# Purification of His-tagged Proteins Under Native Conditions Using PureCube His Affinity Agarose

## Overview

This protocol describes the generation of a cleared lysate from an *E. coli* cell pellet and the subsequent purification of His-tagged proteins under native conditions using our PureCube His Affinity Agaroses, featuring NTA, IDA, or the novel EDTA-stable INDIGO ligands.

Reagent amounts given apply to 200 mL IPTG-induced bacterial culture of a well-expressed protein (approximately 10–50 mg/L). If other culture volumes are processed or protein expression is higher or lower, reagent volumes may need to be adjusted.

In this protocol, cell lysis is done using lysozyme because it is an inexpensive and efficient method for cells that have been frozen. However, lysis methods based on physical disruption (e.g., sonication or homogenization) or detergents (e.g., CHAPS) can also be used. The His-tagged target protein is purified from the cleared lysate under native conditions in a bind-wash-elute procedure. Binding is performed in batch mode (as opposed to on-column binding). This method is most efficient, especially when the target protein is present at low concentrations or the His-tag is not fully accessible. Please contact us if you have questions or need assistance optimizing a protocol for your application (contact@cube-biotech.com); other protocols can also be found at www.cube-biotech.com/protocols.

## Equipment

- ☐ Ice bath
- ☐ Refrigerated centrifuge for 50 mL tube (min 10,000 x g)
- ☐ 50 mL centrifuge tube
- ☐ Micropipettor
- ☐ Micropipetting tips
- ☐ Disposable gravity flow columns with capped bottom outlet, 2 ml, (e.g. Pierce / ThermoScientific #29920)
- ☐ Optional: 15 mL conical propylene tubes (e.g. Falcon)
- ☐ pH meter
- ☐ End-over-end shaker
- ☐ SDS-PAGE equipment
- ☐ Optional: Western Blot equipment

## Materials

- ☐ Cell pellet from a 200 mL culture (approx. 0.5 g)
- ☐ PureCube His Affinity Agarose, e.g.

- PureCube Ni-NTA Agarose (50 mL; #31105)
- PureCube Co-NTA Agarose (50 mL; #31405)
- PureCube 100 Ni-NTA Agarose (50 mL; #74105)
- PureCube 100 Co-NTA Agarose (50 mL; #74405)
- PureCube 100 INDIGO Ni-Agarose (50 mL; #75105)
- PureCube Ni-IDA Agarose (50 mL; #30105)

- ☐ Sodium phosphate monobasic ($NaH_2PO_4$)
- ☐ Sodium chloride (NaCl)
- ☐ Imidazole
- ☐ Sodium hydroxide (NaOH)
- ☐ Lysozyme
- ☐ Benzonase® nuclease (e.g. Merck Milipore, #707464)
- ☐ Dithiothreitol (DTT)
- ☐ Glycerol
- ☐ Sodium dodecyl sulfate (SDS)
- ☐ Bromophenol blue
- ☐ Tris base
- ☐ HCl
- ☐ Optional: Protease inhibitor cocktail (e.g. Roche cOmplete, #04693116001)
- ☐ Optional: PentaHis Antibody (Cube Biotech #40040)

## Solutions and buffers

### Lysis Buffer, 50 mL

| Component | Final concentration | Molecular weight (g/mol) | Stock concentration | Amount needed for stock | Stock needed for buffer |
|---|---|---|---|---|---|
| $NaH_2PO_4$ | 50 mM | 119.98 | 0.5 M | 29.99 g/ 500 mL | 5 mL |
| NaCl | 300 mM | 58.44 | 5 M | 146.1 g/ 500 mL | 3 mL |
| Imidazole | 10 mM | 68.08 | 1 M | 6.8 g/ 100 mL | 0.5 mL |
| **Instructions:** Mix in 40 mL water. Adjust the pH to 8.0 using NaOH and then add water to a total volume of 50 mL. Always prepare fresh. | | | | | |

### Wash Buffer, 100 mL

| Component | Final concentration | Molecular weight (g/mol) | Stock concentration | Amount needed for stock | Stock needed for buffer |
|---|---|---|---|---|---|
| $NaH_2PO_4$ | 50 mM | 119.98 | 0.5 M | 29.99 g/ 500 mL | 10 mL |
| NaCl | 300 mM | 58.44 | 5 M | 146.1 g/ 500 mL | 6 mL |
| Imidazole | 20 mM | 68.08 | 1 M | 6.8 g/ 100 mL | 2 mL |
| **Instructions:** Mix in 80 mL water. Adjust the pH to 8.0 using NaOH and then add water to a total volume of 100 mL. Always prepare fresh. | | | | | |

### Elution Buffer, 50 mL

| Component | Final concentration | Molecular weight (g/mol) | Stock concentration | Amount needed for stock | Stock needed for buffer |
|---|---|---|---|---|---|
| $NaH_2PO_4$ | 50 mM | 119.98 | 0.5 M | 29.99 g/ 500 mL | 5 mL |
| NaCl | 300 mM | 58.44 | 5 M | 146.1 g/ 500 mL | 3 mL |
| Imidazole* | 500 mM | 68.08 | 1 M | 6.8 g/ 100 mL | 25 mL |
| **Instructions:** Mix in 40 mL water. Adjust the pH to 8.0 using NaOH and then add water to a total volume of 50 mL. Always prepare fresh. | | | | | |

* Tag length and protein structure can impact the interaction between His-tag and nickel ion. Therefore, we recommend trying a concentration gradient of imidazole to find the minimum concentration that elutes the desired amount of protein from the column.

