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Live-cell imaging for synaptic vesicle precursors in human iNeuron axons

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ABSTRACT

Here, we describe procedure and equipment used for live-imaging of synaptic vesicle precursors. This was performed using DIV21 human iPSC-derived excitatory glutamatergic neurons. Equipment and software used varied based on scheduled upgrades to microscopy equipment during the course of this study.

MATERIALS

1. GlutaMAX™ SupplementGibco - Thermo FisherCatalog #35050061
2. B-27™ Supplement (50X), serum freeGibco - Thermo FisherCatalog #17504044
3. Hibernate A low fluorescence media (BrainBits, Cat# HALF)
4. Recombinant Human NT-3peprotechCatalog #450-03
5. Recombinant Human/Murine/Rat BDNFpeprotechCatalog #450-02

SAFETY WARNINGS

Safety information

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

Note

Please refer to "Protocol: Culture and transfection of iPSC-derived neurons for live-imaging of axonal cargoes" for plating and transfection instructions.

Image human iNeurons on DIV21, 48-72 hours after transfection with PGK-mScarlet-synaptophysin.

2 Replace culture media with low fluorescence imaging media.

2.1 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). 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 Identify the neuronal soma and measure ~100-150 µm from the soma. Create an ROI that includes a segment of the axon that is in the same Z plane. Image several frames at this field of view prior to photobleaching. Perform one photobleaching cycle with the 405 nm laser for 3 ms/pixel. Dramatically decreased mScarlet-SYP signal should be observed in the ROI following photobleaching, and entry of mScarlet-SYP+ vesicles into the bleached region should be readily observed.

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

Note

- Rapid framerate is preferable due to the high transport speed of SVPs
- Knowledge of the pixel/micron ratio for the specific objective and camera being used is necessary for accurately measuring distances.