Purification of Strep-tagged Proteins Using PureCube HiCap StrepTactin® Agarose and PureCube 1-Step Batch Midi Plus Columns

Overview

This protocol describes the generation of a cleared lysate from an *E. coli* cell pellet and the subsequent purification of Strep-tagged proteins under native conditions using PureCube HiCap StrepTactin Agarose. 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.

The protocol uses the revolutionary 1-step batch Midi Plus Columns that feature the SelfSeal™ membrane technology, saving time and pipetting steps. They replace disposable gravity flow columns used in standard protocols. Volumes up to 20 mL can be applied to a Midi Plus Column. For small scale experiments, 1-step batch Mini 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 Strep-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 Strep-tag is not fully accessible. Batch binding can be done directly in the 1-step batch Midi Plus 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 Midi Plus Columns (Cube Biotech #63203)
- ☐ Ice bath
- ☐ Refrigerated centrifuge for 50 mL tube (min 10,000 x g)
- ☐ 50 mL centrifuge tube
- ☐ Micropipettor
- ☐ Micropipetting tips
- ☐ 50 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 (ca. 0.5 g)
- ☐ PureCube HiCap StrepTactin Agarose (10 mL; Cube Biotech #34103)
- ☐ TRIS base
- ☐ Sodium chloride (NaCl)
- ☐ Sodium hydroxide (NaOH)
- ☐ Lysozyme
- ☐ Benzonase® nuclease (e.g. Merck Milipore, #707464)
- ☐ Dithiothreitol (DTT)
- ☐ Glycerol
- ☐ Sodium dodecyl sulfate (SDS)
- ☐ Bromophenol blue
- ☐ HCl
- ☐ Protease inhibitor cocktail (e.g. Roche cOmplete, #04693116001)
- ☐ Optional: Strep Antibody, Cube Biotech #40070)

Solutions and buffers

Lysis Buffer, 50 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
TRIS base, pH 8.0	100 mM	121.14	1 M	60.57 g/ 500 mL	5 mL
NaCl	150 mM	58.44	5 M	146.1 g/ 500 mL	1.5 mL
Protease inhibitor	1x		follow supplier's instructions		
Lysozyme	1 mg/ml	-	-	-	50 mg
Instructions: Prepare a TRIS base stock solution and set the pH with HCl to 8.0. Add protease inhibitor and lysozyme directly before use.					

Wash Buffer, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
TRIS base, pH 8.0	100 mM	121.14	1 M	60.57 g/ 500 mL	10 mL
NaCl	150 mM	58.44	5 M	146.1 g/ 500 mL	3 mL
Instructions: Prepare a TRIS base stock solution and set the pH with HCl to 8.0.					

Elution Buffer, 10 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
TRIS base, pH 8.0	100 mM	121.14	1 M	60.57 g/ 500 mL	1 mL
NaCl	150 mM	58.44	5 M	146.1 g/ 500 mL	300 µL
Desthiobiotin	2.5 mM	214.26	25 mM	53 mg/10 mL	1 mL
Instructions: Prepare a TRIS base stock solution and set the pH with HCl to 8.0. Add desthiobiotin directly before use.					

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	300 mM	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: Prepare a 1 M Tris-HCl stock solution 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, and pour it into a 50 mL conical centrifuge tube.
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 4°C for 1 h.
5. Centrifuge the lysate for 30 min at 10,000 x g and 4˚C. Carefully collect the supernatant without touching the pellet.

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

6. Resuspend the PureCube HiCap StrepTactin Agarose by inverting the bottle until the suspension is homogeneous. Transfer 1 mL of the 50% suspension (corresponding to 0.5 mL bed volume) into the batch incubation chamber of the spin column barrel. Use the ***clear*** spin push cap to close the chamber and spin the resin at 400 x g for 5 min.
7. Add 10 mL of Wash Buffer and centrifuge again for 5 min at 400 x g.
8. Repeat the step twice to completely remove any residual ethanol that might interfere with protein binding to the affinity resin.

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

9. Immediately before loading, filter the cleared lysate prepared in step 5 through a 0.2 µm filter (e.g. syringe filter) to remove any solid material that might clog the column.
10. Empty the 50 mL centrifuge tube and place the spin column barrel containing the equilibrated purification resin back into it.
11. Load the lysate filtered in step 9. Tightly screw the ***yellow*** batch incubation cap and invert 2-3 times to mix sample and resin. Incubate at 4°C for 1 h on an end-over-end shaker.

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

12. After batch incubation, replace the ***yellow*** cap with the ***clear*** spin push cap. Centrifuge at 400 x g for 5 min, or until the lysate has completely passed through, and collect the flow-through.

    These are the **wash fractions.**

13. Wash at least 4 times with 5 mL each of Wash Buffer.

    These are the **elution fractions.**

14. Replace the 50 mL conical centrifuge tube, and elute the rho1D4-tagged protein by adding 0.5 mL Elution Buffer, and incubating at 4°C for 15 min.
15. Repeat step 9 four times, for a total of five elutions. Collect each elution fraction separately.

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

16. Determine the protein concentration of the elution fractions with Bradford assay, using BSA as protein standard.
17. Analyze all fractions by SDS-PAGE.
18. Optional: Perform Western Blot using Strep Antibody.