# IDA-Ni His-tagged Protein Purification Kit

A polyhistidine tag, also 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 cells 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 variable factors such as pH, temperature, salt type, salt 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.

Although IMAC is a very effective protein purification technique, they are mainly based on traditional affinity chromatography matrices such as agarose resin or column. These solid matrices make the purification process tedious, time-consuming, unable to handle very tiny samples, and challenging to adapt to the automation system. We developed an extremely efficient magnetic IMAC separation system to overcome these limitations.

BcMag™ IDA magnetic beads are 5μm in diameter and highly uniform IMAC magnetic microspheres covalently immobilized with a high density of IDA (iminodiacetate) ligand. It is designed for capture and purification of histidine-tagged proteins from various sample types. The microspheres combine all the advantages of IMAC protein purification (low costs, simplicity, high specificity, and capacity**)** and magnetic properties to perform efficient manual or automatic quick high-throughput purification. Bioclone offers two ion-charged IDA magnetic beads for His tag protein purification; BcMag™ IDA-$Ni^{2+}$ magnetic beads and BcMag™ IDA-$Co^{2+}$ magnetic beads. The most used metal ion is nickel ($Ni^{2+}$) for poly (His) fusion proteins since it gives a high yield, whereas the cobalt ion ($Co^{2+}$) can provide higher purity but with a lower yield.

**Workflow**

The purification with magnetic microparticles is straightforward (Fig.1). Mix the microparticles with the cell lysate and incubate them with continuous rotation for a sufficient time. 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.

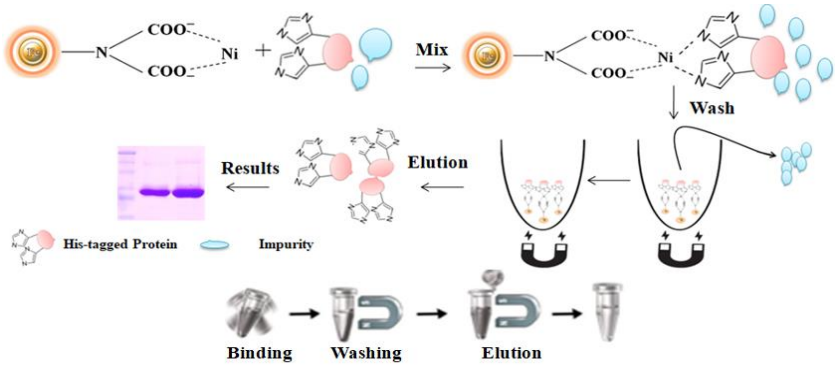


Fig.1 Workflow of IDA His-tagged protein purification



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**Features and Advantages**

- Quick, Easy, and one-step high-throughput procedure; eliminates columns or filters or a laborious repeat of pipetting or centrifugation.
- Stable covalent bond with minimal ligand leakage
- High binding capacity, very low nonspecific binding;
- Scalable -easily adjusts for sample size and automation;
- Reproducible results

**Applications**

- Investigating protein structure and function
- Preparing recombinant proteins for X-ray crystallography
- Ideal for 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 microsphere charged with $Ni^{2+}$ or $Co^{2+}$ |
| Magnetization | ~60 EMU/g |
| Type of Magnetization | Superparamagnetic |
| Effective Density | 2.5 g/ml |
| Concentration | 100 mg/ml (1% $NiSO_4.6H_2O$ or 1% $CoCl_26H_2O$) |
| 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 his-tagged protein from E.coli. For a soluble his-tagged protein with t severely nonspecific binding problem such as low expressed protein, we recommend using BcMag™ IMAC-Ni reducing reagents compatible magnetic beads (Cat. No MHP-101). 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.
- Recombinant protein expressed in E.coli is classified into two groups: soluble and insoluble. Soluble: The Recombinant proteins remain soluble in the cytoplasm. The soluble target protein is found in the supernatant while the insoluble protein (called inclusion bodies) remains in the pellet. Practically, the solubility of the recombinant protein can be determined by the following method. Harvest the bacterial cells by centrifuge and lyse the cell. Separate the insoluble fraction of the lysed cells from the soluble fraction by centrifugation and perform an SDS-PAGE analysis.
- The purification protocol for soluble and insoluble proteins is very similar. The only difference is all the purification buffers contain 8M Urea or 6M Guanidine hydrochloride for insoluble protein.
- Before purifying the His-Tagged fusion protein, you should equilibrate all the reagents contained in the kit at room temperature.

**Equipment**

- Centrifuge
- Ultrasonic processor
- Ice bucket filled with ice
- Magnetic rack (for manual operation)


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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™ IDA-$Ni^{2+}$ His-tagged Protein Purification Magnetic Beads or BcMag™ IDA-$Co^{2+}$ His-tagged Protein Purification Magnetic Beads
- PBS Buffer (137 mM NaCl, 2.7 mM KCl, 4.3 mM , $Na_2HPO_4$, 1.4 mM $KH_2PO_4$, pH 7.5)

**1. Buffer for soluble protein purification**

- 1x Binding/Washing Buffer (100 mM Sodium phosphate, pH 8.0, 0.5 M NaCl, 10 mM imidazole)
  Note: Add detergents such as 0.5% Triton-x 100, 0.5% Tween20, and protease inhibitors such as phenylmethylsulfonyl fluoride (PMSF) in the Binding/Washing Buffer can reduce nonspecific binding and the tagged protein degradation. However, EDTA and reducing reagents such as DTT or 2-Mercaptoehanol should be avoided.
- 1x Elution Buffer (100 Sodium phosphate, pH 8.0, 0.5 M NaCl, 0.3-1 M imidazole)

