

Protein expression and purification of Cas13a Lbu, Lsh and Lwa with Ni-NTA Agarose

Aim of the Experiment

This protocol gives a detailed description how to purify Cas13a with Ni-NTA beads. Always work on ice and cool the devices to 4 °C. The volume of the starting culture is assumed to be 1 l. Depending on the actual volume, change the volumes values accordingly

Materials

- Phenylmethyl sulfonyl fluoride (PMSF, Carl Roth, Germany)
 - cOmplete™ EDTA-free Protease Inhibitor Cocktail (Roche, Switzerland)
 - Tris-HCl (Carl Roth, Germany)
 - NaCl (Carl Roth, Germany)
 - Glycerol (Carl Roth, Germany)
 - Tris(2-carboxyethyl)phosphine (TCEP, Sigma Aldrich, USA)
 - Imidazole (Carl Roth, Germany)
 - TEV protease (from own purification)
 - 5 mL column ()
 - Ni-NTA Agarose (Qiagen, Germany)
 - Bradford assay reagent (Coomassie Protein Assay, Thermo Scientific, USA)
 - Dialyse membrane (Zellu Trans T4, 45 mm, 12-14 kDa, Scienova, Germany)
 - Centrifugal filter (Amicon Ultra, 30 kDa, Millipore, USA)
 - Sonicator (Sonoplus, Bandelin, Germany)
 - Nanophotometer (Implen, Germany)
 - Buffers:
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Table 1: Lysis Buffer

Concentration	Chemicals
50 mM	Tris-HCl [pH 7.0]
500 mM	NaCl
5%	Glycerol (v/v)
1 mM	TCEP

Table 2: Washing Buffer

Concentration	Chemicals
50 mM	Tris-HCl [pH 7.0]
500 mM	NaCl
5%	Glycerol (v/v)
25 mM	Imidazole
1 mM	TCEP

Table 3: Elution Buffer

Concentration	Chemicals
50 mM	Tris-HCl [pH 7.0]
500 mM	NaCl
5%	Glycerol (v/v)
250 mM	Imidazole
1 mM	TCEP

Table 4: Gel Filtration Buffer

Concentration	Chemicals
20 mM	Tris-HCl [pH 7.0]
200 mM	KCl
5%	Glycerol (v/v)
1 mM	TCEP

Procedure

Cell lysis

1. Thaw cell pellet with expressed protein and resuspend in 20 ml lysis buffer (Table 1).
2. Add 0.5 mM PMSF and 1 tablet cOmplete protease inhibitor.
3. Lyse cells using sonication (20 s pulse, 50 % amplitude, 10 s pause).
4. Centrifuge for 30 min at 6000 rcf (Rotana 460 R, Hettich, Germany).
5. Transfer supernatant into a fresh tube .

Prepare Ni-NTA agarose

1. For every 4 ml lysate, pipette 1 ml Ni-NTA agarose into a 15 ml tube. Cut the tip off to avoid shredding the matrix.
2. Briefly spin down the agarose for 15 s.
3. Remove supernatant and add 2 ml lysis buffer. Gently mix by inverting.
4. Again spin down agarose for 15 s and then discard supernatant.

Protein purification

1. Load lysate on the Ni-NTA agarose.
 2. Incubate for 60 min at 4 °C while shaking to allow protein binding.
 3. Load 2.5 ml of the mixture onto a 5 ml column.
 4. Remove the column's bottom cap and collect flow-through in a 15 ml tube, which is placed in ice.
 5. Wait until the column stops dripping and load the remaining mixture in 2.5 ml steps, subsequently.
 6. Put the column in a new Falcon tube.
 7. Wash with 2.5 ml washing buffer.
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8. Again collect flow-through.
 9. Repeat the washing step once.
 10. Elute the protein with 3x 0.5 mL elution buffer in a fresh falcon tube.
 11. Analyze lysate, flow-through, washing and elution fractions on a 10 % SDS-PAGE.
 12. Load the purified proteins in a dialysis membrane and add an appropriate amount of TEV protease to cleave off the His-MBP tag
 13. Dialyse the sample in a large volume of gel filtration buffer (table 4) over night at 4 °C.
 14. Get rid of the cleaved off His-MBP tag by repeating Ni-NTA agarose purification. This time however, the desired protein does not bind to the column and can be collected in the first flow-through. Also elute the bound proteins to clean the Ni-NTA agarose for further use and also for SDS-PAGE analysis.
 15. Analyze all fractions in a 10 % SDS-PAGE.
 16. Pool all fractions that contain Cas13a and concentrate it to 1-2 μ M. Measure the concentration via absorbance at 280 nm with a nanophotometer or using a Bradford assay.
 17. Check purity in a 10 % SDS-PAGE.
 18. Split the sample in 100 μ l aliquots and shock freeze them in liquid N₂ before storing at -80 °C.
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