Dec 19, 2019

# LIVE IMAGING OF i$^3$NEURONS (Support Protocol 5)

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.5w7g7hn

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

Neurodegeneration Method Development Community
Tech. support email: **ndcn-help@chanzuckerberg.com**

Julia Rossmanith
protocols.io



**DOI: dx.doi.org/10.17504/protocols.io.5w7g7hn**

**External link: https://doi.org/10.1002/cpcb.51**

**Protocol Citation:** Michael S. Fernandopulle, Ryan Prestil, Christopher Grunseich, Chao Wang, Li Gan, Michael E. Ward 2019. LIVE IMAGING OF i3NEURONS (Support Protocol 5) . **protocols.io https://dx.doi.org/10.17504/protocols.io.5w7g7hn**

**Manuscript citation:**
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



**Protocol status:** Working
**We use this protocol and it's working**

**Created:** July 27, 2019

**Last Modified:** December 19, 2019

**Protocol Integer ID:** 26303

**Keywords:** i3LMN, i3Neurons, iPSC, iPSC-derived neurons, transcription factor-mediated differentiation

## 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.

## Attachments

PDF

fernandopulle2018.pd...

1.7MB

## 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 **BrainBits** **Catalog #**HALF

SOS® neuronal supplement **Catalog #**M09-50

µ-Slide 8 Well Glass Bottom **Ibidi** **Catalog #**80827

## Safety warnings

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