

Expression and purification of Ubc9 (SUMO E2)

Melchior's lab protocol 2002

Frauke Melchior

Ruprecht-Karls-Universität (ZMBH), Heidelberg, Germany

INTRODUCTION

Sumoylation is a process which requires an enzymatic cascade involving an E1 activating enzyme, an E2 conjugating enzyme and in most cases in vivo an E3 ligase enzyme. Reconstitution of the sumoylation reaction in vitro has the obvious advantage that SUMO isopeptidases, E3 ligases and competing substrates present in eukaryotic extracts can be avoided.

Here, we provide a complete protocol describing the expression and purification of the recombinant E2 enzyme of the SUMO pathway, called Ubc9. Notice that this protocol allows the purification of a non-tagged Ubc9, avoiding any consequences due to the presence of a tag.

METHODS

Day 1

Transformation of 1 μ l pET23Ubc9 in competent BL21(DE3)

Streak out on LB-Amp-plates, 37°C over night

Day 2

Inoculate overnight culture (LB+Amp)

Day 3

Inoculate 2 l LB / Amp with 20 ml overnight culture (for this harvest bacteria by centrifugation and resuspend in fresh LB/Amp prior to inoculation)

Grow at 37°C until OD₆₀₀=0,5 - 0,6, induce with 1mM IPTG

Grow for 3 - 4 h at 37°C

Harvest bacteria and resuspend in 60 ml buffer:

- 50 mM Na-Phosphat pH 6,5
- 50 mM NaCl

Store at -80°C until next day (**freezing is essential!**)

Day 4

Thaw cells, add Protease-Inhibitors (PMSF, Aprotinin, Leupeptin, Pepstatin) and 1 mM DTT

Ultracentrifugation: 1 h 100,000g, 4°C (50.2Ti or 45Ti)

Note: neither lysozyme nor sonification required!!! Ubc9 leaks out just after freeze thawing!!!

Equilibrate 10 ml SP-Sepharose-beads (SIGMA) by resuspension, centrifugation:

- 1x with 0,5 M Na-P pH 6,5
- 2x with 50 mM Na-P, 50 mM NaCl, pH 6,5
- 1x with 50 mM Na-P, 50 mM NaCl, pH 6,5 plus Protease-Inhibitors and DTT

Transfer Bead-Suspension in 50 ml column (cold room)

Run Supernatant from ultracentrifugation over column (discard flow through)

Wash column with 2-3 column volumes of:

- 50 mM Na-P pH 6,5 , 50 mM NaCl + Inhibitors + DTT

Elute Ubc9 with 2 – 3 column volumes of:

- 50 mM NaP pH 6,5 , 300 mM NaCl + Inhibitors + DTT

Collect 2 ml fractions, keep on ice

Test fractions by SDS-PAGE (15% Minigel) and stain with Coomassie

Combine Ubc9 peak fractions, concentrate with 5K-Centriprep concentrator to 2-5 ml,

filtrate through 0.2 µm low protein binding filter or centrifuge,

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
- 1 µg/ml each of Leupeptin and Pepstatin

Run over night and collect 5 ml fractions

Day 5

Test fractions by SDS-PAGE (15% Minigel) and stain with Coomassie

Combine peak fractions, aliquote in small aliquots (2-10 µl) and flash freeze

Ubc9 can be thawed and refrozen several times, however for reproducibility we prefer to use aliquots only once. High dilutions of Ubc9 can be stabilized with 0.05% Tween and 0.2 mg/ml Ovalbumin

BIBLIOGRAPHY

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