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Expression and Purification of His-tagged TEV Protease

The expression vector, pETTev, produces an MBP-His-TEV protease fusion with an internal cleavage site. The product self cleaves intracellularly to yield His-tagged protease. If your expression has gone well, the SDS-PAGE analysis of induced cells will show bands for the MBP tag 43.3 kDa, His-TEV, 28.5 kDa, and the 10 kDa and 60 kDa chaperones from the Arctic Express host strain. Ideally, you will not see any uncleaved fusion protein. Since the protease gene contains rare codons, you need to use a host strain that compensates for them. Arctic Express (DE3) RIL cells (Stratagene) gave better expression than Rosetta (DE3) cells (Novagen), but Rosetta (DE3) is still a suitable host strain and that expression protocol is listed at the very end of this document.

Expression (pETTEV, shaker flasks):

1. Pick a single colony from freshly transformed Arctic Express (DE3) RIL cells and inoculate 10 ml of LB containing 30 ug/ml of Kanamycin and 20 ug/ml of Gentamycin. Incubate O/N at 37°C with 225 rpm shaking.
2. Inoculate 1 liter of LB containing 30 ug/ml Kanamycin and 20 ug/ml Gentamycin with the 10 ml O/N culture and incubate at 30° C with 200 rpm shaking.
3. Grow at 30°C until the cells reach an OD_{600} = 0.4, then drop the temperature to 12°C.
4. During the temperature equilibration the cells will continue to grow and therefore monitor growth until OD_{600} = 0.5-0.6 at which point, induce the cells with IPTG to 1mM.
5. After induction, allow the cells to grow at 12°C for 20 hours then harvest by centrifugation at 6K rpm for 15 minutes. Weigh the cell pellet and store at -80°C.

Purification (all steps are carried out at 4° C):

1. Thaw cell pellet on ice and resuspend in 20 ml per gram weight of lysis buffer (50 mM NaH_2PO_4, 0.3 M NaCl, pH 8.0).
2. Sonicate on ice for 15 min. (Pulse for 5 seconds on/off to avoid overheating).
3. Centrifuge the lysate at 12K rpm for 20 minutes to pellet cellular debris. Save supernatant. While lysate is pelleting, equilibrate 2.5 ml of Ni-NTA slurry per gram weight of cell pellet with 4 column volumes of equilibration buffer (50 mM NaH_2PO_4, 0.3 M NaCl, 10 mM Imidazole, pH 8.0).
4. Add supernatant to Ni-NTA beads and rock on a rotary shaker for at least 1 hour.
5. Pour Ni-NTA slurry onto a column and wash with 20 column volumes of wash buffer (50 mM NaH_2PO_4, 0.3 M NaCl, 10 mM Imidazole, pH 8.0).
6. Elute the protein with 8 fractions x 2.5 ml of elution buffer (50 mM NaH_2PO_4, 0.3 M NaCl, 250 mM Imidazole, pH 8.0).
7. Analyze fractions by SDS-PAGE. Even with extensive washing, it is difficult to remove completely the chaperones and residual MBP tag. These impurities can be removed by cation exchange FPLC using a MonoS column, however, we have noticed that further handling often results in diminished yields and lower activity.
8. Tev protease is not very stable in high concentrations of imidazole (see Ref.), so buffer swapping out the imidazole is performed immediately after elution off of Ni-NTA. Pool the most concentrated elution fractions and dilute 10-fold with sucrose solution (100 mM Tris, 10% Sucrose (v/v), pH 8.0). Concentrate the solution in the 200 ml Amicon stir cell. To avoid precipitation do not let the solution drop below 35 ml in the stir cell. Repeat until the imidazole concentration is < 2.5 mM.
9. Concentrate the final 35 ml His-TEV solution down to 5 ml in a Amicon 15 centrifuge tube (10,000 MWCO).
10. Take a final concentration measurement (ε_{280}=33460 $M^{-1}cm^{-1}$) then store at -80°C in 500 ul aliquots.
11. The typical yield per liter induction is ~11 mg.

Reference: Fang, L., et. al. *Protein Expression and Purification* (2006) 5:102-109.

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Enzymatic Activity Test of His-tagged TEV Protease
Buffer Conditions: 50 mM Tris, 150 mM NaCl, 10 mM EDTA, pH 7.5

The substrate (His-MBP-RPA70AB) has a MW =71.6 kDa and when cleaved yields two products MW=44.6 kDa and 27 kDa. (Unfortunately, the latter runs nearly identical to His-TEV (MW=28.5) by SDS-PAGE.) Two 50uL reactions were set up with different substrate:enzyme A_{280} ratios (50:1 or 25:1) at 2 diffrenent temperatures. As a control, 10 ul of substrate (no enzyme) was incubated overnight. 10 ul aliquots were taken from each reaction after 15 min, 30 min, 1 hr, 2 hr, and overnight incubations and analyzed by SDS-PAGE (see Figures 1&2). The optimal conditions for cleavage were 25:1 at RT.

