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Cell culture, transfection, immunocytochemistry, and imaging

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Cell culture, transfection, immunocytochemistry, and imaging

Michael G G Hanna[1,2], Pietro De Camilli[1,2]

[1]Departments of Neuroscience and of Cell Biology, Howard Hughes Medical Institute, Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, Connecticut 06510, USA;
[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815

Michael G G Hanna
Yale University

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Abstract

This protocol is to help with the maintenance, transfection, immunocytochemistry, and imaging of adherent mammalian cells associated with the publication DOI above.

Attachments

PDF

Cell culture, transf...

67KB

Materials

DMEM, high glucose **Thermo Fisher Catalog #**11965118

Penicillin-Streptomycin (10,000 U/mL) **Gibco - Thermo Fisher Catalog #**15140122

L-Glutamine (200 mM) **Gibco - Thermo Fischer Catalog #**25030081

DMEM/F-12, HEPES **Thermo Fisher Catalog #**11330057

ProLong™ Gold Antifade Mountant with DAPI **Thermo Fisher Catalog #**P36935

Live Cell Imaging Solution **Thermo Fisher Catalog #**A14291DJ

Required buffers

Fixation buffer
400mM Sucrose
5mM KCl
1mM NaH_2PO_4
2mM $MgCl_2$
5mM Glucose
1mM EGTA, 5mM PIPES
Stored at 4°C

Dilute PFA to 4% (Electron Microscopy Sciences, #15710) in fixation buffer just before use

16% Paraformaldehyde Aqueous Solution EM Grade **Fisher Scientific Catalog #**50-980-487

PBS
Diluted to 1x from 10x stock; Sigma # D1408-500mL

Dulbecco's Phosphate Buffered Saline **Sigma Aldrich Catalog #**D1408-500ML

Stored at room temperature

Antibody dilution (AbDil) buffer
1xPBS
4% BSA
0.1% Triton X-100
0.1% NaN_3
Stored at 4°C

Solubilization buffer
1xPBS
0.5% Triton X-100
Stored at room temperature

PBS wash buffer
1xPBS
0.1% Triton X-100
Stored at room temperature

TBS
50mM Tris pH 7.5
150mM NaCl
Stored at room temperature

Protocol materials

L-Glutamine (200 mM) **Gibco - Thermo Fischer Catalog #**25030081 Materials, Step 1

DMEM/F-12, HEPES **Thermo Fisher Catalog #**11330057 Materials, Step 2

ProLong™ Gold Antifade Mountant with DAPI **Thermo Fisher Catalog #**P36935 Materials, Step 15

Live Cell Imaging Solution **Thermo Fisher Catalog #**A14291DJ Materials, Step 18

16% Paraformaldehyde Aqueous Solution EM Grade **Fisher Scientific Catalog #**50-980-487 Materials

Dulbecco's Phosphate Buffered Saline **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D1408-500ML Materials

DMEM, high glucose **Thermo Fisher Catalog #**11965118 Materials, Step 1

Penicillin-Streptomycin (10,000 U/mL) **Gibco - Thermo Fisher Catalog #**15140122 Materials, Step 1

Safety warnings

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

A. General cell culture

1 Culture COS-7 or HeLa (wild-type, parental control, and all knock-in lines) cells at 37 °C and [M] 5 % CO2 in DMEM (Gibco, #11965118) containing [M] 10 % FBS, [M] 1 millimolar (mM) sodium pyruvate, [M] 100 U/mL penicillin, [M] 100 mg/mL streptomycin (Gibco/LifeTech, #15140-122) and [M] 2 millimolar (mM) L-glutamine (Gibco/LifeTech, #25030-081).

DMEM, high glucose **Thermo Fisher Catalog #**11965118

Penicillin-Streptomycin (10,000 U/mL) **Gibco - Thermo Fisher Catalog #**15140122

L-Glutamine (200 mM) **Gibco - Thermo Fischer Catalog #**25030081

2 Culture hTERT-RPE1 (wild-type, parental control, and all knockout lines) cells at 37 °C and [M] 5 % CO2 in DMEM/F12 (Gibco, #11330057) containing [M] 10 % FBS, [M] 1 millimolar (mM) sodium pyruvate, [M] 100 U/mL penicillin, [M] 100 mg/mL streptomycin and [M] 2 millimolar (mM) L-glutamine.

