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Live-imaging of axonal cargoes in human iPSC-derived neurons or mouse primary neurons

Dan Dou[1,2], C. Alexander Boecker[3], Erika L.F. Holzbaur[1,2]

[1]Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA;
[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, USA;
[3]Department of Neurology, University Medical Center Goettingen, 37077 Goettingen, Germany

Dan Dou

ABSTRACT

Here, we describe procedure and equipment used for live-imaging of axonal cargoes. This was performed both using primary mouse cortical neurons and human iPSCderived excitatory glutamatergic neurons. Equipment and software used varied based on laboratory site and scheduled upgrades to microscopy equipment during the course of this study.

ATTACHMENTS

551-1147.pdf

GUIDELINES

Citations:

- Boecker, C.A., Olenick, M.A., Gallagher, E.R., Ward, M.E., and Holzbaur, E.L.F. (2020). ToolBox: Live Imaging of intracellular organelle transport in induced pluripotent stem cell-derived neurons. Traffic 21, 138–155.
- Kaech, S., and Banker, G. (2006). Culturing hippocampal neurons. Nat. Protoc. 1, 2406–2415.

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

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MATERIALS

Reagents

- Hibernate E low fluorescence media (CATALOG)
- GlutaMAX™ Supplement **Gibco - Thermo Fisher Catalog #35050061**
- B-27™ Supplement (50X), serum free **Gibco - Thermo Fisher Catalog #17504044**
- Hibernate A low fluorescence media (BrainBits, Cat# HALF)
- Recombinant Human NT-3 **peprotech Catalog #450-03**
- Recombinant Human/Murine/Rat BDNF **peprotech Catalog #450-02**

Equipment

- Heated environmental imaging chamber (37 °C)
- Spinning disk confocal microscope (see Materials and Methods for specific systems and cameras used)
- 60x 1.40 NA oil immersion objective
- VisiView software

SAFETY WARNINGS

Investigators should be trained and familiar with the confocal microscope to avoid eye damage from lasers.

Live-imaging of axonal cargoes in human iPSC-derived neur..

5m

1

Note

Please refer “Protocol: Primary neuron culture for live-imaging of axonal cargoes” and “Protocol: Culture and transfection of iPSC-derived neurons for live-imaging of axonal cargoes” for plating and transfection instructions.

Image primary mouse cortical neurons on DIV7. Image human iNeurons on DIV21.

2 Replace culture media with low fluorescence imaging media.

2.1 For primary mouse neurons, use Hibernate E medium supplemented with

	A	B
	B-27	2%
	GlutaMAX	2 mM

2.2 For iNeurons, use Hibernate A medium supplemented with

	A	B
	BDNF	10 ng/mL
	NT-3	10 ng/mL
	B-27	2%

3 Image using spinning disk confocal microscope under 60x magnification (oil immersion objective).

Note

See “Materials and Methods” for specific microscopes and cameras used.

4 Identify axons of transfected neurons based on morphological parameters. (Boecker et al., 2020; Kaech and Banker, 2006). For example, axons can most reliably be identified by their length and should span over at least 500 µm.

5 Acquire time lapse recordings at a frame rate of 1 frame per second for 00:05:00. 5m

Note

- Time lapses were taken in the mid-axon, defined as >300 µm from the soma and > 100 µm from the distal axon terminal.
- Knowledge of the pixel/micron ratio for the specific objective and camera being used is necessary for accurately measuring these distances.