

## Purification of untagged recombinant human RanGAP1

### *Melchior's lab protocol*

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#### INTRODUCTION

RanGAP1 was the first SUMO target identified. It is very efficiently sumoylated just in the presence of E1 and E2 enzymes, without the need for an E3 ligase. The sumoylation of RanGAP1 is essential to target it to the cytoplasmic periphery of the nuclear pore complex via interaction with RanBP2 (Nup358).

Bacterially expressed RanGAP1 is purified from inclusion bodies and refolded to its catalytically fully active form during a dialysis step. Properly folded (active) RanGAP1 can be separated from the unfolded (inactive) form by gel filtration.

#### METHODS

##### Day 1

Transform hRanGAP1-pET11 plasmid (Ampicillin resistance) into BL21(DE3)pLysS and start 100 ml over night culture (37°C).

##### Day 2

Centrifuge overnight culture, add 2 l fresh medium (LB + Amp, 1 mM MgCl<sub>2</sub>, 0.1 % glucose) and shake at 37 °C. At OD<sub>600nm</sub> = 1, add 1 mM IPTG, grow for 3 h at 37 °C and harvest bacteria. Resuspend in 60 ml lysis buffer:

- 50 mM Tris pH 8,0
- 100 mM NaCl
- 1 mM EDTA

Store at -80°C until needed.

##### Day 3

Thaw pellet, add Aprotinin, leupeptin, pepstatin (1 µg/ml each) and 1 mM DTT.

Pass through Emulsion flex / French press.

Ultracentrifugation for 1 h, 100.000g, 4°C.

Transfer pellet into large glass dounce, thoroughly resuspend pellet in 50 ml washbuffer on ice:

- 50 mM Tris pH 8,0
- 1 % TritonX100
- Protease inhibitors and DTT

Centrifuge again for 20 min.

Harvest pellet and repeat homogenization and centrifugation step.

Harvest pellet and resuspend in 50 ml 2M-Urea buffer (freshly made, 4°C):

- 50 mM Tris pH 7,4
- 2 M Urea
- Protease inhibitors and DTT

Ultracentrifugation for 20 min, 100.000g, 4°C.

Transfer pellet into glass vial and solubilize with **50 ml** 8M urea buffer (freshly made, keep at room temperature, do not heat urea containing buffer to dissolve urea):

- 50 mM Tris, pH 7,4
- 8 M Urea
- Protease inhibitors including PMSF, and 1 mM DTT

Ultracentrifugation for 20 min, 100.000g, at room temperature !

Collect supernatant and dialyse against 2 l Dialysis buffer at 4°C (buffer should have room temperature at beginning of dialysis):

- 50 mM Tris pH 7,4
- 150 mM NaCl
- Protease inhibitors including PMSF, and 1 mM DTT

Replace buffer once after 2-3 hours (with 4°C cold buffer) and dialyse overnight.

#### Day 4

Replace buffer and continue dialysis for 2-3 hours.

Collect sample from dialysis tubing, and centrifuge ca. 45 min 100.000g, 4°C.

Equilibrate 12 ml Q-Sepharose in 50 ml column at 4°C with:

- 50 mM Tris pH 7,4
- 150 mM NaCl
- + Protease inhibitors and 1 mM DTT

Load supernatant onto column, discard flow through and wash extensively with:

- 50 mM Tris pH 7,4
- 300 mM NaCl
- Protease inhibitors, 1 mM DTT

Elute RanGAP1 with:

- 50 mM Tris pH 7,4
- 1 M NaCl
- Protease inhibitors, 1 mM DTT

Collect 5ml-Fractions, analyze samples by SDS-PAGE on 8% Minigel.

(RanGAP1 smears over many fractions, degradation products visible).

Combine RanGAP1 containing fractions, concentrate Fractions in 30kDa-Centriprep to 2-5 ml, centrifuge in tabletop ultracentrifuge, filter through 0.2 µm low protein binding filter.

Load onto preparative S200 column (FPLC) equilibrated in TB:

- 20 mM Hepes/KOH pH 7.3
- 110 mM potassium acetate
- 2 mM magnesium acetate
- 0.5 mM EGTA
- 1 mM DTT
- 1 µg/ml Aprotinin, Leupeptin, Pepstatin

Run over night, collect 5 ml fractions

### Day 5

Test fractions by SDS-PAGE (8 % Minigel) and staining with Coomassie.

Combine peak fractions, aliquote and flash freeze.

Properly folded RanGAP1 elutes from column (HiLoad 26/60 Superdex 200) at about 180-200 ml as sharp peak. This RanGAP1 is catalytically highly active.

Discard any protein that elutes in the void volume (unfolded and inactive) !!!

*Note: 6 mg/ml RanGAP1 has an OD280 of 1 (it has very few aromatic amino acids)*

### BIBLIOGRAPHY

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