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

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We use this protocol and it's working

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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 × 5 ml (Cytiva, #17524802)

Superdex 200 Increase 10/300 GL column (Cytiva, #28990946)



Before start

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

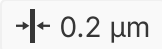
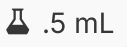
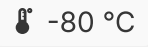
- 1 Grow *E. coli* Rosetta pLysS cells in  8 L Luria broth medium at  37 °C until an OD_{600 nm} of 0.8 is reached.
- 2 Induce protein expression with [M] 150 micromolar (μM) IPTG for  05:00:00 at  25 °C .
- 3 Centrifuge cells at 3000 rcf,  4 °C ,  00:15:00
- 4 Aspirate media and resuspend pellet in  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
- 8.1 Repeat sonication for a total of three times
- 9 Centrifuge sample 140,000 rcf at  4 °C for  00:30:00
- 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 
- 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 
- 18 Protein purity can be determined by SDS PAGE analysis