Purification of TEVS219V\_L56V\_S135G and activity test

6xHisTEVS219V\_L56V\_S135G-MBP expression: MBP will be cut off by TEV itself during the purification steps

# Monday

* Media preparation for Lsc and TEV: 3 x 500 ml 2xYT
* Preparation buffers for Lsc purification:
	+ PBS pH 7.3, 150 mM NaCl: 250/500 ml
	+ Elution buffer: 50 mM TrisHCl pH 8.0, 10mM Red-Glu: 100 ml
* Preculture Lsc (Kan 30mg/l): 6 ml medium

# Tuesday

* Culture Lsc (1x500 ml 2xYT, Kan 30mg/l): Preculture 1/100 culture volume(5 ml). 37°C 220 rpm ~ 2.5h (OD600~1.2) (chill on ice if OD higher) and grow for another hour at 20°C. Induce at 20°C with 1 mM IPTG, o/n (~16h)
* Preparation buffers for TEV purification
	+ 25 mM PBS pH 8.0, 200 mM NaCl, Glycerol 10% (v/v), 25 mM Imidazol 25: 250 ml
	+ 25 mM PBS pH 8.0, 200 mM NaCl, Glycerol 10% (v/v), 500 mM Imidazol: 100 ml
	+ 25 mM PBS pH 8.0, 200 mM, Glycerol 10% (v/v), 5 mM DTT: 500 ml
* Preculture of TEV\_L56V/S135G (Amp 50/100 mg/l+Cam 34/40 mg/l): 20 ml

# Wednesday

* TEV culture (2x500 ml LB, Amp 50/100 mg/l+Cam 34/40 mg/l): Preculture 1/100 culture volume(5 ml), 37°C 220 rpm for ~3h, then at OD600 ~0.6 chill on ice and induce at 20°C with 1 mM IPTG, o/n
* Lsc GSTrap:
	+ Centrifugation (4500g, 20’, 4°C)
	+ Resuspension 100 ml buf
	+ Centrifugation
	+ Resuspension in 50 ml Lysis buffer (Lysozime 0.25 mg/ml and P.I. AEBSF 0.5 mM final)
	+ Sonication: 10s sonication/50s pause for > of 2’ of a sonication
	+ Centrifugation: 18000 g 20’ 4°C
	+ Filter the sample: before with 0.45um membrane, then 0.2um
	+ GSTrap chromatography: 5 ml column, 1 step elution.
* SDS-PAGE of Lsc only if chromatogram is unclear
* Pull the fractions and aliquot Lsc: ~1 mg/ml aliquots of 1 ml each (εc~ 86.5, MW~73). Fresh freeze in LN and put at -80°C
* Put S75 under water and equilibration o/n (program)

# Thursday

* TEV 1st purification step: IMAC
	+ Centrifugation (4500g, 20’, 4°C)
	+ Resuspension 100 ml buf
	+ Centrifugation
	+ Resuspension in 50 ml Lysis buffer (Lysozime 0.25 mg/ml)
	+ Sonication: 10s sonication/50s pause for > of 2’ of a sonication
	+ Centrifugation: 18000 g 20’ 4°C, collect surnatant
	+ Filter the sample: before with 0.45um membrane, then 0.2um
	+ IMAC chromatography: 5 ml column, direct elution with 500mM on
* TEV 2nd step purification: SEC S75
	+ Concentration: to 6 ml with 10KDa centricon.
	+ Filtration through 0.2 um membrane
	+ S75 run
	+ Pull fractions, measure concentration (εc~33, MW~30)
	+ Make a 0.4 mg/ml fraction of 200 ul volume and fresh freeze in LN and put at -80°C.

# Friday

* Thaw 1 aliquot Lsc (1ml 1mg/ml) and 1 aliquot of TEV (200 ul 0.3 mg/ml)
* Divide the Lsc aliquot in 2 x 500 ul
* Add 125 ul of TEV (1:10 TEV protein ratio). “Lsc 1:10 cut” sample
* Add to the second aliquot 12.5 ul of TEV (1:100 TEV protein ratio). “Lsc 1:100 cut” sample. Keep the leftover TEV for SDS-PAGE
* Collect 13 ul from sample 1:10 and 10.7 ul (in order to have same quantity of 1:10) from sample 1:100 at intervals of 1, 2, 3h and heat inactivate TEV by preparing the SDS-PAGE sample (thus by sample buffer, DTT and a ~90°C step, better if using the Incubate option of the PCR machine!)
* Stocks of DTT and Sample loading buffer should be 10X (1M) and 4X, respectively. Prepare 20 ul SDS-PAGE samples (for 10 ul loads, thus keeping ~10 ul as back up): 2 ul DTT, 5 ul Sample loading buffer and 13 ul Sample+H2O.
* Make at least a SDS-PAGE of
	+ TEV after IMAC
	+ TEV after SEC/GF (you should see only pure TEV without the MBP protein part)
	+ Lsc-GST
	+ Lsc 1:10 cut 1h
	+ Lsc 1:100 cut 1h (10.7 ul + 2.3 ul H2O)
	+ Lsc 1:10 cut 2h
	+ Lsc 1:100 cut 2h (10.7 ul + 2.3 ul H2O)
	+ Lsc 1:10 cut 3h
	+ Lsc 1:100 cut 3h (10.7 ul + 2.3 ul H2O)
* MWs expected:
	+ Lsc-GST6xHis ~76 KDa
	+ Lsc ~46 KDa
	+ GST6xHis ~29 KDa
	+ TEV-MBP ~72 KDa (TEV self digests the linker with MBP; MBP is probably used to help TEV getting its proper fold)
	+ TEV ~ 30 KDa
	+ MBP ~ 42 KDa
* Make vial good for 10/20 mg of protein to digest o/n at RT