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Purification of FIP200 CTR-GFP

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We use this protocol and it's working

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Abstract

This protocol details the purification GFP-FIP200 (CTR).

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%
	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
	HEPES, pH 7.4	25 mM
	NaCl	150 mM
	DTT	1 mM

Purification procedure

1d 1h 45m 30s

1 To purify GFP-FIP200(CTR), as described previously (Turco et al. 2019 Mol Cell, PMID: 30853400), fuse the C-terminal domain of FIP200 (1458-1594aa) to a N-terminal 6xHis-TEV-GFP-tag through cloning into a pET-DUET1 vector (available on Addgene).

2 After the transformation of the pET-DUET1 vector encoding 6xHis-TEV-GFP-FIP200(CTR) in E. coli Rosetta pLysS cells (Novagen Cat# 70956-4), grow cells in 2x Tryptone Yeast extract (TY) medium at 37 °C until an OD_{600} of 0.4 and then continued at 18 °C.

3 Once the cells reached 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%
	Imidazole	10 mM
	β-mercaptoethanol	2 mM

5 Sonicate cell lysates twice for 00:00:30. 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 loaded onto a pre-equilibrated 5 ml His-Trap HP column (Cytiva).

8 After binding of 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 and incuabte the fractions containing the 6xHis-TEV-GFP-FIP200(CTR) Overnight with TEV protease at 4 °C .

8h

11 After cleave off the 6xHis tag , recapture 6xHis tag and His-tagged TEV protease with nickel beads for 01:00:00 at 4 °C .

1h

12 Pellet the beads by centrifugation and the supernatant, concentrate containing the GFP-FIP200(CTR) protein using a 30 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.

SEC buffer:

	A	B
	HEPES, pH 7.4	25 mM
	NaCl	150 mM
	DTT	1 mM

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

15 Pool fractions containing purified GFP-FIP200(CTR).

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

17 Store proteins at -80 °C .