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

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Expression and purification of PI3KC3-C1 complex

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ABSTRACT

Expression and purification of PI3KC3-C1 complex

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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 Pellets were homogenized 20 times by Pyrex douncer (Corning) in lysis buffer (25 mM HEPES pH 7.5, 200 mM NaCl, 2 mM MgCl2, 10% Glycerol) with 25mM TCEP/proteinase inhibitors (Thermo Scientific), and then add 10% Triton X-100 stock to final 1% concentration.

9 Rocking at 4 °C for 01:00:00 1h

10 Clarify lysate for 17000 rpm for 00:40:00 at 4 °C. 40m

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

12 Rock supernatant with equilibrated Strep-Tactin Sepharose resin for Overnight at 4 °C 1h

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

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

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

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