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Cell culture, transfection, immunocytochemistry, and imaging

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We use this protocol and it's working

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Abstract

This protocol describes the maintenance, transfection, immunocytochemistry, and imaging of RPE1 and also transfection, immunocytochemistry, and imaging of iPSCs, i^3 Neurons and DA neurons.

Attachments

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Materials

Reagents:

- Lipofectamine™ 2000 Transfection Reagent **Thermo Fisher Scientific Catalog #**11668019
- Lipofectamine™ Stem Transfection Reagent **Thermo Fisher Scientific Catalog #**STEM00008

Troubleshooting

General cell culture for RPE1

1 Grow hTERT-RPE1 cells in DMEM/F12 (Thermo Fisher Scientific) supplemented with 10% FBS (Thermo Fisher Scientific), 1% glutaMAX, 1% penicillin-streptomycin. Keep cells at 37 °C with 5% CO2 in an enclosed incubator.

Note

For general maintenance, when cells reach 80-90% confluency, detach them from the dish with Trypsin and dilute 1:10-20 in a new dish.

Cell transfection for RPE1 5d

2 For live-cell imaging experiments, seed the cells on glass-bottom dishes (MatTek; 35mm) at the concentrations ranging from 1-2 × 10^5 cells.

3 For RPE1: allow the cells to adhere for 08:00:00 - 24:00:00 before being transiently transfected using 4 µL Lipofectamine™ 2000 Transfection Reagent (Invitrogen) in Opti-MEM media, mix them with the respective plasmids (1 µg - 2 µg) and visualize after 48:00:00 . 3d

4 For cilia generation, serum-starve the cells in DMEM/F12 media (without FBS) for 48:00:00 . 2d

Cell transfection for iPSCs, i^{3}Neurons and DA Neurons 2d

5 For live-cell imaging experiments, seed cells on glass-bottom dishes (MatTek; 35mm) at the concentrations ranging from 3-5 × 10^5 cells.

6 Use 2.4 µL of Lipofectamine™ Stem Transfection Reagent (Invitrogen) in Opti-MEM media with respective plasmids (1 µg - 3 µg) and visualize at least 48:00:00 later. 2d

Immunocytochemistry of RPE1, iPSCs, i^{3}Neurons and DA Neurons 2h 7m

7 For fixed-cell imaging experiments, seed cells on glass-bottom dishes (MatTek; 35mm) at the concentrations ranging from 1-3 × 10^5 cells. 20m

- For immunofluorescence visualization, fix the cells with 4% (v/v) paraformaldehyde (Electron Microscopy Sciences) in 1× phosphate-buffered saline (PBS) for 00:20:00 .

8 Wash cells thrice in PBS.

9 Perform cell extraction with 0.25-0.5% (v/v) Triton X-100 in PBS for 00:10:00 . 10m

10 Wash cells thrice in PBS.

11 For removal of free aldehyde groups, quench the cells with fresh 1 µL sodium borohydride (Sigma-Aldrich) in PBS for 00:07:00 , and then wash thrice in PBS. 7m

12 Further block the cells for 00:30:00 in 5% bovine serum albumin (BSA, Sigma-Aldrich) in PBS and then incubate Overnight at 4 °C with the respective antibodies listed in Table S1. 30m

13 Wash the cells with PBS thrice the following day and incubate with Alexa Fluor-conjugated secondary antibodies (Thermo Fisher Scientific) for 01:00:00 at Room temperature . 1h

14 Wash the cells thrice in 1×PBS.

15 Use DAPI (Thermo Fisher Scientific) for nuclear staining, when necessary.

Imaging

16 For live imaging, maintain cells in a caged incubator with humidified atmosphere (5% CO_2) at 37 °C .

Note

The Yokogawa spinning disk field scanning confocal system with microlensing (CSU-W1 SoRa, Nikon) controlled by NIS elements (Nikon) software was used for imaging. Excitation wavelengths between 405-640 nm, CFI SR Plan ApoIR 60XC WI objective lens and SoRa lens-switched light path at 1x, 2.8x or 4x were used. SoRa images were deconvolved using the Batch Deconvolution (Nikon) software.