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Strep pull down assay

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We use this protocol and it's working

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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

Expression

2d 0h 40m

1 Transfect 10 ml HEK GNTi cells at concentration of 2 × 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