**2. Buffer for insoluble protein purification**

- 1x Binding/Washing Buffer (100 mM Sodium phosphate, pH 8.0, 0.5 M NaCl, 10 mM imidazole, 8M Urea, or 6M guanidine hydrochloride)
- 1x Elution Buffer (100 Sodium phosphate, pH 8.0, 0.5 M NaCl, 1 M imidazole, 8M Urea or 6M guanidine hydrochloride)

**Procedure**

**A Cell Extract Preparation**

1. Harvest cells by centrifugation at 6000 rpm for 6 minutes, remove the supernatant and wash the pellet with 1 x PBS buffer.
2. Remove the supernatant entirely and freeze the cell pellet at -80ºC for 6 hours or overnight.
3. Thaw the cell pellets at room temperature for 15-30 minutes until they detach from the wall.
4. Suspend the cell pellets with an appropriate volume of 1x Binding/Washing Buffer (30 ul Binding/Washing Buffer per 1ml bacteria culture) and incubate at room temperature for 30-60 minutes with gentle stirring.
5. Lyse cell by French press or sonication at 4°C.
   - Lyse cell by French press: Pass through French press 2-3x to ensure efficient lysis. French press should be chilled and operated at 4°C.
   - Lyse cell by Sonication: Sonicate the sample on ice using three 10-second bursts at high intensity and let the mixture cool down for 30 seconds on ice until the sample is not sticky.
6. Centrifuge at 16000 rpm for 15 minutes at 4°C.
7. Remove the supernatant containing soluble recombinant protein and transfer it to a new tube.
8. Save the cell pellet that contains insoluble protein.
9. Aspirate 10 µl of the supernatant from soluble and insoluble fraction, add an equal volume of 2x SDS sample loading buffer, boil for 5 minutes and run SDS-PAGE to determine the expression level and solubility of His-tagged protein.
10. Once the solubility of the tagged protein is determined, follow the protocol for the purification of the tagged protein.

**B. Procedure for Soluble protein purification**

**a. Magnetic beads preparation**


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1. Vigorously shake the bottle until the magnetic beads become homogeneous and transfer an appropriate volume of the magnetic beads (20 ul beads per 1ml bacteria culture) from the bottle to a new tube or flask (large-scale purification).

   ***Note:***

   - ***Optimize the number 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 2 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 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 soluble 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 rack. The beads are ready for purification.

**b. Purification**

1. Add the equilibrated beads to the cell lysates (Step A(7) and incubate on Mini BlotBoy 3D Rocker with continuous rotation for 5-10 minutes.
2. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack. Add ten bead-bed volumes of soluble Binding/Washing 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.
3. Repeat step (2) by washing the beads with ten bead-bed volumes of soluble Binding/Washing Buffer until the absorbance of eluting at 280 nm approaches the background level ($OD_{280} < 0.05$).
4. Elute protein with an appropriate volume of elution buffer (10 ul elution buffer per 1ml bacteria culture) by pipetting up and down 10-15 times or vortex mixer for 5 minutes. Collect the sample by a 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.***
5. Collect and Transfer the supernatant to a new tube.

**C. Procedure for insoluble protein purification**

1. Resuspend the pellet from step A (8) **in 1x Insoluble Binding/Washing Buffer** (30 ul insoluble Binding/Washing Buffer per 1ml bacteria culture) by stirring at 4° C for 1 to 2 hours and centrifuge the suspension at 20,000 rpm for 20 min. Transfer the supernatant to a new tube or culture flask (large-scale purification).
2. Prepare the magnetic beads with **1x Insoluble Binding/Washing Buffer** as described in Procedure for Soluble protein purification.
3. Follow the procedure for soluble protein purification, but use all insoluble protein purification buffers.

**D. Troubleshooting**

| Problem | Probable cause | Suggestion |
|---|---|---|
| | The open reading frame is wrong. | • Confirm sequence to ensure that the reading frame is correct.<br>• Reclone and move the tag to the other end of the protein.<br>• Purify the fusion protein under denaturing conditions. |
| | His tag is not accessible due to protein conformation | • Purify the fusion protein under denaturing conditions.<br>• 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 yield of the purified | The protein is unstable. | • Add protease inhibitor to Binding/Washing Buffer. |


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| | | |
|---|---|---|
| protein is too low or undetectable in eluted protein solution by SDS-PAGE. | The protein is not efficiently eluted from beads. | • Increase the concentration of imidazole to 0.5-1.0 M in the Elution Buffer<br>• 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.<br>Increase pH slightly in Binding/Washing Buffer. |
| | The beads used are insufficient for purification | • Increase the number of magnetic beads. |
| Observe multiple bands in the eluted protein. | Degradation of the fusion protein | • Add appropriate protease inhibitor.<br>• Use protease-deficient expression host. |
| | The washing condition is not optimized. | • Increase the concentration of imidazole.<br>• Increase washing time and washing volume |
| Eluted proteins lose activity | Some proteins will lose or decrease activity when fused to His-tag. | • Try to fuse His-tag at the other end of the protein.<br>• 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 | |


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