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Purification mCherry-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 mCherry-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%
	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 OD₆₀₀ of 0.4 and then continue at 18 °C .
- 3 Once the cells reaches an OD₆₀₀ of 0.8, induce the protein expression with 100 micromolar (μM) isopropyl β-D-1-thiogalactopyranoside (IPTG) for 16:00:00 at 18 °C .
- 4 Collect cells by centrifugation and resuspend in lysis buffer.



16h



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%
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-8×50Y 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



	A	B
	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 .

