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Purification of FUNDC1-GFP

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We use this protocol and it's working

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Abstract

This protocol details the purification of FUNDC1-GFP.

Materials

Lysis buffer:

Tris-HCl	50 mM
pH	7.4
NaCl	300 mM
Triton X-100	1%
glycerol	5%
MgCl2	2 mM
DTT	1 mM
β-mercaptoethanol	2mM
cOmplete EDTA-free protease inhibitors (Roche)	
CIP protease inhibitor (Sigma)	
DNase (Sigma)	

Wash buffer:

Tris-HCl	50 mM
pH	7.4
NaCl	300 mM
DTT	1 mM

High salt buffer:

Tris-HCl	50 mM
pH	7.4
NaCl	700 mM
DTT	1 mM

SEC buffer:

Tris-HCl	25 mM
pH	7.4
NaCl	300 mM

DTT	1 mM

- FUNDC1-GFP (available from Addgene) or FUNDC1(Y18A/L21A)-GFP (ΔLIR) (available from Addgene)
- pET-DUET1 vector (available on Addgene) pETDuet-1 TIM9,10 **addgene Catalog #**170280
- FUNDC1 Y18A/L21A (ΔLIR)(available on Addgene)
- Rosetta pLysS cells (Novagen Cat# 70956-4)
  Rosetta™(DE3)pLysS Competent Cells - Novagen **Merck Catalog #**70956-4
- SORVAL RC6+ centrifuge with an F21S-8x50Y rotor (Thermo Scientific)
- 10 kDa cut-off Amicon filter (Merck Millipore)
  Amicon® Ultra Centrifugal Filter, 10 kDa MWCO **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**UFC801008

Purification - FUNDC1-GFP

20h 46m

1 To purify GFP-tagged

- FUNDC1-GFP (available from Addgene) or FUNDC1(Y18A/L21A)-GFP (ΔLIR) (available from Addgene),

fuse the cytosol-exposed domain of FUNDC1 (1-50aa) to a C-terminal GFP-tag through cloning into a pET-DUET1 vector (available on Addgene).

2 Introduce the point mutants by in vitro mutagenesis to generate

- FUNDC1 Y18A/L21A (ΔLIR)(available on Addgene).

3 After the transformation of the pET-DUET1 vector encoding FUNDC1-GFP wild-type or mutants 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.

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

16h

5 Collect the cells by centrifugation and resuspend in lysis buffer.

Lysis buffer:

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	300 mM
	Triton X-100	1%
	Glycerol	5%
	MgCl2	2 mM
	DTT	1 mM
	β-mercaptoethanol	2mM
	cOmplete EDTA-free protease inhibitors (Roche)	
	CIP protease inhibitor (Sigma)	
	DNase (Sigma)	

6 Sonicate the cell lysates twice for 30 s and clear by centrifugation at 18000 rpm, 4°C, 00:45:00 in a SORVAL RC6+ centrifuge with an F21S-8x50Y rotor (Thermo Scientific). 45m

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

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

7 Collect the supernatant and incubate with pre-equilibrated Glutathione Sepharose 4B beads (GE Healthcare) for 02:00:00 at 4 °C with gentle shaking to bind FUNDC1-GFP. 2h

8 Centrifuge the samples to pellet the beads and remove the unbound lysate.

9 Wash the beads twice with wash buffer, once with high salt wash buffer, and two more times with wash buffer.

Wash buffer:

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	300 mM
	DTT	1 mM

High salt buffer:

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	700 mM
	DTT	1 mM

10 Cleave off the GST-tag Overnight by eluting the GFP-tagged cargo receptor from the GSH beads by the addition of TEV protease in wash buffer at 4 °C

11 To collect the supernatant, collect the beads by centrifugation.

12 Wash the beads twice with 4 mL of wash buffer, and collect the supernatant.

13 Pool the supernatant fractions, filtered through a 0.45 µm syringe filter, concentrated with 10 kDa cut-off Amicon filter (Merck Millipore), and load onto a pre-equilibrated Superdex 200 Increase 10/300 GL column (Cytiva).

14 Elute the proteins with SEC buffer.

SEC buffer:

	A	B
	Tris-HCl pH 7.4	25 mM
	NaCl	300 mM
	DTT	1 mM

15 Analyze fractions by SDS-PAGE and Coomassie staining. Pool the fractions containing purified FUNDC1-GFP.

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

Note

Store the proteins at -80 °C.