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BODIPY 558/568 C12 (Red-C12) Tracing in Hippocampal Neurons

In 1 collection

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We use this protocol and it's working

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Abstract

This protocol provides a detailed method for tracing BODIPY 558/568 labelled fatty acid (Red-C12) from lipid droplets to mitochondria in hippocampal neurons.

Guidelines

The protocol needs prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.

Materials

Materials:

- Hippocampal neurons (Isolated from neonatal P0-1 rat pups and cultured sparsely)
- BODIPY 558/568 C12 **Cayman Chemical Company** **Catalog #**27014
- KLH45 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**SML1998
- HBSS (Hank's Balanced Salt Solution)
- Etomoxir **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**E1905
- TTX (Tetrodotoxin) (Abcam, ab120055)
- MitoTracker™ Green FM - Special Packaging **Thermo Fisher** **Catalog #**M7514

Equipments:

- Tissue culture incubator
- Heating block
- Confocal microscope: Zeiss 880

Red-C12 Pulse Treatment

1d

1 Add [M] 5 micromolar (µM) KLH45 and [M] 10 micromolar (µM) BODIPY 558/568 C12 (Red-C12) to the feeding media.

2 Transfer cover slips with sparsely cultured hippocampal neurons to the prepared media and incubate for 24:00:00 in a tissue culture incubator at 37 °C with 5% CO_2.

1d

Incorporation Period

1h

3 After 24 hours, transfer the neurons to complete feeding media without Red-C12.

4 Incubate for 01:00:00 to allow complete incorporation of Red-C12 into neuronal lipid droplets (LDs).

1h

Wash and Chase

5 Wash the neurons gently with HBSS to remove excess media and non-incorporated Red-C12.

6 Transfer the neurons to fresh HBSS.

7 Chase the neurons for the defined period (0 to 4.5 hours) under the following conditions:

7.1 Control: HBSS alone

7.2 Etomoxir Treatment: [M] 10 micromolar (µM) Etomoxir in HBSS

7.3 TTX Treatment: [M] 5 micromolar (µM) TTX in HBSS

MitoTracker Staining

30m

8 At 30 minutes before the end of the chase period, add [M] 50 nanomolar (nM) MitoTracker Green FM to the media.

9 Return the neurons to the incubator for 00:30:00 .

30m

Final Wash

10 Wash the neurons once with HBSS to remove excess dye.

11 Transfer the neurons to fresh HBSS (containing the same inhibitors as in the chase condition, if applicable) preconditioned in the incubator.

Imaging Preparation

12 Mount the coverslip containing neurons onto a custom-designed imaging chamber.

13 Ensure the chamber is appropriately sealed and filled with fresh HBSS to maintain neuronal viability.

Imaging and Analysis

14 Place the imaging chamber on the stage of a confocal microscope.

15 Use appropriate settings to image:

15.1 Red-C12: Excitation/emission at ~558/568 nm.

15.2 MitoTracker Green FM: Excitation/emission at ~490/516 nm.

16 Capture images at the desired time points and fields of view.

17 Select distinctly isolated mitochondria on axons and capture fluorescence intensity of Red-C12.

Note

- Protect Red-C12 and MitoTracker from light to prevent photobleaching.
- Maintain consistent temperature and conditions during imaging to ensure neuronal viability.
- Ensure inhibitors (Etomoxir and TTX) are freshly prepared.
- Avoid analysis of the mitochondria from somatic and dendritic regions of neurons.