Sep 23, 2023

Expression and purification of mCherry-OPTN

Nature Structural & Molecular Biology

DOI
https://dx.doi.org/10.17504/protocols.io.4r3l225djl1y/v1

Elias Adriaenssens[1]

[1]Sascha Martens lab, University of Vienna, Max Perutz Labs - Vienna (AT)

Elias Adriaenssens
Sascha Martens lab, University of Vienna, Max Perutz Labs - ...

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.4r3l225djl1y/v1

External link: https://doi.org/10.1038/s41594-024-01338-y

Protocol Citation: Elias Adriaenssens 2023. Expression and purification of mCherry-OPTN. **protocols.io** https://dx.doi.org/10.17504/protocols.io.4r3l225djl1y/v1

Manuscript citation:
Elias Adriaenssens, Thanh Ngoc Nguyen, Justyna Sawa-Makarska, Grace Khuu, Martina Schuschnig, Stephen Shoebridge, Marvin Skulsuppaisarn, Emily Maria Watts, Kitti Dora Csalyi, Benjamin Scott Padman, Michael Lazarou, Sascha Martens (2024) Control of mitophagy initiation and progression by the TBK1 adaptors NAP1 and SINTBAD.Nature Structural & Molecular Biology doi: 10.1038/s41594-024-01338-y

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

Created: June 27, 2023

Last Modified: May 31, 2024

Protocol Integer ID: 84067

Keywords: mCherry-OPTN, ASAPCRN, purification of mcherry, purification, optn this protocol, optn, mcherry

Funders Acknowledgements:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-000350
Marie Skłodowska-Curie MSCA Postdoctoral fellowship
Grant ID: 101062916

Abstract

This protocol describes the purification of mCherry-OPTN.

Attachments

PDF

753-1921.pdf
48KB

Materials

Lysis Buffer

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	300 mM
	MgCl2	2 mM
	glycerol	5%
	Imidazole	10 mM
	β-mercaptoethanol	2 mM
	cOmplete EDTA-free protease inhibitors (Roche)	
	CIP protease inhibitor (Sigma)	
	DNase (Sigma)	

Wash buffer A

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	300 mM
	Imidazole	10 mM
	β-mercaptoethanol	2 mM

Wash buffer B

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	300 mM
	Imidazole	300 mM
	Wash buffer A	2 mM

SEC buffer

	A	B
	Tris-HCl pH 7.4	25 mM
	NaCl	150 mM
	DTT	1 mM

mCherry-OPTN

16h 0m 30s

1 For mCherry-OPTN, clone human OPTN cDNA in a pETDuet-1 vector with an N-terminal 6x His tag follow it by a TEV cleavage site (Addgene #190191).

2 After the transformation of the pETDuet-1 vector encoding 6xHis-TEV-mCherry-OPTN in *E. coli* Rosetta pLySS cells, grow cells in 2x TY medium at 37 °C until an OD_{600} of 0.4 and then continue at 18 °C.

3 Once the cells reached an OD_{600} of 0.8, induce protein expression with [M] 50 micromolar (µM) IPTG for 16:00:00 at 18 °C. 16h

4 Collect cells by centrifugation and resuspend in lysis buffer.

5 Sonicate the cell lysates.

5.1 Sonicate the cell lysates for 00:00:30. (1/2) 30s

5.2 Sonicate the cell lysates for 00:00:30. (2/2) 30s

6 Clear Lysates by centrifugation at 18000 rpm, 4°C, 00:45:00 in a SORVAL RC6+ centrifuge with an F21S-8×50Y rotor (Thermo Scientific). 45m

7 Filter the supernatant through an 0.45 µm filter and load onto a preequilibrated 5 mL His-Trap HP column (Cytiva).

8 After His tagged proteins were bound to the column, wash the column with three column volumes of wash buffer A.

9 Elute proteins with a stepwise imidazole gradient (30, 75, 100, 150, 225, 300 mM) by increasing addition of buffer B.

10 Pool the fractions at 75-100 mM imidazole that contains the 6xHis-TEV-mCherry-OPTN.

11 Incubate the pooled samples Overnight with TEV protease at 4 °C . 30s

12 After the 6xHis tag was cleaved off, concentrate the protein using a 50 kDa cut-off Amicon filter (Merck Millipore) and load onto a pre-equilibrated Superdex 200 Increase 10/300 GL column (Cytiva).

13 Elute the proteins with SEC buffer.

14 Analyze fractions by SDS-PAGE and Coomassie staining.

15 Pool fractions containing purified mCherry-OPTN.

16 After concentrating the purified protein, aliquot the protein and snap-freeze in liquid nitrogen.

17 Store the proteins at -80 °C .