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Purification of mCherry-WIPI2d/WIPI3

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We use this protocol and it's working

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Abstract

This protocol details the purification of mCherry- WIPI2d/WIPI3.

Materials

Rosetta™(DE3)pLysS Competent Cells - Novagen **Merck Catalog #**70956-4

Lysis buffer:

	A	B
	Tris-HCl, pH 7.4	50 mM
	NaCl	300 mM
	MgCl2	2 mM
	Glycerol	5%
	Triton X-100	1%
	Imidazole	10 mM
	β-mercaptoethanol	2 mM

Wash buffer:

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

SEC buffer:

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

Purification procedure

16h 45m 30s

1 To purify mCherry-WIPI2d and mCherry-WIPI3, as described previously for WIPI2d (Fracchiolla et al. 2020, J Cell Biol, PMID: 32437499), fuse the coding sequence of WIPI2d or WIPI3 to a N-terminal 6xHis-TEV-mCherry-tag through cloning into a pET-DUET1 vector (available from Addgene).

2 After the transformation of the pET-DUET1 vector encoding 6xHis-TEV-mCherry-WIPI2d/WIPI3 in E. coli Rosetta pLysS cells (Novagen Cat# 70956-4), grow the cells in 2x Tryptone Yeast extract (TY) medium at 37 °C until an OD_{600} of 0.4 and then continue at 18 °C .

3 Once the cells reach an OD_{600} of 0.8, induce the protein expression with [M] 100 micromolar (µM) isopropyl β-D-1-thiogalactopyranoside (IPTG) for 16:00:00 at 18 °C . 16h

4 Collect the cells by centrifugation and resuspend in lysis buffer, complete EDTA-free protease inhibitors (Roche), CIP protease inhibitor (Sigma), and DNase (Sigma)).

Lysis buffer:

	A	B
	Tris-HCl, pH 7.4	50 mM
	NaCl	300 mM
	MgCl2	2 mM
	Glycerol	5%
	Triton X-100	1%
	Imidazole	10 mM
	β-mercaptoethanol	2 mM

5 Sonicate cell lysates twice for 00:00:30 . 30s

6 Clear the 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 pre-equilibrated 5 ml His-Trap HP column (Cytiva).

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

Wash buffer:

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

9 Elute the proteins with a stepwise imidazole gradient (30, 75, 100, 150, 225, 300 mM).

10 Pool the fractions containing the 6xHis-TEV-mCherry-WIPI2d/WIPI3, concentrate using a 30 kDa cut-off Amicon filter (Merck Millipore) and loade onto a pre-equilibrated Superdex 200 Increase 10/300 GL column (Cytiva).

11 Elute the proteins with SEC buffer.

SEC buffer:

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

12 Analyse the fractions by SDS-PAGE and Coomassie staining.

13 Pool the fractions containing purified mCherry-WIPI2d or mCherry-WIPI3.

14 After concentrating the purified protein, aliquot the protein and snap-frozen in liquid nitrogen.

15 Store the proteins at -80 °C .