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Assay for lysosomal activity using Magic Red

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We use this protocol and it's working

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Abstract

Assay for lysosomal Cathepsin B activity in neurons using Magic Red

Protocol materials

Magic Red substrate **Bio-Rad Laboratories Catalog #**ICT937

Plate 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

(DIV) 0

Preparing reagents

2 Reconstitute Magic Red substrate upon arrival in 50 µL of DMSO

Magic Red substrate **Bio-Rad Laboratories Catalog #**ICT937

3 On the day of imaging prepare 1:10 fresh dilution of reconstituted Magic Red solution in water

4 On the day of imaging equilibrate 4 ml of fresh maintenance media (MM) and 4 ml of imaging media (IM) per imaging dish in an incubator

Note

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

Imaging media composition can be obtained from:
dx.doi.org/10.17504/protocols.io.36wgqqkz3gk5/v1

Imaging steps

15m

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

6 Add 2 mL of fresh MM and then add 5 µL of the diluted Magic Red solution (prepared in step 3)

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

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

9 Add 2 mL of fresh IM and then add 5 µL of the diluted Magic Red solution (prepared in step 3)

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