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We use this protocol and it's working

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Expression and purification of mCherry-OPTN

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ABSTRACT

This protocol describes the purification of mCherry-OPTN.

ATTACHMENTS

753-1921.pdf

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-8x50Y 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.