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

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Expression and purification for cryo-EM samples of FIP200NTD:ATG13(363-517)-ULK1(MIT) complexes

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ABSTRACT

Expression and purification for cryo-EM samples of FIP200NTD:ATG13(363-517)-ULK1(MIT) complexes

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Expression

2d 0h 40m

1 Transfect 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.

Purification

2h 50m

8 Cell pellets were lysed at room temperature for 20 min with lysis buffer (25 mM HEPES pH 7.5, 200 mM NaCl, 2 mM MgCl2, 1 mM TCEP, 10% Glycerol) with 5 mM EDTA, 1% Triton X-100 and protease inhibitor cocktail (Thermo Scientific) for 00:15:00 15m

9 Clarify lysate for 17000 rpm for 00:35:00 at 4 °C. 35m

10 Wash strep-tactin resin (IBA Lifesciences, Germany) into lysis buffer (without Triton). Load clarified lysate onto resin

11 Rock supernatant with equilibrated Strep-Tactin Sepharose resin for 01:00:00 at 4 °C 1h

12 Wash with 5CV lysis buffer (25 mM HEPES pH 7.5, 200 mM NaCl, 2 mM MgCl2, 1 mM TCEP, 5 mM EDTA, 10% Glycerol)

13 Elute with lysis buffer plus 4 mM desthiobiotin for STREP resin

14 His6-TEV cleavage at 4 °C Overnight , 1h

15 Concentrate elution and inject onto pre-equilibrated Superose 6 Increase 10/300 GL column (Cytiva)
(25 mM HEPES pH 7.5, 150 mM NaCl, 1 mM MgCl2, 1 mM TCEP)

16 Pool peak fractions, concentrate, snap freeze, and store at -80 °C