Senp1 Purification Protocol

Liv Jensen, Al-Sady Lab

Adapted from Reverter and Lima, *Methods Mol Biol.* (2009)

Buffers:

Lysis Buffer: 20% sucrose, 20mM Tris pH 8.0, 200mM NaCl, 1mM PMSF

A: 20mM Tris pH8.0, 200mM NaCl, 10mM Imidazole

B: 20mM Tris pH8.0, 200mM NaCl, 1mM BME, 400mM Imidazole

Protocol:

1. Express His-thrombin-Senp1 (BAS 327) in BL21s. Spin down cells at 4000 x g. Snap freeze and store pellets at -80˚C.
2. Suspend pellets in 75ml lysis buffer per 1L of expression culture. Lyse by sonication on ice (1s ON/ 4s OFF; 2min total ON), and spin down cell debris at 25,000 x g, 20 min.
3. Apply supernatant to 5ml (per 1L expression culture) CV TALON column equilibrated in A. Nutate 1 hr at 4˚C.
4. Wash 3X 3CV with A.
5. Elute with 3X 2CV of B.
6. Pool fractions containing Senp1 by SDS-PAGE (MW: 29.2 kDa), calculate concentration, add glycerol to final concentration of 10%, and snap freeze at -80˚C in 50-100µl aliquots.
7. Test cleavage efficiency – typically use 0.03mg/ml final concentration to cleave SUMO-tagged proteins.