Apr 18, 2022

# Cell culture, transfection, and imaging

Will Hancock-Cerutti[1,2,3], Pietro De Camilli[1,3]

[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]Interdisciplinary Neuroscience Program and MD-PhD Program, Yale University School of Medicine, New Haven, Connecticut 06510, USA;

[3]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815

1

dx.doi.org/10.17504/protocols.io.36wgq41j3vk5/v1

William Hancock-Cerutti

This protocol describes general procedures for culturing HeLa cells, transient transfection, and imaging using an Andor Dragonfly spinning disk confocal system.

dxwubkjz7.pdf

DOI

dx.doi.org/10.17504/protocols.io.36wgq41j3vk5/v1

Will Hancock-Cerutti, Pietro De Camilli 2022. Cell culture, transfection, and imaging. **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgq41j3vk5/v1

Cell culture, Transfection, Spinning-disk confocal, Fluorescent microscopy, ASAPCRN

protocol ,

Jul 08, 2021

Apr 18, 2022

Jul 08, 2021 Urmilas

Jul 14, 2021 William Hancock-Cerutti

51390

**DMEM Solution:**

| A | B |
|---|---|
| FBS | 10% |
| Penicillin | 100 U/ml |
| Streptomycin | 100 mg/ml |
| L-glutamine | 2 mM |

* (all from Gibco)

## General preparation

1 Culture the HeLa-M cells at **37 °C** in 5% $CO_2$ and DMEM containing 10% FBS, **100 U/ml** penicillin, **100 mg/mL** streptomycin, and **2 Milimolar (mM)** L-glutamine (all from Gibco).

Note: For general maintenance, when cells reached 80-90% confluency, they were deattached from the dish with Trypsin and diluted 1:20 in a new dish.

2 For live-cell imaging experiments, seed the cells on glass-bottomed dishes (MatTek) at a concentration of 35,000 cells per dish and transfect after **24:00:00** using FuGene HD (Promega) in Opti-MEM (Gibco). 1d

3 1d

Image the cells **24:00:00** after transfection.

4 Just before imaging, remove the growth medium and replace with prewarmed live-cell imaging solution (Life Technologies).

5 30m



**Citation:** Will Hancock-Cerutti, Pietro De Camilli Cell culture, transfection, and imaging https://dx.doi.org/10.17504/protocols.io.36wgq41j3vk5/v1

For lysotracker experiments, incubate the cells in **50 Nanomolar (nM)** LysoTracker Red DND-99 (ThermoFisher) in complete DMEM for **00:30:00** , wash twice with media, then image in live-cell imaging solution.

6 Perform all live-cell imaging at **37 °C** and 5% $CO_2$.

7 

Perform spinning-disk confocal microscopy using an Andor Dragonfly system equipped with a plan apochromat objective (63×, 1.4 NA, oil) and a Zyla scientific CMOS camera.

8 For any given experiment, use the same exposure time, laser power, and gain for image acquisition to allow for quantitative comparison.

## Imaging of cells stably expressing STING-GFP 3d 14h

9 Generate the cells stably expressing STING-GFP as described elsewhere.

10 Culture the stable STING-GFP HeLa-M cells at **37 °C** in 5% $CO_2$ and DMEM containing 10% FBS, **100 U/ml** penicillin, **100 mg/mL** streptomycin, and **2 Milimolar (mM)** Lglutamine (all from Gibco).

3d

11 

For experiments using siRNA, transfect 60 pmols of the indicated siRNA using **6 µL** Lipofectamine RNAiMax (ThermoFisher) in Opti-MEM (Gibco) per dish according to manufacturer protocol. Image the cells **72:00:00** after siRNA transfection.

14h

12 

For experiments using cGAMP, transfect **50 µg/L** of cGAMP using **18 µL** Lipofectamine RNAiMax (ThermoFisher) in Opti-MEM (Gibco) per dish according to manufacturer protocol. Image the cells **14:00:00** after transfection.



**Citation:** Will Hancock-Cerutti, Pietro De Camilli Cell culture, transfection, and imaging
https://dx.doi.org/10.17504/protocols.io.36wgq41j3vk5/v1

**Citation:** Will Hancock-Cerutti, Pietro De Camilli Cell culture, transfection, and imaging
https://dx.doi.org/10.17504/protocols.io.36wgq41j3vk5/v1