USER GUIDE: Immobilized Metal Ion Affinity Chromatography (IMAC)

IMAC utilizes the surface bonds between the proteins and the histidine residues (or Trp and Cys) binding with divalent metal ions (e.g. $Ni^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Co^{2+}$) immobilized via a chelating ligand.

This material is for EBL mam-50 series products induced **His_IDA resin**, **His_NTA resin, His_Super resin**, and **IMAC resin.**

Purifying His-tagged recombinant proteins by immobilized metal affinity chromatography (IMAC) continues to grow in popularity. Nickel ($Ni^{2+}$) is the most commonly used metal ion in IMAC purifications. Both Ni-IDA resin and Ni-NTA resin have higher binding capacities for histidine than other resins with cobalt or other divalent cations. EBL mam-50 series provides different type resin available for the changeable samples.

EBL mam-50 His_Super has lower metal ion leaking, can be compatible with solutions containing EDTA or other chelators and reducers and let us directly purify target protein without dialyzing or buffer changing.

EBL AM-50 series use polyacrylate medium and fully optimize the arm technology for coupling ligands to this matrix. By this, an excellent combination of high binding capacity, high performance and high flow rate addressed. Furthermore, leakage of $Ni^{2+}$ ions is minimized.

## Key Features:

- An excellent mechanical and chemical stability.
- Extremely high dynamic binding capacity (DBC), DOUBLED comparing to the products provided by other manufacturer.
- High protein binding capacity and minimal leakage of $Ni^{2+}$ ions
- Extremely stable in high and low pH with excellent mechanical property.
- Compatible with a very wide range of reducing agents, detergents, and other additives
- EBL mam-50 His_Super has high tolerability to EDTA (up to 0.1M) and reduct reagents such as 2-Mer (up to 30mM)

**Specifications:**

| | |
|---|---|
| Particle Size (μm) | 50 ± 5 % |
| Pore Size (Å) | 800 |
| Bead Matrix | Highly cross-linked polyacrylate polymer |
| Ligand Density | ≈150 μmol $Ni^{2+}$ |
| Dynamic Binding Capacity (g/ml) | ≈90 mg His-tagged protein/ml medium |
| Max. Linear Flow Rate (cm/h) | ≦1800 |
| Suggested Flow Rate (cm/h) | 150-700 |
| pH Stability | 3-13 |
| Pressure (MPa) | 10 |
| Chemical Stability | 0.01 M HCl, 0.5 M NaOH. Sustained for 1 week at 40 °C.<br>1 M NaOH, 70 % acetic acid. Sustained for 12 h.<br>2 % SDS. Sustained for 1 h.<br>30 % 2-propanol. Sustained for 30 min. |
| Storage buffer | 20 % ethanol |
| Storage temperature | +4- 28 °C |

**Quick start protocol:**

**Preparation of cleared E. coli lysates under native conditions**

Notes before starting:
Prepare a bacterial cell pellet (frozen at ~20°C or ~80°C)
Lysis may include Nucleic acid-Cut (CAT NO: C300102, equivalent to Benzonase nuclease ).

Procedure
1. Thaw cell pellet for 15 min on ice. Re-suspend cells in lysis buffer with 10 mM imidazole (CAT NO: C300103) at 2~5 ml per gram wet weight.
Note: If the tagged protein does not bind under these conditions, imidazole should be reduced to 1~5 mM. For proteins exhibiting high binding affinities, the imidazole concentration can be increased to 20 mM.

2. Add lysozyme (CAT NO: C300106) to 1 mg/ml and Nucleic acid-Cut (5 units per ml

of original cell culture volume processed) and incubate on ice for 10 min. Alternative lysis methods can be used, such as ultrasonic, etc.

3. Centrifuge lysate with 10,000 g for 20~30 min at 4 °C to pellet the cellular debris. Supernatant is collected and kept on ice.

4. Any insoluble material must be solubilized before purification under denaturing conditions. Add 5 µl 2 × SDS-PAGE sample buffer to 5µl cleared lysate supernatant for SDS-PAGE analysis.

Purification of 6 × His-tagged proteins under native conditions

Notes before starting
During centrifugation in step 3 of “Preparation of cleared E. coli lysates under native conditions” (see above), equilibrate the Ni-NTA matrix.

Procedure：
1. Pipet 1 ml of Ni-NTA slurry (0.5 ml bed volume) to a 15 ml tube and briefly centrifuge. Remove supernatant and add 2 ml of lysis buffer. Mix by gently inverting. Repeat the centrifugation step and remove the supernatant.

2. Add 4 ml cleared lysate (from step 3 above) to this equilibrated matrix and mix gently by shaking (200 rpm on a rotary shaker) at 4 °C for 60 min.

3. Load the lysate/Ni-NTA mixture into a column with the bottom outlet capped.

4. Remove bottom cap and collect the column flow-through. Save flow through for SDS-PAGE analysis.

5. Wash twice with 5 x bed volume (2.5 ml) wash buffer. Collect wash fractions for SDS-PAGE analysis.

6. Elute the protein 4 times with 0.5 ml elution buffer. Collect the eluations into four tubes and perform SDS-PAGE analysis.

