Äkta Protein Purification of Cas13a Lbu, Lsh and Lwa and TEV protease

Aim of the Experiment

This protocols provides a detailed description of Cas13a and TEV protease purification using an Äkta protein purification system. In this process, affinity and size exclusion chromatography are used.

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, Germany)
- Imidazole (Carl Roth, Germany)
- TEV protease (from own purification)
- Dialysis membrane (Zellu Trans T4, 45 mm, 12-14 kDa, Scienova, Germany)
- Centrifugal filter (Amicon Ultra, 30 kDa, Millipore, USA)
- Membrane filter (Nylon, ø 0.2 μm, 45 mm, Thermo Scientific, USA)
- Protein purification system (Äkta pure, GE Healthcare, UK)
- Gel filtration column (Superdex 200 Increase, 10/300 GL, GE Healthcare, UK)
- Affinity column (HisTrap FF crude, 1 ml, GE Healthcare, UK)
- Sonicator (Sonoplus, Bandelin, Germany)
- Buffers:

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

Always work on ice and cool down devices to 4 °C. Consider that EDTA inhibits Cas13a. Filter (ø 0.2 μm) and degas buffers before using them in the protein purification system. Flow-rates and pressure control of columns were adjusted according to the manufacturer's instructions.

Cell Lysis

1. Unfreeze 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.

Affinity Chromatography

1. Wash column with 2 column volumes H_2O, elution buffer (table 3) and washing buffer each(table 2), respectively.
2. Load supernatant on column with a maximum speed of 1 ml/min.
3. Wash column with 5 column volumes washing buffer to get rid of non-specific binders.
4. Elute bound Cas13a with a linear gradient from 25 mM to 250 mM in 10 column volumes and thereby collect fractions.
5. Pool all fractions that contain Cas13a.

Gel Filtration

1. Load the purified proteins in a dialysis membrane and add an appropriate amount of TEV protease (for Lbu and Lsh) or SUMO protease (for Lwa) to cleave off the His-MBP tag or the His-SUMO tag, respectively.

2. Dialyse the sample against a large volume of gel filtration buffer (Table 4) over night at 4 °C.
3. Up-concentrate the sample to 500 µl with a centrifugal filter (MWCO: 30 kDa) at 5000 rcf. Pipette up and down all 20 min to avoid aggregation. Wash the filter two times with 10 ml gel filtration buffer.
4. Equilibrate the S200 size exclusion column with 1.5 column volumes gel filtration buffer.
5. Load 500 µl concentrated protein on the column via a 500 µl injection loop and inject by flushing the loop with 1 ml gel filtration buffer.
6. Elute with 20 column volumes and thereby collect fractions.
7. Pool all fractions that contain Cas13a and concentrate it to 1-2 µM. Measure the concentration via absorbance at 280 nm with a nanophotometer or unsing a Bradford assay.
8. Check purity in a 10 % SDS-PAGE.
9. Split the sample in 100 µl aliquots and shock freeze them in liquid N_2 before storing at -80 °C.

We also tried to purify the Cas13a proteins by cation exchange chromatography. However, this did not work out. Here, we want to provide the used buffers (tables 5, 6). We used following columns.

- Heparin column (HiTrap Heparin HP, 1 ml, GE Healthcare, UK)
- Cation exchange column (HiTrap SP HP, 1 ml, GE Healthcare, UK)

Table 5: Wash buffer for cation exchange chromatography

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

Table 6: Elution buffer for cation exchange chromatography

Concentration	**Chemicals**
50 mM	Tris-HCl [pH 7.0]
1500 mM	KCl
5%	Glycerol (v/v)
1 mM	TCEP

The TEV protease was expressed and purified in the same way as the Cas13a proteins (expect for overnight dialysis). This buffers were used (table 7, 8, 9):

Table 7: Lysis buffer for TEV purification

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

Table 8: Wash buffer for TEV purification

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

Table 9: Elution buffer for TEV purification

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

The TEV protease was stored in storage buffer (table 10) at -80 °C.

Table 10: Storage buffer for TEV purification

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