to raise antibodies against a protein of interest
- Effective screening protein expression, even with crude cell lysates
- Microscale purification of his-tagged proteins

Specificities	
Composition	Magnetic beads charged with Ni^{2+}
Magnetization	~60 EMU/g
Type of Magnetization	Superparamagnetic
Stability	pH 4-11,100% Ethanol, 100% Methanol,8M Urea, 6M guanidine hydrochloride, 20 mM DTT, 20mM EDTA
Concentration	100 mg/ml (1% $NiSO_4.6H_2O$)
Binding Capacity	>2mg His-tagged GFP /ml of Beads
Storage	Store at 4°C upon receipt

Protocol

Note

- This protocol is optimized to capture and purify soluble intracellular protein extract, HeLa, CHO mammalian cells, or Sf9 insect cells culture supernatant. For insoluble his-tagged protein, we recommend using BcMag™ IDA--Ni His-tagged protein purification magnetic beads (Cat #, MHN-1). Creating a universal kit for protein purification is difficult because each protein has a different composition and structure. To get the best results, the user should optimize the working conditions for the purification of individual proteins based on the suggestions described in the troubleshooting section. For purification from other expression systems, optimizing the working condition is necessary.
- Protein yield and purity are affected by the expression level, structure, and solubility of the recombinant fusion protein; thus, these parameters must be optimized. Perform a small-scale test to evaluate the expression level and determine the solubility of each His-tagged protein for the best results.

- Protein extraction methods include employing commercially available detergent-based reagents and mechanical methods such as freeze/thaw cycles, sonication, or French press. These instructions are effective for many samples; however, further optimization may be required to reduce nonspecific binding. The lysis technique must be optimized to maximize protein yield. Adjust the suggested imidazole concentrations in the Equilibration, Wash, and Elution Buffers to optimize conditions.
- Before purifying the His-Tagged fusion protein, you should equilibrate all the reagents contained in the kit at room temperature.

Materials Required

Equipment

- Centrifuge
- Ultrasonic processor
- Ice bucket filled with ice
- Magnetic rack (for manual operation)
- Based on sample volume, the user can choose one of the following magnetic Racks: BcMag rack-2 for holding two individual 1.5 ml centrifuge tubes (Cat.# MS-01); BcMag rack-6 for holding six individual 1.5 ml centrifuge tubes (Cat. # MS-02); BcMag rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Cat. # MS-03); BcMag rack-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Cat. # MS-04); BcMag™ rack-96 for holding a 96 ELISA plate or PCR plate (Cat. # MS-05). For larger scale purification, Ceramic magnets Block for large scale purification (6 in x 4 in x 1 in block ferrite magnet, Applied Magnets, Cat# CERAMIC-B8)
- Corning 430825 cell culture flask for large-scale purification (Cole-Parmer, Cat#EW-01936-22)
- Mini BlotBoy 3D Rocker, fixed speed, small 10" x 7.5" platform w/ flat mat (Benchmark Scientific, Inc. Cat# B3D1008) or compatible

Buffer

- BcMag™ culture medium compatible His-tagged protein purification magnetic beads
- 1x Binding/Washing Buffer I (0.5 M NaCl, 100 mM Sodium phosphate, 10 mM imidazole, pH 8.0)
- 1x Elution Buffer I (100 Sodium phosphate , 0.5 M NaCl , 0.5-1 M imidazole, pH 8.0)
- NH_4OH

a. Magnetic beads preparation

1. Vigorously shake the bottle until the magnetic beads become homogeneous, and transfer an appropriate volume of the magnetic beads based on the estimated amount of the target proteins (>2mg His-tagged GFP /ml of Beads) from the bottle to a new tube or flask.

   Note:

   - **Optimize the amount of beads used for each application. Too many beads will cause higher background. Insufficient beads will lead to lower yields.**
   - **Do not allow the beads to sit for more than 3 minutes before dispensing. Resuspend the magnetic beads every 3 minutes.**
2. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the magnetic rack. Add ten bead-bed volumes of H_2O_2 and shake it to mix the beads. Again, place the tube on the magnetic rack for 1-3 minutes and remove the supernatant while the tube remains on the rack.
3. Repeat step (2) one more time.
4. Equilibrate the beads by adding ten bead-bed volumes of 1x Binding/Washing Buffer and shake it to mix the beads. Incubate at room temperature with continuous rotation for 2 minutes. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the magnetic rack. The beads are ready for purification.

Procedure for His-tagged protein purification.

b. Purification

1. Transfer the appropriate volume of cell culture supernatant or the clarified sample to a fresh tube.
2. Use diluted ammonium hydroxide to adjust the pH of the cell culture supernatant to 7-7.5.
3. Add the equilibrated beads, mix and incubate on Mini BlotBoy 3D Rocker with continuous rotation for 5-10 minutes.
4. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the magnetic rack. Add ten bead-bed volumes of Wash Buffer and shake it ten times to wash the beads. Again, place the tube on the magnetic rack for 1-3 minutes and remove the supernatant while the tube remains on the rack.
5. Repeat step (4) six times.
   Note: It may be necessary to wash the beads more than six times for some proteins to reduce the nonspecific binding.
6. Elute protein with an appropriate volume of elution buffer by vigorously shaking by magnetic rack.
   Note: Optimize elution condition by adjusting the imidazole concentration for each protein, and it may be necessary to elute the protein 2-3 times.
7. Transfer the supernatant to a new tube.

C. Troubleshooting

Problem	Probable cause	Suggestion
The yield of the purified protein is too low or undetectable in eluted protein solution by SDS-PAGE.	The open reading frame is wrong.	• Confirm sequence to ensure that the reading frame is correct. • Reclone and move the tag to the other end of the protein. • Purify the fusion protein under denaturing conditions.
	His tag is not accessible due to protein conformation	• Purify the fusion protein under denaturing conditions. • Reclone and move the tag to the other end of the protein.
	The protein does not bind to the Magnetic Beads.	• Check the pH of all the buffers and solutions.
	The protein is unstable.	• Add protease inhibitor to Binding/Washing Buffer.
	The protein is not efficiently eluted from beads.	• Increase imidazole concentration to 0.5-1.0 M in the Elution Buffer • Use 0.5-1.0 M sodium citrate (pH 4.0) to elute protein. After elution, immediately adjust pH to 7.0 using 1.0 M Tris-HCl (pH 9.0).
	The target protein appears in the washing buffer.	Reduce the concentration of imidazole. Increase pH slightly in the Binding/Washing Buffer.
	The beads used are insufficient for purification	• Increase the number of magnetic beads.
	Inadequate lysis and extraction of cells	• Improve the cell lysis protocol.
	Soluble protein expression is low.	• Improve expression conditions.
Observe multiple bands in the eluted protein.	Degradation of the fusion protein	• Add appropriate protease inhibitor. • Use protease-deficient expression host.
	The washing condition is not optimized.	• Increase the concentration of imidazole. • Increase washing time and washing volume
Eluted proteins lose activity	Some proteins will lose or decrease activity when fused toHis-tag.	• Try to fuse His-tag at the other end of the protein. • Switch to other fusion tags, such as GST

Related Products	
IDA Magnetic Beads	Secreted His-tagged protein purification Kit
IDA-His-tagged protein purification Kit	Low Expression His-tagged Protein Purification Kit
GST-tagged Protein Purification Kit	