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## Purification of FUNDC1-GFP

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**Protocol status:** Working

**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%
MgCl <sub>2</sub>	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
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- FUNDC1-GFP (available from Addgene) or FUNDC1(Y18A/L21A)-GFP ( $\Delta$ LIR) (available from Addgene)
- pET-DUET1 vector (available on Addgene)  pETDuet-1 TIM9,10 **addgene Catalog #170280**
- FUNDC1 Y18A/L21A ( $\Delta$ LIR)(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)



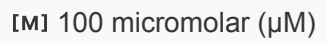


 Amicon® Ultra Centrifugal Filter, 10 kDa MWCO **Merck MilliporeSigma (Sigma-Aldrich) Catalog #UFC801008**



## Purification - FUNDC1-GFP

20h 46m


- 1 To purify GFP-tagged
  - FUNDC1-GFP (available from Addgene) or FUNDC1(Y18A/L21A)-GFP ( $\Delta$ LIR) (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 ( $\Delta$ LIR)(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<sub>600</sub> of 0.4 and then continue at  18 °C .
- 4 Once the cells reaches an OD<sub>600</sub> of 0.8, induce protein expression with  100 micromolar ( $\mu$ M) isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG) for  16:00:00 at  18 °C .
- 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%
MgCl <sub>2</sub>	2 mM
DTT	1 mM
$\beta$ -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 .