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Cell culture, transfection, and imaging

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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

William Hancock-Cerutti

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We use this protocol and it's working

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Abstract

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

Attachments

dxwubkjz7.pdf

142KB

Materials

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, [M] 100 mg/mL streptomycin, and [M] 2 millimolar (mM) L-glutamine (all from Gibco).

Note

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 Image the cells 24:00:00 after transfection. 1d

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

5 For lysotracker experiments, incubate the cells in [M] 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. 30m

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, [M] 100 mg/mL streptomycin, and [M] 2 millimolar (mM) Lglutamine (all from Gibco).

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.

3d

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.

14h