Aug 29, 2024

Purification mCherry-ATG13 IDR

DOI
dx.doi.org/10.17504/protocols.io.81wgbz4m1gpk/v1

Elias Adriaenssens[1]

[1]Sascha Martens lab, University of Vienna, Max Perutz Labs - Vienna

Elias Adriaenssens
Sascha Martens lab, University of Vienna, Max Perutz Labs - ...

DOI: dx.doi.org/10.17504/protocols.io.81wgbz4m1gpk/v1

Protocol Citation: Elias Adriaenssens 2024. Purification mCherry-ATG13 IDR. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.81wgbz4m1gpk/v1**

Protocol status: Working
We use this protocol and it's working

Created: July 02, 2024

Last Modified: August 29, 2024

Protocol Integer ID: 103081

Keywords: ASAPCRN

Funders Acknowledgement:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-000350
Marie Skłodowska-Curie MSCA Postdoctoral fellowship
Grant ID: 101062916

Abstract

This protocol details the purification of mCherry-ATG13 IDR.

Materials

Lysis buffer:

	A	B
	Tris-HCl pH 7.4	50 mM
	pH	7.4
	NaCl	300 mM
	MgCl2	2 mM
	glycerol	5%
	Triton X-100	1%
	Imidazole	10 mM
	β-mercaptoethanol	2 mM
	cOmplete EDTA-free protease inhibitors (Roche)	
	CIP protease inhibitor (Sigma)	
	DNase (Sigma)	

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

16h

1 To purify mCherry-tagged ATG13 IDR, fuse the coding sequence for ATG13 (190-517aa) or ATG13 (230-517aa) to a N-terminal 6xHis-TEV-mCherry-tag through cloning into a pET-DUET1 vector (plasmids available from Addgene).

2 After the transformation of the pET-DUET1 vectors encoding the mCherry-tagged ATG13 IDR 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 the protein expression with [M] 100 micromolar (µM) isopropyl β-D-1-thiogalactopyranoside (IPTG) for 16:00:00 at 18 °C.

16h

4 Collect cells by centrifugation and resuspend in lysis buffer.

Lysis buffer:

	A	B
	Tris-HCl pH 7.4	50 mM
	pH	7.4
	NaCl	300 mM
	MgCl2	2 mM
	glycerol	5%
	Triton X-100	1%
	Imidazole	10 mM
	β-mercaptoethanol	2 mM
	cOmplete EDTA-free protease inhibitors (Roche)	
	CIP protease inhibitor (Sigma)	
	DNase (Sigma)	

5 Sonicate the 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-8x50Y 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 His-tagged proteins are bound 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-ATG13 IDR, concentrated using a 30 kDa cut-off Amicon filter (Merck Millipore).

11 Load the samples were ed onto a pre-equilibrated Superose 200 Increase 10/300 GL column (Cytiva). Elute the proteins with SEC buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM DTT).

SEC Buffer:

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

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

13 Pool the fractions containing purified ATG13 IDR.

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

15 Store the proteins at -80 °C .