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Purification GFP-ATG13 IDR

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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
	MgCl ₂	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:

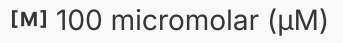


	A	B
	Tris-HCl pH 7.4	25 mM
	NaCl	150 mM
	DTT	1 mM

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 OD₆₀₀ of 0.4 and then continue at  18 °C .
- 4 Once the cells reaches an OD₆₀₀ 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 .
- 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
MgCl ₂	2 mM
glycerol	5%
Triton X-100	1%
β-mercaptoethanol	2 mM



	A	B
	cComplete EDTA-free protease inhibitors (Roche)	
	CIP protease inhibitor (Sigma)	
	DNase (Sigma)	

- 6 Sonicate the cell lysates twice for  00:00:30 . 
- 7 Clear the lysates by centrifugation at  18000 rpm, 4°C, 00:45:00 in a SORVAL RC6+ centrifuge with an F21S-8×50Y rotor (Thermo Scientific). 
- 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. 
  
- 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 .