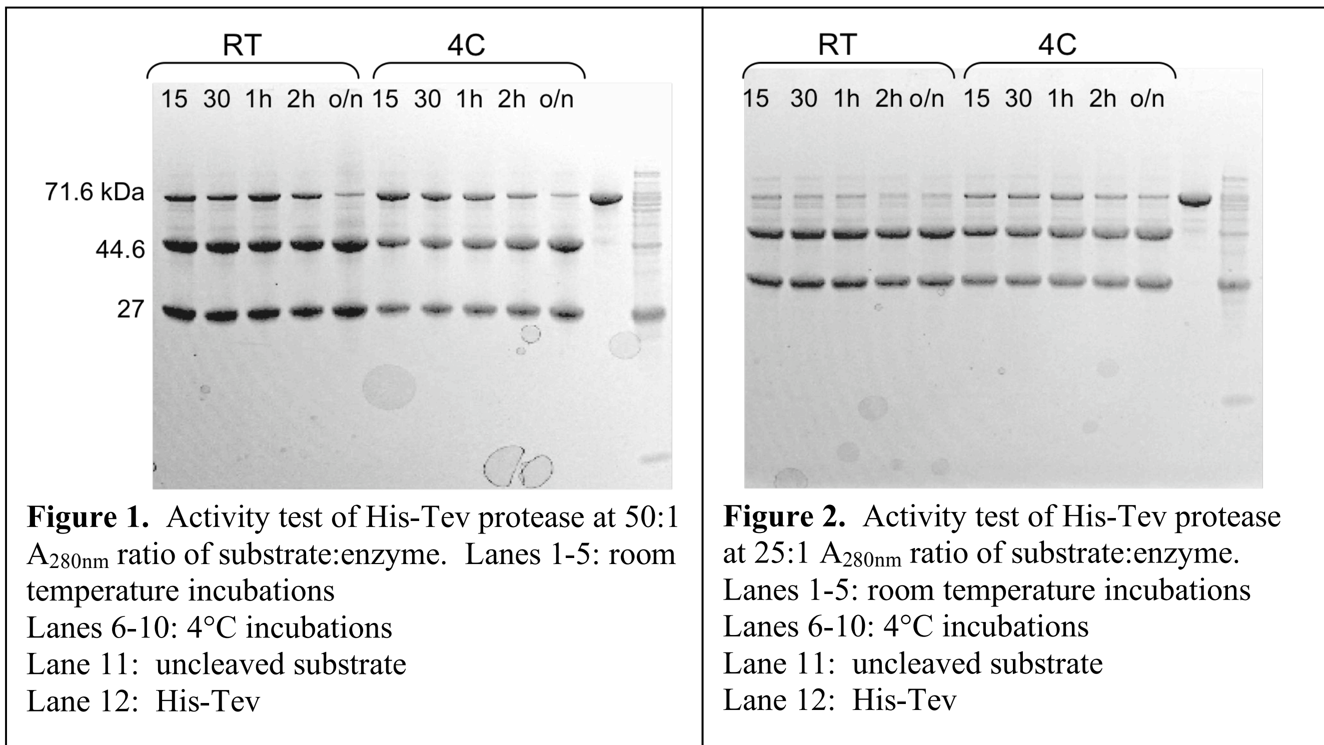

Figure 1. Activity test of His-Tev protease at 50:1 A_{280nm} ratio of substrate:enzyme. Lanes 1-5: room temperature incubations
Lanes 6-10: 4°C incubations
Lane 11: uncleaved substrate
Lane 12: His-Tev

Figure 2. Activity test of His-Tev protease at 25:1 A_{280nm} ratio of substrate:enzyme.
Lanes 1-5: room temperature incubations
Lanes 6-10: 4°C incubations
Lane 11: uncleaved substrate
Lane 12: His-Tev

Expression Rosetta (DE3) host strain:

1. Pick a single colony from either freshly transformed Rosetta(DE3) cells, or from a glycerol stock, and inoculate 20 ml of LB containing 30 ug/ml of Kanamycin (KAN) and 34 ug/ml of Chloramphenicol (CAP). Incubate O/N in the 37°C shaker at ~200rpm
2. Make a 1:100 dilution of O/N culture in 1 liter of LB containing 30 ug/ml Kan and 34 ug/ml CAP and incubate shaking at 37°C.
3. Grow cells until an OD_{600} ~0.35 is reached and drop temperature down to 16°C. (This allows for the cultures adjustment to 16°C before the induction point).
4. When an OD_{600} of ~0.5 is reached, induce cells with 0.5 mM IPTG and continue to incubate shaking at 16°C for another 12-18 hours.
5. Harvest cells by centrifugation for 20 minutes and freeze at -80°C