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Purification of FUNDC1-GST

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We use this protocol and it's working

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Abstract

This protocol details the purification of FUNDC1-GST.

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

- 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-GST

16h

1 To purify FUNDC1-GST, fuse the cytosol-exposed domain of FUNDC1 (1-50aa) to a C-terminal GST-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). After the transformation of the pET-DUET1 vector encoding FUNDC1-GST 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.

3 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

4 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)	

5 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

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 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-GST. 2h

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

8 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

9 Incubate the beads Overnight with 4 mL of [M] 50 millimolar (mM) reduced glutathione dissolves in wash buffer at 4 °C, to elute FUNDC1-GST from the beads. 2h

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

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

12 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).

13 Elute the proteins with SEC buffer.

SEC buffer:

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

14 Analyze fractions by SDS-PAGE and Coomassie staining. Pool the fractions containing purified FUNDC1-GST.

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

Note

Store the proteins at -80 °C.