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

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 MagBeads.
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.
Magnetic bead purification is easily scalable. To minimize unspecific binding and reduce cost, the volume magnetic bead suspension used should be adjusted to the expression level of interest. See Table 1 for more details.

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 using detergents (e.g., CHAPS) can also be used. The Strep-tagged target protein is purified from cleared lysate under native conditions in a bind-wash-elute procedure.

Magnetic beads are well-suited to purify proteins from dilute solutions, such as cell culture or medium supernatants. 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 microcentrifuge (min 10,000 x g)
- ☐ Micropipettor
- ☐ Micropipetting tips
- ☐ 1.5 mL conical microcentrifuge tubes
- ☐ Magnetic holder for microcentrifuge tubes (for separation of magnetic beads)
- ☐ pH meter
- ☐ End-over-end shaker
- ☐ SDS-PAGE equipment
- ☐ Optional: Western Blot equipment

Materials

- ☐ Cell pellet from expression screen (e.g., from 10 mL culture)
- ☐ PureCube HiCap StrepTactin MagBeads (1 mL; Cube Biotech #34201)
- ☐ 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, 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
Protease inhibitor	1x		follow supplier‘s instructions		
Lysozyme	1 mg/ml	-	-	-	10 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 pellet on ice.
2. Resuspend the cell pellet in 1 mL Lysis Buffer.
3. Add 6 U Benzonase® (3 units/mL bacterial culture) to the lysate to reduce viscosity caused by genomic DNA.
4. Incubate for 30 min on ice.
5. Centrifuge the lysate for 30 min at 10,000xg and 4°C. Collect the supernatant.
6. Resuspend the PureCube HiCap StrepTactin MagBeads by vortexing. Transfer 200 µL of the 5% magnetic bead suspension into a conical microcentrifuge tube (or the volume adjusted to the expression level; see Table 1).
7. Add 1 mL Wash Buffer and mix by vortexing. Place the tube on a magnetic microtube stand until the beads are separated and discard the supernatant.
8. Repeat step 7 twice.
9. Pipet 1 mL of the cleared lysate onto the equilibrated magnetic beads, and incubate the lysate-magnetic bead mixture at 4°C for 1 h on an end-over-end shaker.
10. Place the tube on the magnetic microtube stand until the beads separate and remove the supernatant.
11. Remove the tube from the magnet. Add 500 µL Wash Buffer and mix by vortexing. Place the tube again on the magnetic microtube stand and allow the beads to separate. Remove the supernatant.
12. Repeat step 11 twice.
13. Elute the Strep-tagged protein using 100 µL Elution Buffer.
14. Repeat step 13 five times. Collect each elution fraction in a separate tube and determine the protein concentration of each fraction.
15. Analyze all fractions by SDS-PAGE.
16. 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.**

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.