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

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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 iPSC-derived 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:

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.
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.


2 Replace culture media with low fluorescence imaging media.

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

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
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<tbody>
<tr>
<td>B-27</td>
<td>2%</td>
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<tr>
<td>GlutaMAX</td>
<td>2 mM</td>
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2.2 For iNeurons, use Hibernate A medium supplemented with

<table>
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<tr>
<th>A</th>
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<tbody>
<tr>
<td>BDNF</td>
<td>10 ng/mL</td>
</tr>
<tr>
<td>NT-3</td>
<td>10 ng/mL</td>
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<tr>
<td>B-27</td>
<td>2%</td>
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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 5 minutes.

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