Modified method for purification of SUMO fusion proteins

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Method scaled for purification of protein from 1 liter of cells

Protein expression and Ni-bead purification

Inoculate 6-8 ml overnight culture per liter of YT + selection drugs. Grow 37 deg 3-4 hrs till A600 ~ 0.6

Add 0.2 ml of 1M IPTG per liter culture (0.2 mM final). Reduce temperature to 30 deg and incubate 3 hrs (temp and induction time is protein-specific). Save 1 ml of induced and uninduced cells for protein gel analysis.

Harvest cells. Wash cells with 25 ml cold BB. Freeze cell pellet at -70 deg.

Resuspend in 20 ml BB +DTT and PMSF. Can also add NP40 to 0.05% if needed.

Add 1 ml 10 mg/ml lysozyme. Incubate 30-45 min (will become viscous).

Disrupt with sonicator (large or small tip, depending on sample size) 3 x 30 sec on ice (small tip in 50 ml screwcap tube works fine).

Spin 13K in Sorvall F21 rotor, 30 min.

Prepare Ni Beads: wash 2x with 10x bed vol BB (w/o DTT).

Add ~1 ml Ni-bead slurry (~0.8 ml bed vol [per liter of culture]) to extract. Incubate 45 min – 1 hr on roller @ 4 deg.

Wash beads: 3 x 5 column vols of BB (5' incubation between washes).

Elute protein with 3 elutions of 2 CV elution buffer (4 deg or RT elutions are OK). Can also do more elutions if desired.

Analyze purified protein by SDS PAGE (4-12 microliters/lane depending on expression level).

Sumo protease cleavage and clean up

Change protein buffer to Sumo cleavage buffer. Either dialyze O/N or concentrate and dilute protein in cleavage buffer. If protein becomes cloudy, spin in tabletop centrifuge 4100 rpm, 15 min.

Add 30 microliters SUMO protease (0.148 mg/ml; current prep) per liter of starting culture. Incubate RT 3.5 hrs (RT) or 5 hrs to O/N (4 deg).

Spin again as above if cloudy after cleavage is complete.

Bind cleaved protein to same volume of Ni Beads used above. Desired protein should be in the flow through. Can wash beads with 1-2 CV of SUMO cleavage buffer if desired.

Analyze by SDS PAGE (2-10 microliters depending on protein concentration). Beads may turn brown during incubation with DTT-containing buffers. Proceed with purification as long as beads still have blue color.

Elute Ni-beads, discard eluate and store beads in 20% ethanol for further cleaning and regeneration.

If required, further purify the protein using other chromatography columns .

Ni-bead binding buffer (BB)	1 liter
50 mM HEPES	11.9 g HEPES
0.5 M NaCl (can use lower if desired)	29.22 g NaCl
10% glycerol	100 ml
40 mM Imidazole	2.7 g Imidazole
pH 7 w/ HCl	

Ni elution buffer	200 ml
50 mM HEPES	2.38g HEPES
0.5 M NaCl	5.84 g NaCl
10% glycerol	200 ml glycerol
0.5 M Imidazole	6.8 g Imidazole
pH7 w/ HCl	

SUMO cleavage buffer	2.5 liter
50 mM HEPES	29.8 g HEPES
300 mM NaCl	48.25 g NaCl
10% glycerol	1250 ml glycerol
pH7 w/KOH	

Can add NP40 to all buffers at 0.05% if desired

Add DTT to 1 mM
Add standard set of protease inhibitors to BB and Ni-elution buffer
Add only PMSF + DTT to SUMO cleavage buffer