**Super-Tev Transformation and Purification**

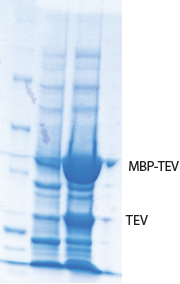
Transformation and Expression (1L per prep)

Transformation:

15 ul Rosetta (DE3)pLysS +0.5 μl pMHT238Δ (Super TEV plasmid, Blommel & Fox, 2007)

- 30min on ice

- 45 s @42°C O/N Expression

- 5min on ice

- add 1mL LB

- shake 1h @37°C

- plate on LBCm, Kan

- O/N @37°C

O/N culture:

- 1 colony in 50mL LBCm-34ug/ml, Kan-50ug/ml

- shake O/N @37°C

Expression:

To check for expression: compare sample of O/N culture with sample after 12h of expression

- inoculate 1L auto-induction media (Cm-34ug/ml, Kan-50ug/ml) with 5mL O/N culture

**1L Auto-induction media:** (modified terrific broth)

47.6 g Terrific Broth powder + 0.8% Glycerol (8ml)

=> Leave stir bar in bottle & autoclave

+ 2mM MgSO4

+ 0.375% Aspartic Acid

+ 0.5% (w/v) Lactose

+ 0.015% (w/v) Glucose

=> add to autoclaved media as powder, stir (takes a while to dissolve) and sterile filter (0.2 um, plastic bottles)

- shake for 12h @37°C

- Spin down cells: 5000rpm, 15min, 4°C

- Freeze pellet in liquid N2 & store @ -80°C

**Buffers:**

**Lysis buffer**

50mM HEPES pH 7.5

300mM NaCl

0.3mM TCEP (buffered to pH 7.4)

20% Ethylenglycol

1mM PMSF (fresh)

**Buffer A**

20mM HEPES pH 7.4

500mM NaCl

0.3mM TCEP (buffered to pH 7.4)

**Buffer B**

20mM HEPES pH 7.4

500mM NaCl

0.3mM TCEP

500mM Imidazole (buffered to pH 7.4)

The protocol shown here is a 2L culture purification.

**1) Lysis**

1. Add 2 mini-protease tablet and 500 ul 100 mM PMSF stock solution to 50 ml lysis buffer. Resuspend cells in 20 ml lysis buffer. Thaw the pellet in ice/cold water and vortex to mix. Use the dounce homogenizer on ice to more effectively resuspend the cells. The left 30 ml lysis buffer is used to rinse falcon-tube and homogenizer. Keep the suspension in a metal beaker on ice.
2. Use homogenizer to lyse the cells. Place beaker in ice bucket. Lower probe into the cell suspension, leaving at least ½ inch between probe tip and bottom of the beaker. Sonicator settings: amplitude=35%, 30 sec on 30 sec off, for 10 min total. When cells are lysed the solution will become darker and thinner. If cells are not lysed, perform sonication with above settings for longer.
3. Spin down the cell debris at 17,000 rpm for 40 min at 4°C.

**2) Purification with** **TALON cobalt resin**

1. Take out cobalt resin from the stock bottle and 50 ml resin is used for 2L culture. Wash the resin in filter funnel with vacuum by 100 ml ddH2O (3 times) and 100 ml buffer A (3 times).
2. Transfer the supernatant of cell lysate after centrifugation into new tubes (3 here) and mix it with the pre-equilibrated cobalt resin, incubate at 4°C for 1 hr with rocking.
3. Pour the cell lysate+resin mixture into filter funnel, apply vacuum and collect flowthrough

**(Note: For the following wash and elution steps make sure that the resin gets completely resuspended during each step. Use a plastic stirrer to scrape the resin stuck to the funnel bottom and stir to mix)**

1. Wash with 500 ml buffer A (3 times), apply vacuum, collect Wash 1.
2. Wash with 500 ml 15% buffer B (3 times). Let the buffer incubate with resin for 5 min. Apply vacuum, collect Wash 2.
3. Pour in buffer B and allow the protein to elute off resin by gravity for 10 min. Apply vacuum to collect residual buffer. 6 washes were done here. 1. 40 ml (collected 35 ml, 0.85mg/ml) 2. 20 ml (collected 20 ml, 1.23 mg/ml) 3. 20 ml (collected 15 ml, 1.35 mg/ml) 4. 20 ml (collected 20 ml, 1.44mg/ml) 5. 20 ml (collected 15 ml, 1.28 mg/ml) 6. 30 ml (collected 30 ml, 1.02 mg/ml). Run gel on flowthrough, washes, and elution to check purification.



Use Nanodrop to determine protein concentrations.

\* MWCO=28.14kDa

\*ε=31.74=31740 L mol-1 cm-1

Fraction 1-2 were combined and Fractions 4-5-6 were combined. Fraction 3 was buffer exchanged using desalting column.

\* Dilute pooled fractions with 10X freezing buffer and glycerol (30% final).

10X freezing buffer:

100mM Tris pH 7.5

3mM TCEP

5mM EDTA

Fractions 1 and 2:

5 ml buffer A, 55 ml protein, 30 ml glycerol and 10 ml 10X freezing buffer

Fractions 3, 4 and 6

65 ml protein, 33 ml glycerol and 11 ml 10X freezing buffer.

\* Freeze TEV protease (1ml aliquots) in liquid N2

\* Store @ -80°C

\* regenerate TALON column

**Regeneration Talon Column**

Strip, A: 1M NaCl, 50mM EDTA pH 8, B : water

Recharge, A:50mM CoCl2, 0.5M NaCl