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Mitophagy detection assay

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

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Abstract

Detection of mitophagy flux in neurons using Dojindo Mtphagy dye and Lysotracker Green

Protocol materials

Mtphagy Dye **Dojindo Catalog #**MD01

Lysotracker Green **Thermo Fisher Scientific Catalog #**L7526

Before start

Make maintenance media (MM) and imaging media (IM) for cortical neurons

1 Plate 120,000-150,000 neurons in an imaging dish at *days in vitro* (DIV) 0

Note

Protocol for primary neuron isolation and in vitro culture can be obtained from:
dx.doi.org/10.17504/protocols.io.81wgby723vpk/v1

Preparing reagants

2 On the day of imaging, equilibrate 12 ml of fresh maintenance media (MM) per imaging dish in an incubator at 37 °C

Note

Maintence media composition can be obtained from:
dx.doi.org/10.17504/protocols.io.81wgby723vpk/v1

3 Prepare intermediate dilution of DMP Red (commercially available as Mtphagy Dye **Dojindo Catalog #**MD01) in MM: for each imaging dish, make 500 µL of fresh MM containing 0.5 µL of DMP Red

4 Prepare intermediate dilution of Lysotracker Green in MM: for each imaging dish make 50 µL of fresh MM containing 0.1 µL of Lysotracker Green
Lysotracker Green **Thermo Fisher Scientific Catalog #**L7526

5 Prepare imaging media (IM) with Lysotracker Green- for each imaging dish make 50 µL of fresh MM containing 0.1 µL of of Lysotracker Green

Note

Imaging media composition for 50 ml:

45% Glucose 660 µL
B-27 1 mL
Hibernate E Up to 50 mL

Imaging steps

3h 30m

6 At DIV 6 or 7, aspirate out conditioned media from the imaging dish, wash the neurons once with fresh MM

7 Add 1.6 mL of fresh MM and then add 0.4 mL of MM with the intermediate dilution of DMP Red (prepared in step 3)

8 Incubate the neurons in an incubator at 37 °C for 00:30:00 30m

9 Post incubation, aspirate out the media, and wash once with fresh MM

10 Add 2 mL of fresh MM containing 3 nM Antimycin A or an equivalent volume of 100% ethanol

11 Incubate the neurons in an incubator at 37 °C for 01:00:00 1h

12 Post incubation, aspirate out the media, and replace it with fresh MM **with no Antimycin A or ethanol**

13 Incubate the neurons in an incubator at 37 °C for 01:30:00 1h 30m

14 Post incubation add 50 µL of MM with the intermediate dilution of Lysotracker Green (prepared in step 4)

15 Incubate the neurons in an incubator at 37 °C for 00:30:00 30m

16 Post incubation, aspirate out the media, and wash once with fresh IM

17 Add 2 mL of fresh IM followed by 50 µL of IM with the intermediate dilution of Lysotracker Green (prepared in step 5)

18 Image neurons live under a confocal microscope with a heating chamber kept at 37 °C