iPSCs

Dec 18, 2019

LIVE IMAGING OF i^{3}NEURONS (Support Protocol 5)

In 1 collection

Michael S. Fernandopulle[1], Ryan Prestil[1], Christopher Grunseich[1], Chao Wang[2], Li Gan[2], Michael E. Ward[1]

[1]National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, [2]Gladstone Institute of Neurological Disease, Gladstone Institutes, San Francisco, California

1 Works for me dx.doi.org/10.17504/protocols.io.5w7g7hn

Neurodegeneration Method Development Community

ABSTRACT

Live imaging permits visualization of molecular and organellar dynamics within the neuron. While a standard confocal microscope is sufficient for short imaging experiments, extended imaging applications (>1 hr) are best served by a 37 °C live imaging chamber outfitted onto the microscope. CM should also be changed to Hibernate A Low Fluorescence Medium (BrainBits LLC, cat. no. SKU#HAPR) for extended imaging.

EXTERNAL LINK

https://doi.org/10.1002/cpcb.51

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Fernandopulle, M. S., Prestil, R., Grunseich, C., Wang, C., Gan, L., & Ward, M. E. (2018). Transcription-factor mediated differentiation of human iPSCs into neurons. Current Protocols in Cell Biology, e51. doi:https://doi.org/10.1002/cpcb.51

fernandopulle2018.pdf

GUIDELINES

Live imaging permits visualization of molecular and organellar dynamics within the neuron. While a standard confocal microscope is sufficient for short imaging experiments, extended imaging applications (>1 hr) are best served by a 37 °C live imaging chamber outfitted onto the microscope. CM should also be changed to Hibernate A Low Fluorescence Medium (BrainBits LLC, cat. no. SKU#HAPR) for extended imaging. Hibernate A permits long-term maintenance of neuronal cultures in ambient carbon dioxide levels (0.04 % vs. 5 % for standard cell culture incubators) and provides a better imaging environment by reducing autofluorescence from phenol red-containing medium. Finally, medium (either CM for short imaging or Hibernate A for extended imaging) should be supplemented with SOS neuronal supplement (Cell Guidance Systems, M09-50) instead of B27. SOS supplement does not contain phototoxic components present in B27 and other neuronal supplements. Imaging is best done on glass-bottom slides, such as Ibidi µ-slides (Ibidi, cat. no. 80827).

Hibernate A Low Fluorescence
by BrainBits
Catalog #: HALF

SOS® neuronal supplement
Catalog #: M09-50

µ-Slide 8 Well Glass Bottom
by Ibidi
Catalog #: 80827

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.