Purification of His-tagged Proteins Under Native Conditions Using PureCube His Affinity Agarose and PureCube 1-step batch Mini Columns

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 10 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.
The protocol uses the revolutionary 1-step batch Mini Columns that feature the SelfSeal™ membrane technology, saving time and pipetting steps. They replace disposable gravity flow columns used in standard protocols. Volumes of up to 600 µL can be applied to a Mini Column. For larger scale experiments, 1-step batch Midi Plus Columns are available.

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. Batch binding can be done directly in the 1-step batch Mini column to simplify the procedure.

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

- ☐ PureCube 1-step batch Mini Columns (Cube Biotech #63103)
- ☐ Ice bath
- ☐ Microcentrifuge with rotor suitable for 2 mL centrifuge tubes (min 10,000 xg)
- ☐ 2 mL centrifuge tubes
- ☐ Micropipettor
- ☐ Micropipetting tips
- ☐ 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 10 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 1 mL Lysis Buffer and pour it into a 2 mL microcentrifuge tube. 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.

   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. 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.

4. Centrifuge the lysate at 10.000 x g for 30 min and 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.

5. Resuspend the PureCube His Affinity Agarose by inverting the bottle until the suspension is homogeneous. Transfer 200 µL of the 50% suspension (corresponding to 100 µL bed volume) into the batch incubation chamber of the spin column barrel. Close the chamber and spin the resin at 10.000-14.000 x g for 20 sec.

6. Add 600 µL of Lysis Buffer and centrifuge again at 10.000-14.000 x g for 20 sec.

7. Repeat the step to completely remove any residual ethanol that might interfere with protein binding to the affinity resin.

8. Immediately before loading, filter the cleared lysate prepared in step 4 through a 0.2 µm filter (e.g. syringe filter) to remove any solid material that might clog the column.

   Note: It is critical to perform this filter step immediately before loading the column.

9. Empty the 2 mL centrifuge tube and place the spin column barrel containing the equilibrated purification resin back into it.

10. Load the lysate filtered in step 8. The maximum loading volume is 600 µL. Invert 2-3 times to mix sample and resin. Incubate at 4°C for 1 h on an end-over-end shaker.

11. Centrifuge at at 10.000-14.000 x g for 20 sec, or until the lysate has completely passed through, and collect the flow-through.

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

12. Wash twice with 600 µL each of Wash Buffer.

    These are the **wash fractions.**

13. Replace the 2 mL microcentrifuge tube with a fresh one, and elute the His-tagged protein by adding 50-600 µL Elution Buffer and centrifuging for 20 sec at 10.000-14.000 x g.

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

14. Repeat step 13 four times, for a total of five elutions. Collect each elution fraction separately.

15. Determine the protein concentration of the elution fractions with Bradford assay, using BSA as protein standard.

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

16. Analyze all fractions by SDS-PAGE.

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