DMEM/F-12, HEPES **Thermo Fisher Catalog #**11330057

Note

Note: For general maintenance, when cells reache 80-90% confluency, detach from the dish with Trypsin (Gibco, #25300054) and dilute 1:10-20 in a new dish.

Trypsin-EDTA (0.05%), phenol red **Thermo Fisher Scientific Catalog #**25300054

B. Cell transfection

3 For live-cell imaging experiments, seed cells on glass-bottom dishes (MatTek; 35mm) at the following concentrations: COS-7, $8.5x10^4$; HeLa, $7.5x10^4$; RPE1, $1.0x10^5$.

4 Allow cells to adhere for 8-24 hours before being transiently transfected using FuGene HD (Promega) following manufacturer's instructions.

Note

Note: Replace Media just before addition of the transfection reagent that contains no antibiotics to lower toxicity.

5 Incubate transfection reagents with cells for 08:00:00 - 36:00:00 before imaging. 1d 12h

C. Immunocytochemistry 2h 30m

6 For fixed-cell imaging experiments, seed cells on to glass coverslips (FisherBrand # 12542A) in a well of a 6-well plate (Corning #3516).

i. Perform seeding at the following concentrations for all fixed-cell experiments where cells are not additionally transfected: HeLa, $1.5x10^5$; RPE1, $2.0x10^5$.

ii. Perform seeding at the following concentrations for all fixed-cell experiments where cells are additionally transfected: HeLa, $7.5x10^4$; RPE1, $1.25x10^5$. Perform transfection as described above before fixation.

7 Just before fixation, wash cells with 1 mL PBS quickly.

8 To each well, add 1 mL fixation buffer + 4% PFA equilibrated at 37 °C. Perform fixation at 37 °C for a total of 00:10:00. 10m

9 Remove Fixation solution, and wash cells with TBS three 10-minute washes to ensure the stoppage and removal of fixation solution.

9.1 Remove Fixation solution.

9.2 Wash cells with TBS for 00:10:00. 10m

9.3 Wash cells with TBS for 00:10:00. 10m

9.4 Wash cells with TBS for 00:10:00 . 10m

10 After the last TBS wash, add 1 mL solubilization buffer to each well for 00:10:00 at Room temperature . 10m

11 After solubilization is complete, add 1 mL AbDil buffer for 01:00:00 at Room temperature . 1h

12 Dilute antibodies in AbDil buffer. 100 µL AbDil buffer containing the diluted antibody is used for each coverslip. Pipette the diluted antibody solution as a droplet onto parafilm in a humidity-controlled chamber.

13 Invert coverslips onto the antibody droplet (cells facing down into solution) and place at 4 °C for 16:00:00 - 24:00:00 . 1d

14 Move coverslips into individual wells of a clean 6-well plate (cells now facing upwards) and wash three times with PBS wash buffer for 00:10:00 each. 10m

14.1 Move coverslips into individual wells of a clean 6-well plate (cells now facing upwards).

14.2 Wash PBS wash buffer for 00:10:00 . 10m

14.3 Wash PBS wash buffer for 00:10:00 . 10m

14.4 Wash PBS wash buffer for 00:10:00 . 10m

15 After the final wash, partially dry coverslips using an aspirator and invert (cells facing down) onto a drop of mounting medium (Invitrogen #P36935) on a glass slide (Electron Microscopy Sciences # 4951-001T). (Allow slides to cure as directed by the manufacturer of the mounting medium).

ProLong™ Gold Antifade Mountant with DAPI **Thermo Fisher Catalog #**P36935

16 After curing, permanently immobilize coverslips onto the glass slide using nail polish (Sally Hansen Advanced Hair and Nail).

D. Imaging

17 Perform all imaging using an Andor Dragonfly system equipped with a plan apochromat objective (60x, 1.4 NA, oil) and a Zyla scientific CMOS camera.

18 Perform all live-cell imaging at 37 °C and [M] 5 % CO2 in live cell imaging buffer (Invitrogen #A14291DJ).

Live Cell Imaging Solution **Thermo Fisher Catalog #**A14291DJ