

Expression and Purification of His-tagged Ulp1 (SUMO Protease) Under Native Conditions

All steps are carried out at 4°C.

Expression (pH6Ulp1):

1. Pick a single colony from either freshly transformed BL 21(DE3) cells, or from a glycerol stock, and inoculate 20 ml of LB containing 30 ug/ml of Kanamycin (Kan). 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 of Kan and incubate shaking at 37°C.
3. Grow cells until an OD₆₀₀ ~0.5 is reached.
4. Induce cells with 0.5 mM IPTG and continue to incubate shaking at 37°C for another 3 hours.
5. Harvest cells by centrifugation and freeze at -80°C

Purification:

1. Thaw cell pellet on ice and resuspend in 4mL per gram weight of lysis buffer (50mM NaH₂PO₄, 300mM NaCl, 10mM Imidazole, pH 7.5).
2. Add lysozyme to resuspension (1mg/ml) and incubate on ice for 30 minutes.
3. Sonicate on ice for 10 minutes.
4. Centrifuge lysate at 10,000 rpm for 25 minutes to pellet cellular debris. Save supernatant.
5. Apply supernatant to QIAGEN Ni-NTA column equilibrated in lysis buffer (1ml of beads per 4ml of lysate).
6. Wash the column with 10 column volumes of wash buffer (50mM NaH₂PO₄, 300mM NaCl, 20mM Imidazole, pH 7.5).
7. Elute the protein with at least 3 column volumes of elution buffer (50mM NaH₂PO₄, 300mM NaCl, 250mM Imidazole, pH 7.5).
8. Analyze fractions by SDS-PAGE. Some small amount of high MW impurities will be present. This was common during purifications.
9. Pool fractions containing the protease and dialyze against 50mM Tris-HCl pH 8.0, 200mM NaCl, 2mM BME. Yield is ~20 mg/L of induced media.
10. The protease is relatively pure at this point, although further purification can be achieved by passage over Superdex75 gel filtration resin.
11. Protease may be stored at -80°C as a 50% stock in glycerol.

Amino Acid Sequence of H6Ulp1:

M G S S H H H H H G G G L V P E L N E K D D D Q V Q K A L A S R E N T Q L M
N R D N I E I T V R D F K T L A P R R W L N D T I I E F F M K Y I E K S T P N T V
A F N S F F Y T N L S E R G Y Q G V R R W M K R K K T Q I D K L D K I F T P I N
L N Q S H W A L G I I D L K K K T I G Y V D S L S N G P N A M S F A I L T D L Q
K Y V M E E S K H T I G E D F D L I H L D C P Q Q P N G Y D C G I Y V C M N T L
Y G S A D A P L D F D Y K D A I R M R R F I A H L I L T D A L K

Molecular Weight: 26829.5 Da

Extinction Coefficient: 28950 M⁻¹cm⁻¹

pI: 6.79