Purification of Strep-tagged Proteins Using PureCube HiCap StrepTactin® Agarose

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.

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. 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 (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 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 3 mL Wash Buffer and gently resuspend the slurry to equilibrate the resin. Allow the resin to settle by gravity and remove the supernatant. Repeat this step twice.
8. Add 10 mL cleared lysate to the equilibrated PureCube HiCap StrepTactin Agarose resin and incubate at 4°C for 1 h on an end-over-end shaker.
9. Transfer the binding suspension to a disposable gravity flow column with a capped bottom outlet. Use Wash Buffer to rinse the centrifuge tube and remove resin adhered to the wall.

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

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

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

11. Wash the column with 1 mL Wash Buffer. Repeat the washing step at least 3 times.

    These are the **wash fractions.**

12. Elute the Strep-tagged protein 5 times using 250 µL 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 using Strep Antibody.