Jun 29, 2024

Crude Subcellular fractionation of FAM177A1-GFP expressing cells

The Journal of Cell Biology

DOI
https://dx.doi.org/10.17504/protocols.io.n2bvjn2ypgk5/v1

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.n2bvjn2ypgk5/v1

Protocol Citation: Berrak Ugur, Michael G. Hanna, Pietro De Camilli 2024. Crude Subcellular fractionation of FAM177A1-GFP expressing cells. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvjn2ypgk5/v1

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

Created: June 26, 2024

Last Modified: June 29, 2024

Protocol Integer ID: 102587

Keywords: ASAPCRN, crude subcellular fractionation of fam177a1, crude subcellular fractionation, fam177a1, expressing cell, cells this protocol detail, cell, gfp

Funders Acknowledgements:
ASAP
Grant ID: 000580

Abstract

This protocol details the crude subcellular fractionation of FAM177A1-GFP expressing cells.

Materials

Fractionation buffer:

	A	B
	Tris, pH 7.4	25 mM
	NaCl	150 mM
	Protease inhibitor	

Crude Subcellular fractionation

2d 1h 5m

1

> Note
>
> All preparations were performed On ice .

Culture and transfect HeLa cells as described in
dx.doi.org/10.17504/protocols.io.eq2lyp55mlx9/v1

2 24:00:00 - 48:00:00 after transfection, wash cells in 6 well plates with PBS. 2d

2.1 Add 200 µL PBS (or fractionation buffer) to each well.

2.2 Scrape cells to release from well and transfer to a 1.7 mL eppendorph tube with additional 100 µL fractionation buffer wash.

3 Spin the lysate at 1500 rpm, 00:05:00 in a benchtop centrifuge. 5m

4 Remove the supernatant and resuspend cell pellet in 1 mL of cold fractionation buffer.

5 Homogenize resuspended cells with cell cracker (Isobiotec; 8-12 strokes), 1 mL at a time.

5.1 Wash with 1 mL fractionation buffer between samples.

5.2 Always use new syringes for each sample.

6 Ultracentrifuge lysates in a Beckman-Coulter table-top ultracentrifuge (TLA100 rotor) at 50000 rpm, 4°C, 01:00:00 to pellet membrane.

1h

7 After the centrifugation, transfer the supernatant to a new 1.7 mL Eppendorf tube and save it for western blot analysis.

8 Solubilize the membrane fractions from the bottom of the tubes using 4X Laemni buffer for western blot analysis.