



JUL 31, 2023

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

Gabriele

Zaffagnini¹,

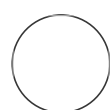
Elisabeth Holzer^{2,3}

¹Oocyte Biology & Cellular Dormancy Group, Centre for Genomic Regulation, Barcelona, Spain;

²Max Perutz Labs, University of Vienna, Vienna Biocenter, Vienna, Austria ;

³Vienna Biocenter PhD Program, Doctoral School of the University of Vienna and Medical University of Vienna, Vienna, Austria

OPEN ACCESS



OLIVIA HARDING

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)

DOI:

dx.doi.org/10.17504/protocol.s.io.j8nlkob61v5r/v1

Protocol Citation: Gabriele Zaffagnini, Elisabeth Holzer 2023. EXPRESSION AND PURIFICATION OF HUMAN p62 (HIS-TEV-mCherry-p62).

protocols.io

<https://dx.doi.org/10.17504/protocols.io.j8nlkob61v5r/v1>

MANUSCRIPT CITATION:

[Wurzer et al., 2015](#)

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: Jun 12, 2023

BEFORE START INSTRUCTIONS

Last Modified: Jul 31, 2023

PROTOCOL integer ID:
83273

Keywords: ASAPCRN

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  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



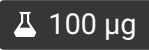
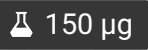


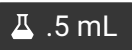

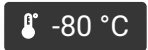
1m 30s

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  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  to  TEV protease to pooled protein-containing fractions and incubate  at  to cleave. 1d
- 15 Concentrate resulting protein with a 30 kDa MWCO concentrator (Millipore) to between  and 
- 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

