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FIP200-eGFP expression and purification

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ABSTRACT

This protocol describes how to express and purify human FIP200 tagged C-terminally with eGFP.

ATTACHMENTS

protocols io FIP200.pdf

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PROTOCOL CITATION

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MATERIALS TEXT

Expression:
pGB-GST-3C-FIP200-GFP (Addgene ID: 187832)
Sf9 insect cells
SF921 medium with antibiotics 100 IU/ml Penicillin and 100 µg/ml Streptomycin

Lysis Buffer:
50 mM HEPES pH 7.5
300 mM NaCl
1 mM $MgCl_2$
10% glycerol
0.5% CHAPS
5 U/ml Benzonase (Sigma)
1 mM DTT
CIP protease inhibitor (Sigma)
cOmplete EDTA-free protease inhibitor cocktail (Roche)

Wash Buffer:
50 mM HEPES pH 7.5
200 mM NaCl
1 mM $MgCl_2$
1 mM DTT

Gel-filtration buffer:
25 mM HEPES pH 7.5
200 mM NaCl
1 mM DTT

Columns/Resin:
Glutathione Sepharose 4B (Cytiva)
Superose 6 increase 10/300 column (Cytiva)

Expression

1 To generate FIP200-GFP constructs the insect codon optimized FIP200 gene was purchased from GenScript and cloned with respective tags into pGB-02-03 (pGB-GST-3C-FIP200-GFP - Addgene ID: 187830). Generated construct was used for expression in Sf9 insect cells using the Bac-to-Bac system (ThermoFischer Scientific) .

2 Transfect 2.5 µg of bacmid DNA into Sf9 insect cells in 6-well plate using FuGene transfection reagent (Promega).

3 About 7 days after transfection the V0 virus should be ready for harvesting. Use the V0 to produce a V1 virus stock by infecting 30 ml of Sf9 cells (1 million/ml). Collect V1 about 4-5 days later. Monitor viability of the cells and green fluorescence to decide when to collect V1.

4 Infect 1L culture of Sf9 cells at 1-1.5 million/ml cells/volume at 99-100% viability in log phase with 1 ml of Virus 1 (V1).

5 After infection monitor cells for viability and fluorescence. Harvest by centrifugation when the viability drops to 80–95% and clear green fluorescence is present.

6 To harvest spin down the cells at 2000 rpm, for 15 min at RT (Sorvall RC6+ centrifuge, Thermo Scientific). Gently wash the cell pellets with PBS, flash-freeze in liquid nitrogen, and store at −80 °C until purification.

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Purification

7 Thaw a cell pellet corresponding to 1L culture by re-suspending it in 40 ml lysis buffer (50 mM HEPES pH 7.5, 300 mM NaCl, 1 mM $MgCl_2$, 10% glycerol, 0.5% CHAPS, 5 U/ml Benzonase (Sigma), 1 mM DTT, CIP protease inhibitor (Sigma), cOmplete EDTA-free protease inhibitor cocktail (Roche)) and rolling or stirring in the cold room.

8 Additionally disrupt the cells with a Dounce homogenizer followed by 1 min sonication at 50% cycles and 50–60% power.

9 Clear the lysate by centrifugation at 72,000 × *g* for 45 min at 4 °C (Beckman Ti45 rotor)

10 Incubate the cleared supernatant with 5 ml of Glutathione Sepharose 4B beads slurry (Cytiva) overnight at 4°C rolling gently. The GSH slurry should be washed with water and then pre-equillibrated with lysis buffer before incubating with the lysate.

11 Wash the beads seven times with wash buffer (50 mM HEPES pH 7.5, 200 mM NaCl, 1 mM $MgCl_2$, 1 mM DTT).

12 Incubate the beads overnight with preCission 3C protease at 4°C (40 ul of 6 mg/ml home-made protease).

13 Spin down the beads (4000 rpm, 3 min, 4°C) and collect the supernatant containing cleaved FIP200-eGFP.

14 Filter the supernatant through a 0.45 µm syringe filter to remove any residual beads.

15 Concentrate the protein down to 0.5 ml using a 100 kDa cut-off Amicon filter and apply onto a Superose 6 Increase 10/300 column (Cytiva) pre-equillibrated with a SEC buffer containing 25 mM HEPES pH 7.5, 200 mM NaCl, 1 mM DTT. Pool fractions containing pure proteins (see attached pdf), concentrate, snap freeze in liquid nitrogen, and store at −80°C.

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