### 5X SDS-PAGE Buffer, 10 mL

| Component | Final concentration | Molecular weight (g/mol) | Stock concentration | Amount needed for stock | Stock needed for buffer |
|---|---|---|---|---|---|
| Tris-HCl, pH 6.8–7.0 | 300mM | 121.14 | 1 M | 121.14 g/ 1 L | 3 mL |
| Glycerol | 50% (v/v) | – | 100% (v/v) | – | 5 mL |
| SDS | 5% (w/v) | – | – | – | 0.5 g |
| Bromophenol blue | 0.05% (w/v) | – | 4% | – | 125 µL |
| DTT | 250 mM | 154.25 | 1 M | 1.54 g/ 10 mL | 125 µL/aliquot |
| **Instructions:** Make sure to prepare a 1 M Tris-HCl stock by dissolving Tris base in 500 mL deionized water, adding HCl to a pH of 6.8–7.0, and adding water to a final volume of 1 L. For the SDS-PAGE Buffer, mix all components listed **except DTT** and add water to a total of 10 mL. Freeze 20 aliquots (375 µL each) at –20°C. Before use, add DTT to the needed single aliquots. | | | | | |

## Procedure

1. Thaw the *E. coli* cell pellets corresponding to 200 mL bacterial culture on ice for 15 min.

   **Optional:** Freezing the cell pellet at –20°C for 30 min prior to incubation at room temperature improves lysis by lysozyme.

2. Resuspend the cell pellet in 10 mL Lysis Buffer supplemented with 1 mg/ml lysozyme, and pour it into a 50 mL conical centrifuge tube.

   **Optional:** Add 1 tablet protease inhibitor cocktail to the Lysis Buffer. **Up to 20 mM EDTA and 20 mM DTT can be used with INDIGO Ni-Agarose; NTA and IDA agaroses tolerate up to 1 mM EDTA and 10 mM DTT.**

3. If the solution is very viscous, add 3 units Benzonase® per mL *E.coli* culture volume to the lysis buffer. Alternatively or additionally, sonicate the lysate to improve cell disruption.

4. Incubate on an end-over-end shaker at room temperature for 30 min, or at 4°C for 1 h, depending on the temperature stability of the protein.

   **Tip:** Lysis Buffer contains 10 mM imidazole to prevent binding of untagged proteins. If His-tagged proteins do not bind under these conditions, reduce the imidazole concentration to 1–5 mM.

5. Centrifuge the lysate for 30 min at 10,000 x g and 2–8°C. Carefully collect the supernatant without touching the pellet.

   **Note:** The supernatant contains the **cleared lysate fraction.**
   **We recommend to take aliquots of all fractions for SDS-PAGE analysis.**

6. Resuspend the PureCube His Affinity Agarose by inverting the bottle until the suspension is homogeneous. Transfer 1 mL of the 50% suspension (corresponding to 500 µL bed volume) to a 15 mL conical centrifuge tube. Allow the resin to settle by gravity and remove the supernatant.

   **Tip:** Alternatively, resin equilibration can be performed directly in the disposable gravity flow column.

7. Add 2.5 mL Lysis Buffer and gently resuspend the slurry to equilibrate the resin. Allow the resin to settle by gravity and remove 2 mL supernatant.

8. Add 10 mL cleared lysate to the equilibrated PureCube His Affinity Agarose resin and incubate at 4°C for 1 h on an end-over-end shaker.

   **Tip:** Alternatively, batch binding can be performed dirctly in a gravity flow column with closed bottom and top outlets.

9. Transfer the binding suspension to a disposable gravity flow column with a capped bottom outlet. Use Lysis Buffer to rinse the centrifuge tube and remove resin adhered to the wall.

10. Remove the bottom cap of the column and collect the flow-through.

    This is the **flow-through fraction.**

11. Wash the column with 5 mL Wash Buffer. Repeat the washing step at least 3 times.

    These are the **wash fractions.**

12. Elute the His-tagged protein 5 times using 0.5 mL Elution Buffer. Collect each eluate in a separate tube and determine the protein concentration of each fraction.

    These are the **elution fractions.**
    **Optional:** Incubate the resin for 15 min in Elution Buffer before collecting the eluate to increase protein yields.

13. Analyze all fractions by SDS-PAGE.

    **Note:** Do not boil membrane proteins. Instead, incubate samples at 46°C for 30 min in preparation for SDS-PAGE analysis.

14. Optional: Perform Western Blot experiment using PentaHis Antibody.