**Preparation of cleared E. coli lysates under denaturing conditions**

1. Thaw the cell pellet for 15 min on ice and re-suspend in Lysis buffer A or buffer B at 5 ml per gram wet weight.

2. Cells can be lysed in either 6 M Gua-HCl (buffer A) or 8 M Urea (buffer B). It is preferable to lyse the cells in the milder denaturant Urea, so that the cell lysate can be analyzed directly by SDS-PAGE. Gua-HCl is a more efficient solubilization and cell lysis reagent, however, and may be required to solubilize some proteins. Prior to SDS-PAGE analysis, samples containing guanidine must be treated as described in the appendix.

3. The amount of cells required depends on the expression level of the 6 × His-tagged protein and the expression system used. The binding capacity of Ni-resins is protein-dependent and the product provided by EBL normally lies between 40～90 mg/ml medium, doubled comparing to the products provided by other manufacturers.

4. Stir cells for 15～20 min at room temperature or lyse them by gently vortexing, taking care to avoid foaming. Lysis is completed when the solution becomes translucent.

5. Centrifuge lysate with 10,000 × g for 20～30 min at room temperature to pellet the cellular debris. Save supernatant (cleared lysate).
A certain proportion of the cellular protein, including the 6 × His-tagged protein, may remain insoluble and will be located in the pellet. For more complete recovery of the tagged protein, this material must be solubilized as described in this Protocol before purification under denaturing conditions.

6. Add 5 µl 2 × SDS-PAGE sample buffer to 5 µl supernatant and store at ~20°C for future SDS-PAGE analysis.

7. Proceed to protocols for purification under denaturing conditions below.

<u>Prification of 6 x His-tagged proteins from E. coli under denaturing conditions</u>

1. One ml of the EBL Ni-NTA slurry is added to 4 ml lysate and mix gently by shaking (e.g. 200 rpm on a rotary shaker) for 15~60 min at room temperature.

The amount of lysate required depends on the expression level of the 6 × His-tagged protein and the expression system used. The binding capacity of EBL NTA and IDA resins is protein-dependent and normally lies between 40~90 mg/ml medium. For proteins that are expressed at very high levels (50~100 mg of 6 × His-tagged protein per liter of cell culture), a 5 × concentrated cell lysate (resuspend the pellet from a 20 ml culture in 4 ml buffer B) can be used. 4 ml of a 5 × concentrated cell lysate in buffer B will contain approximately 1~2 mg of 6 × His-tagged protein. For much lower expression levels (1~5 mg/liter), 200 ml of cell culture should be used for a 50 × concentrated cell lysate (4 ml cell lysate = 0.2~1 mg of 6 × His-tagged protein).

2. Load lysate–resin mixture carefully into an empty column with the bottom cap still attached.

3. Remove the bottom cap and collect the flow-through. Collect flow-through for SDS-PAGE analysis.

4. Wash twice with 4 ml buffer C. Keep washing fractions for SDS-PAGE analysis.

5. Elute the recombinant protein 4 times with 0.5 ml buffer D, followed by 4 times with 0.5 ml buffer E. Collect fractions and analyze by SDS-PAGE. Monomers generally elute in buffer D, while multimers, aggregates, and proteins with two 6 × His tags will generally elute in buffer E.

**General suggestions**

※ Keep cells and protein solutions at 0–4 °C at all times to prevent protein degradation. The addition of protease inhibitors may also be necessary.

※ Low concentrations of imidazole in lysis and wash buffers minimize nonspecific binding and reduce the amount of contaminating proteins.

※ The capacity of this product is doubled comparing with those provided by other suppliers. Because of this, more imidazole should be needed to complete elution of the target protein. For this purpose, you can use the following strategies or combination of them: I. more volumes of the elution buffer, or II. to increase the concentration of imidazole in clution buffer. III. An adjustment of elution pH buffer can be very effective in many cases. The pH should be optimized via assays

※ Elution conditions are highly reproducible, but optimum conditions must be determined for each His-Tag fusion protein purified. Although several elution methods are effective (imidazole, pH, and EDTA), imidazole is the mildest and is recommended under native conditions, when the protein would be damaged by a reduction in pH, or when the presence of metal ions in the elution may have an adverse effect on the purified protein. Monomers generally elute at approximately pH 5.9, whereas aggregates and proteins that contain more than one His-Tag sequence elute at approximately pH 4.5.

**Appendix: Buffers recipe**

Buffers for purification under **native conditions** (from *E. coli* and insect cells)

--Lysis buffer (1 liter):
50 mM $NaH_2PO_4$, 300 mM NaCl, 10 mM imidazole, adjust pH to 8.0 using NaOH.
--Wash buffer (1 liter):
50 mM $NaH_2PO_4$, 300 mM NaCl, 20 mM imidazole, adjust pH to 8.0 using NaOH.
--Elution buffer (1 liter):
50 mM $NaH_2PO_4$, 300 mM NaCl, 250 mM imidazole, adjust pH to 8.0 using NaOH.

