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Immunofluorescence microscopy of R1441C or VPS35 D620N MEF cells

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Chloe A Hecht[1,2], Herschel Dhekne[1,2], Wondwossen Yeshaw[1,2], Suzanne Pfeffer[1,2]

[1]Department of Biochemistry, Stanford University School of Medicine;
[2]Aligning Science Across Parkinson's Disease

Chloe A Hecht

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We use this protocol and it's working

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Abstract

We present here a method to culture, fix, permeabilize, and stain R1441C or VPS35 D620N MEF cells to visualize transfected Myc-RILPL1 and endogenous TMEM55b or pRab10.

Materials

Paraformaldehyde 16% (Electron Microscopy Sciences 50-980-487)
PBS (Cold Spring Harbor protocol)
TMEM55B polyclonal antibody (Proteintech 23992-1-AP)
Recombinant Anti-Rab10 (Phospho T73) antibody (Abcam ab241060)
Myc antibody (Biolegend 626802)
Donkey anti-Rabbit 568 Alexa Fluor (Thermo Fisher A10042)
Donkey anti-Mouse 488 Alexa Fluor (Thermo Fisher A-21202)
DMEM high glucose (Cytiva SH30243.01)
Fetal bovine serum (Sigma #F0926)
Microscope cover glass (Fisher 12-545-81)
FuGENE 6 transfection reagent (Promega E2691)

Troubleshooting

Culturing, plating, and treating cells

1 Seed R1441C MEF cells or VPS35 D620N MEF cells at 50-60% confluency in a six well plate in 2 mL of complete DMEM (DMEM containing 10% FBS and 1% penicillin-streptomycin) 24 hours before transfection.

2 Transfect with Myc-RILPL1 plasmid with FuGENE 6 transfection reagent (E2691) at a 3:1 FuGENE:plasmid ratio using 2µg per well according to the manufacturer's guidelines for 24 hours.

3 Trypsinize cells and plate onto 12 mm glass coverslips resting in a six well plate at 60% confluency and leave cells to attach to coverslips in a 37ºC incubator with 5% CO2 for 24 hours.

4 If needed for MLi-2 conditions, add 200nM MLi-2 and leave in the incubator for 1.5 -2 hours.

5 For Nocodazole, add 20µM Nocodazole and leave in the incubator for 1.5-2 hours.

Fixing, permeabalizing, and mounting coverslips

6 Solutions needed:
- 4% PFA in 1x PBS
- 0.2% Saponin in 1x PBS
- 2% BSA in 1x PBS (blocking solution)

7 Transfer each coverslip from the 6-well plate to a 24 well plate filled with ~400 µl 4% PFA per well and fix for 10 minutes at room temperature (all subsequent steps and solutions are at room temperature).

8 Wash 3x with 1x PBS.

9 Permeabilize cells with 0.2% Saponin in 1x PBS for 10 minutes.

10 Wash 3x in 1x PBS.

11 Block with 2% BSA in 1x PBS for 30 minutes.

12 Incubate primary antibody in blocking buffer for 2 hours.

12.1 Dilute antibodies (see Materials) as follows:
Rabbit anti-TMEM55B PA5-61760 (1:4000)
Rabbit anti-pRab10 (1:1000)
Mouse anti-Myc (1:1000)

13 Wash 3x with 1x PBS.

14 Incubate with 2º Antibody for 1 hour in the dark in blocking buffer.

14.1 Dilute antibodies (see Materials) as follows:
Donkey anti-Rabbit 568 for TMEM55b and pRab10 (1:2000) and
Donkey anti mouse 488 for Myc (1:2000)
DAPI (1:1000)

15 Wash 3x with 1x PBS.

16 Use a Kimwipe held on the edge of the coverslip to remove excess liquid; Invert the coverslip onto 3µl Mowiol on a Gold Seal glass slide (size 1×3'', 1 mm thick).

17 Allow coverslips to dry at RT overnight.

18 Image