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# Functional assays via live–cell imaging

In 1 collection

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**We use this protocol and it's working**

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## Abstract

Functional assays via live–cell imaging

## Functional assays via live–cell imaging

1 Incubate cells with specific fluorescent dyes tailored to each desired measurement for 30 minutes at 37°C.

2 Wash cells twice with PBS

3 Conduct imaging using a Leica TCS SP8 confocal microscope equipped with a 63×/1.4 numerical aperture oil immersion objective

4 Perform all measurements and analyses on data obtained from over 40 cells across three independent experiments

## For mitochondrial function assessment

5 Use 100 nM tetramethylrhodamine methyl ester perchlorate (TMRM) and 100 nM MitoTracker Green (Invitrogen)

6 Use an excitation wavelength of 568 nm for TMRM and 488 nm for MitoTracker Green

7 Measure emitted fluorescence at wavelengths shorter than 574 nm for TMRM and between 490-530 nm for MitoTracker Green

8 Quantify TMRM intensity in mitochondria stained with MitoTracker Green using Fiji (version 2.7.0, RRID: SCR_002285

9 Set basal TMRM fluorescence intensity at 100% and determine background fluorescence after mitochondrial depolarization with FCCP. Subtract the background value from the basal value for corrected intensity

## For calcium measurements

10 Incubate iPSC-derived neurons and astrocytes with 1 µM Fluo-4 AM (Invitrogen)

11 Use an excitation wavelength of 488 nm and emission range of 490-530 nm for Fluo-4 AM

12 Acquire images every 25 seconds

13 Stimulate neurons with 0.6 mM KCl and astrocytes with 1 mM glutamate for 15 minutes following a 1-minute equilibration period to establish baseline fluorescence intensity

## For oxidative stress evaluation

14 Incubate cells with 1 µM DCF (Invitrogen)

15 Use an excitation wavelength of 488 nm and emission range of 490-530 nm

16 Measure fluorescence intensity using Fiji (version 2.7.0, RRID: SCR_002285)

17 Normalize fluorescence intensity values to baseline values.