Buffers for purification under **denaturing conditions**

--Lysis buffers A (1 liter):
100 mM $NaH_2PO_4$, 10 mM Tris, 6 M Gua-HCl, adjust pH to 8.0 using NaOH.
--Lysis buffer B (1 liter):
100 mM $NaH_2PO_4$, 10 mM Tris, 8 M urea, adjust pH to 8.0 using NaOH.
--Buffer C (1 liter):
100 mM $NaH_2PO_4$, 10 mM Tris, 8 M Urea, adjust pH to 6.3 using HCl.
--Buffer D (1 liter):
100 mM $NaH_2PO_4$, 10 mM Tris, 8 M Urea, adjust pH to 5.9 using HCl.
--Buffer E (1 liter):
100 mM $NaH_2PO_4$, 10 mM Tris, 8 M Urea, adjust pH to 4.5 using HCl.

Note: Due to the dissociation of urea, the pH of Buffers B, C, D, and E should be adjusted immediately prior to use. Do not autoclave.

Buffers for purification from mammalian cells under native conditions

PBS: 50 mM potassium phosphate, pH 7.2; 150 mM NaCl
Lysis buffer (1 liter):
50 mM $NaH_2PO_4$, 300 mM NaCl, 10 mM Imidazole, 0.0 5% Tween 20, adjust pH to 8.0 using NaOH.
Wash buffer (1 liter):
50 mM $NaH_2PO_4$, 300 mM NaCl, 20 mM imidazole, 0.05 % Tween 20, adjust pH to 8.0 using NaOH.
Elution buffer (1 liter):
50 mM $NaH_2PO_4$, 300 mM NaCl, 250 mM imidazole, 0.05 % Tween 20, adjust pH to 8.0 using NaOH

Preparation of guanidine-containing samples for SDS-PAGE

Since fractions that contain Gua-HCl will form a precipitate when treated with SDS, they must either be diluted with $H_2O$ (1:6), dialyzed before analysis, or separated from the Gua-HCl by TCA precipitation.

TCA precipitation:

1. Dilute 10–25 µl protein samples to 100 µl with $ddH_2O$
2. Add 100 µl of 10 % TCA.
3. Leave on ice for 20 min; centrifuge for 15 min in a microcentrifuge.
4. Wash pellet with 100 µl of ice-cold ethanol, dry, and resuspend in sample buffer.

If there is any trace of Gua-HCl present, samples must be loaded immediately after heating for 7 min at 95 °C.

**Reuse of Ni-NTA Resin:**

The reuse of EBL mam-50 series resin depends on the nature of the sample and should only be performed with identical recombinant proteins. We recommend a maximum of 5 runs.

If the resin changes from light blue to brownish-gray, the following regeneration procedure is recommended.

Wash the resin / column successively with:

1. 2 vol regeneration buffer (6 M Gua-HCl, 0.2 M acetic acid).
2. 5 vol water.
3. 3 vol 2% SDS.
4. 1 vol 25 % ethanol.
5. 1 vol 50 % ethanol.
6. 1 vol 75 % ethanol.
7. 5 vol 100 % ethanol.
8. 1 vol 75 % ethanol.
9. 1 vol 50 % ethanol.
10. 1 vol 25 % ethanol.
11. 1 vol water.
12. 5 vol 100 mM EDTA, pH 8.0.
13. 10 vol water.
14. Recharge the column with 2 vol 100 mM $NiSO_4$.
15. Wash the column with 2 vol water.
16. Wash the column with 2 vol regeneration buffer.
17. Equilibrate with 2 vol of a suitable buffer (e.g. Lysis Buffer).

## Ordering information:

| CAT NO: | Product | Quantity |
|---|---|---|
| pct-m500115 | His_IDA resin prepacking colume | 1mlx1/ 1mlx5/ 5mlx1 |
| mam-5001005 | MAM-50 His_IDA resin | 5ml/ 20ml/ 100ml |
| pct-m500215 | IMACA resin prepacking colume | 1mlx1/ 1mlx5/ 5mlx1 |
| mam-5002005 | MAM-50 IMAC resin | 5ml/ 20ml/ 100ml |
| pct-m500315 | His_NTA resin prepacking colume | 1mlx1/ 1mlx5/ 5mlx1 |
| mam-5003005 | MAM-50 His_NTA resin | 5ml/ 20ml/ 100ml |
| pct-m500515 | His_Co++ resin prepacking colume | 1mlx1/ 1mlx5/ 5mlx1 |
| mam-5005005 | MAM-50 His_Co++ resin | 5ml/ 20ml/ 100ml |
| pct-m500615 | His_Super resin prepacking colume | 1mlx1/ 1mlx5/ 5mlx1 |
| mam-5006005 | MAM-50 His_Super resin | 5ml/ 20ml/ 100ml |

## Related product:

| CAT NO: | Product | Quantity |
|---|---|---|
| C300102 | Nucleic acid-Cut | 5KU/ 25KU |
| C300103 | imidazole | 100g/ 500g |
| C300106 | lysozyme | 10g/ 50g |
| C103500 | Tris base | 500g |