

Purification of Strep-tagged Proteins Using PureCube HiCap StrepTactin[®] 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 Strep-tagged proteins under native conditions using PureCube HiCap StrepTactin Agarose. 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 μ L 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 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 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
- Refrigerated microcentrifuge
- 2 mL microcentrifuge tube
- Micropipettor
- Micropipetting tips
- pH meter
- End-over-end shaker
- SDS-PAGE equipment
- Optional: Western Blot equipment

Materials

- Cell pellet from a 10 mL culture (ca. 25 mg)
- 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, 20 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	2 mL
NaCl	150 mM	58.44	5 M	146.1 g/ 500 mL	0.6 mL
Protease inhibitor	1x		follow supplier's instructions		
Lysozyme	1 mg/ml	-	-	-	20 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, 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

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 10 mL bacterial culture on ice for 15 min.
2. Resuspend the cell pellet in 1 mL Lysis Buffer, and pour it into a 2 mL microcentrifuge 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.
6. Resuspend the PureCube HiCap StrepTactin 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.
7. Add 600 µL of Wash Buffer and centrifuge again at 10,000-14,000 x g for 20 sec.
8. Repeat the step to completely remove any residual ethanol that might interfere with protein binding to the affinity resin.
9. 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.
10. Empty the 2 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. 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.
12. Centrifuge at 10,000-14,000 x g for 20 sec, or until the lysate has completely passed through, and collect the flow-through.
13. Wash the column with 600 µL Wash Buffer. Repeat the washing step at least 3 times.
14. Replace the 2 mL microcentrifuge tube with a fresh one, and elute the Strep-tagged protein by adding 50-200 µL Elution Buffer and centrifuging for 20 sec at 10,000-14,000 x g.
15. Repeat step 13 four times, for a total of five elutions. Collect each elution fraction separately.
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.

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

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.**

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

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

These are the **wash fractions**.

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

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

