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## Strep pull down assay

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### ABSTRACT

Strep pull down assay for FIP200(NTD)-TSF WT or mutants were co-transfected with GST-ATG13(363-517) and/or ULK1(MIT)-MBP

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**protocols.io**

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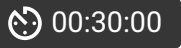
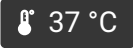
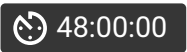


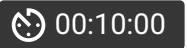
**Protocol status:** Working  
We use this protocol and it's working


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**Keywords:** ASAPCRN



**Funders**  
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Expression		2d 0h 40m
1	Transfect 10 ml HEK GNTi cells at concentration of $2 \times 10^6$ cells/ml	
2	Dilute PEI with Warm Hybridoma-SFM(1X)	
3	In a separate tube, dilute DNA with Hybridoma-SFM(1X)	
4	Add PEI to DNA dilution. Incubate mixture for  00:30:00 at  37 °C	30m
5	Add mixture to cells. Let cells grow for  48:00:00	2d
6	Harvest Cells  500 rpm ,  4 °C ,  00:10:00	10m



7 Wash pellet with cold PBS. Store pellet at  -80 °C until purification.

## Pull down

2h 50m



8 Homogenize the pellets in 0.5 ml of lysis buffer/protease inhibitors/1% TritonX-100, and clarified after  40000 x g ,  00:15:00 .

15m

9 Incubate the lysate with 30 µl Strep-Tactin Sepharose resin (IBA-Lifesciences) at  4 °C for  03:00:00

3h

10 Wash the beads four times, and eluted in 50 µl lysis buffer/4 mM desthiobiotin.

11 Mix 18 µl eluent with lithium dodecylsulfate (LDS)/BME buffer, heated at  60 °C for  00:05:00 and subjected to SDS/PAGE gel. The gel was then stained with Coomassie brilliant blue G250.

5m