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Lysosomal and mitochondrial functional assays in human neurons

In 2 collections

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Ching-Chieh Chou[1], Judith Frydman[1]

[1]Stanford University

Ching-Chieh Chou
Stanford University

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Abstract

Cellular assays to test lysosomal and mitochondrial functions in human neurons at basal and stressful conditions.

Lysosomal acidification

1 Dextran, Fluorescein, 40,000 MW, Anionic, Lysine Fixable (Thermo Fisher Scientific)
Dextran, Tetramethylrhodamine, 70,000 MW, Lysine Fixable (Thermo Fisher Scientific)

2 Conjugated Dextrans are reconstituted at stock concentration of 25 mg/ml in 1 ml H_2O and stored at -20°C.

3 Human tNeurons are seeded at 5 × 10^4 per well of a 24-well plate or 5 ×10^3 per well of a 96-well plate.

4 Cells are incubated with conjugated Dextrans at 0.5 mg/mL for 4 hr at 37°C.

5 Remove medium and rinse with PBS once.

6 Add fresh culture media and chase for additional 20 hr to accumulate Dextran in late endosomes and lysosomes.

7 Cells are treated with DMSO or any pharmacological perturbations.

8 Cells are fixed by 4% PFA for 15 min and prepared for imaging by the confocal microscope or CLARIOstar plate reader.

Lysosomal degradation/proteolysis

9 Magic Red Cathepsin-B substrate, MR-(RR)2, (ImmunoChemistry, #938).

10 Magic Red Cathepsin-B substrate is reconstituted in DMSO and stored at -20°C.

11 When cells are available for experiments, Magic Red Cathepsin-B substrate is diluted with H_2O at
1:10 ratio and added to cell culture medium at a dilution of 1:25 to form 1X staining solution.

12 Human tNeurons are incubated with Magic Red Cathepsin-B substrate for 30 min at 37°C.

13 To test toxic effects on lysosomal degradation revealed by Cathepsin-B activity, cells are treated with DMSO or any pharmacological perturbations.

14 Remove the media and rinse twice with PBS.

15 Cells are fixed by 4% PFA for 15 min and prepared for imaging by the confocal microscope or CLARIOstar plate reader

Lysosomal calcium

16 Cal-520-Dextran Conjugates at 3 kDa (AAT Bioquest).

17 Cal-520 is reconstituted in DMSO, aliquoted into single-use volumes and stored at -20°C.

18 Human tNeurons are plated on sterile multi-chamber glass bottom slides for 5 weeks and incubated with 5 µM Cal-520 and 0.1 µM LysoTracker Red DND-99 (Thermo Fisher Scientific) for 2 hr at 37°C.

19 After washing off excess dye by PBS, cells are treated with DMSO or any pharmacological perturbations.

20 Cells are prepared for live-cell imaging by the confocal microscope in a 37°C incubation chamber with 5% CO_2.

Mitochondrial membrane potential

21 Tetramethylrhodamine ethyl ester (TMRE) reagent (Abcam) accumulates in functional and
polarized mitochondria according to Δψm.

22 The TMRE reagent is reconstituted in DMSO for a stock solution at 1 mM and stored at -20°C.

23 When tNeurons are in culture for 5 weeks in a 96-well plate, the cells are pre-treated with DMSO, 50 µM FCCP or 0.25 mM LLOME for 10 min.

24 Then TMRE reagent is added to fresh cell culture medium at a dilution of 1:1000 along with FCCP or LLOME. Half of the old culture medium is replaced with the TMRE-containing medium in order to incubate the cells with TMRE at a final concentration of 500 nM for 30 min at 37°C.

25 Cells are rinsed with pre-warmed 0.2% bovine serum albumin (BSA)/PBS twice and positioned in the CLARIOstar plate reader for fluorescence measurements.