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Purification GFP-ATG13 IDR

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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 - ...

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We use this protocol and it's working

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Abstract

This protocol details the purification of GFP-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%
	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
	DTT	1 mM

High salt buffer:

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

SEC Buffer:

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

Purification

16h

1 To purify GFP-tagged ATG13 IDR, the coding sequence for ATG13 (190-517aa), (206-517aa), (231-517aa), (190-205_231-517aa), (190-230aa), (190-205aa), or (206-230aa) into GST-TEV-EGFP-insert through cloning into a pGEX-4T1 vector (Plasmids available from Addgene).

2 Mutants

- 3A (M196A/S197A/R199A) and
- 11A (M196A/S197A/R199A/G202A/T204A/P205A/I207A/M208A/I210A/D213A/H214A)

are also expressed according to the protocol below.

3 After the transformation of the pGEX-4T1 vectors encoding the GFP-tagged ATG13 IDR 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 continue at 18 °C.

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

16h

5 Collect cells by centrifugation and resuspend in lysis buffer for GST-tagged proteins (50 mM Tris-HCl pH 7.4, 300 mM NaCl, 2 mM MgCl2, 5% glycerol, 1% Triton X-100, 2 mM β-mercaptoethanol, cOmplete EDTA-free protease inhibitors (Roche), CIP protease inhibitor (Sigma), and DNase (Sigma)).

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%
	β-mercaptoethanol	2 mM
	cOmplete EDTA-free protease inhibitors (Roche)	
	CIP protease inhibitor (Sigma)	
	DNase (Sigma)	

6 Sonicate the cell lysates twice for 00:00:30 . 30s

7 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

8 Collect the supernatant after centrifugation and incubate with pre-equilibrated Glutathione Sepharose 4B beads (GE Healthcare) for 02:00:00 at 4 °C on a roller to bind GST-TEV-EGFP-ATG13 IDR. 2h

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

10 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

11 Incubate the beads Overnight with TEV protease at 4 °C , to elute GFP-tagged ATG13 IDR from the beads. 2h

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

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

14 Pool the supernatant fractions, filter through a 0.45 µm syringe filter, concentrated with 10 or 30 kDa cut-off Amicon filter (Merck Millipore).

15 Load the samples onto a pre-equilibrated Superose 200 Increase 10/300 GL column (Cytiva) or S75 Increase 10/300 column (Cytiva) in case of the smaller peptides (190-230aa and smaller variants thereof).

16 Elute the proteins with SEC buffer.

SEC Buffer:

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

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

18 Pool the fractions containing purified ATG13 IDR. After concentrating the purified protein, aliquote the protein was and snap-frozen in liquid nitrogen.

19 Store the proteins at -80 °C .