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Wurzer et al., 2015

Protocol status: Working
We use this protocol and it's working

EXPRESSION AND PURIFICATION OF HUMAN p62 (HIS-TEV-mCherry-p62)

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ABSTRACT

This protocol describes how to express and purify human p62 tagged N-terminally with HIS and TEV. The expression is performed with *E. coli* Rosetta pLysS cells. The protein is purified via a HisTrap column and gel filtration (SEC).

MATERIALS

E. coli Rosetta (DE3) pLysS cells
LB medium with antibiotics: 50 µl/ml ampicillin and 34 µl/ml chloramphenicol
IPTG (Isopropyl-β-D-thiogalactopyranosid)

Columns/Resin:
HisTrap HP column, 1 x 5 ml (Cytiva, #17524802)
Superdex 200 Increase 10/300 GL column (Cytiva, #28990946)

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BEFORE START INSTRUCTIONS

Lysis Buffer:
25 mM HEPES pH 7.5
150 mM NaCl
25 mM Imidazole
2 mM MgCl2
Freshly added: 2 mM β-Mercaptoethanol, Roche Protease Inhibitor (Merck, #5056489001), DNAse I (Sigma, #DN25-1G)

HisTrap Buffer A (filtered, degassed):
25 mM HEPES pH 7.5
500 mM NaCl
25 mM Imidazole
Freshly added: 2 mM β-Mercaptoethanol

HisTrap Buffer B (filtered, degassed):
25 mM HEPES pH 7.5
500 mM NaCl
400 mM Imidazole
Freshly added: 2 mM β-Mercaptoethanol

SEC Buffer (filtered, degassed):
25 mM HEPES pH 7.5
500 mM NaCl
Freshly added: 1 mM DTT

Expression of p62

5h 15m

1 Grow *E. coli* Rosetta pLysS cells in 8 L Luria broth medium at 37 °C until an $OD_{600\ nm}$ of 0.8 is reached.

2 Induce protein expression with [M] 150 micromolar (µM) IPTG for 05:00:00 at 25 °C. 5h

3 Centrifuge cells at 3000 rcf, 4 °C, 00:15:00 15m

4 Aspirate media and resuspend pellet in 10 mL Lysis Buffer

5 Flash freeze sample in liquid nitrogen

6 Store at -80 °C until use.

Purification of p62

1d 0h 31m 30s

7 Thaw sample at 37 °C

8 Sonicate sample 5 cycles at 65% power for 00:00:30

1m 30s

8.1 Repeat sonication for a total of three times

9 Centrifuge sample 140,000 rcf at 4 °C for 00:30:00

30m

9.1 During centrifugation perform equilibration

10 Equilibrate a HisTrap HP column (GE Healthcare) with 5 column volumes of water and 5 of HisTrap Buffer A

11 Filter the supernatant through 0.2 µm syringe filter

12 Load sample onto the equilibrated HisTrap column at 4 °C

13 Elute protein via a stepwise imidazole gradient using HisTrap Buffers A and B and elution steps: 25 mM, 62.5 mM, 118.75 mM, 148.75 mM, 212.mM, 306.25 mM and 400 mM imidazole

14 Add 100 µg to 150 µg TEV protease to pooled protein-containing fractions and incubate Overnight at 4 °C to cleave. 1d

15 Concentrate resulting protein with a 30 kDa MWCO concentrator (Millipore) to between .5 mL and 1 mL

16 Apply protein to a Superdex 200 Increase 10/300 column (Cytiva) pre-equilibrated with SEC Buffer.

17 Freeze purified protein in liquid nitrogen and store at -80 °C

18 Protein purity can be determined by SDS PAGE analysis