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Live-Imaging of GCase Activity in iPSC-Derived Dopaminergic Neurons Using the Lyso-FQ Probe

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

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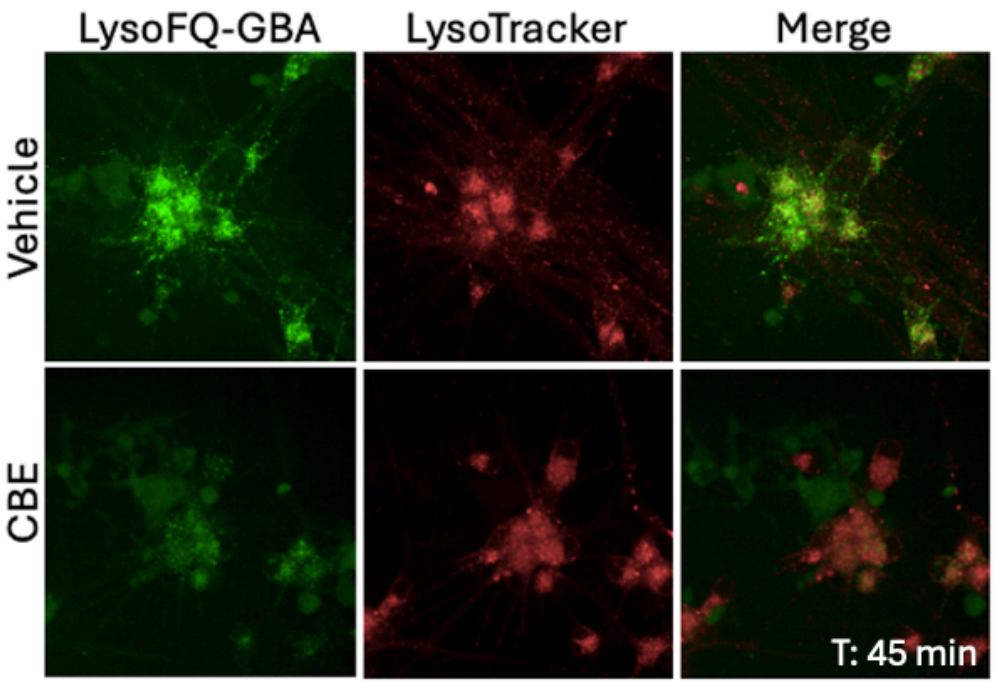
Abstract

This protocol describes the procedure for assessing GCase activity in iPSC-derived dopaminergic neurons using the Lyso-FQ GCase substrate (Biolegend 421948). The assessment is performed via live-cell imaging with the Opera Phenix high-content imaging system and analyzed using Harmony software.

This LysoFQ-GBA (Lysosomal GCase Probe Green) probe, a cell-permeable reporter for GCase activity, fluoresces upon glucocerebrosidase activation, facilitating the detection and monitoring of GBA1 activity.



Materials

Reagents	Company	Cat. Number
LysoTracker Deep Red	ThermoFisher Scientific	L12492
Lyso-FQ	Biolegend	421948
FluoroBrite DMEM	ThermoFisher Scientific	A1896701
CBE (Conduritol B epoxide)	Millipore Sigma	C5424
DMSO	FisherScientific	BP231-100

Safety warnings

Be extremely gentle when dispensing into the wells, as dopaminergic neurons are prone to detaching with forceful pipetting

CBE treatment

16h

1 For the negative control, incubate neurons overnight at 37 °C with [M] 25 micromolar (µM) of the GCase inhibitor Conduritol B epoxide (CBE) 16h

Labeling lysosomes with LysoTracker Deep Red

30m

2 Prepare a LysoTracker Deep Red working solution at [M] 50 nanomolar (nM) in media. Remove the existing media from the wells and add 60 µL of the LysoTracker solution. Incubate for 00:30:00 at 37 °C . 30m

Preparing LysoFQ stock solution

3 While the cells are incubating, prepare the Lyso-FQ stock solution (Biolegend 421948). Reconstitute one 500 µg vial of Lyso-FQ in 77.3 µL of DMSO for a [M] 5 millimolar (mM) stock, then aliquot into 3 µL single-use stock vials. Store aliquots at -80 °C, protected from light, and avoid repeated freeze-thaw cycles.

Addition of Lyso-FQ

4 1. Prepare the Lyso-FQ working solution by diluting the stock 1:1000 in FluoroBrite DMEM.
2. Carefully remove the LysoTracker Deep Red solution from the wells and gently add 50 µL of the Lyso-FQ working solution to each well.
3. Proceed with imaging

Imaging using the Opera Phenix high-content imaging system

2h 30m

5 1. Insert the prepared plate into the Opera Phenix chamber.
2. Utilize a 40× water immersion objective. Capture images using the 488 nm and 647 nm laser channels. Acquire 15-18 fields of view per well every 00:15:00 for a total duration of 02:30:00 . Maintain the plate under standard cell culture conditions (37 °C, 5% CO_2) throughout the imaging session. 2h 30m

Image Analysis Workflow in Harmony Software

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1. **Load Images**
   Open the Data Analysis tab in the Harmony software and load the desired image dataset.
2. Create an Analysis Sequence
3. **Identify Cells**
   Use the **Alexa 647** channel with the **P method** to detect cells. Parameters: Area > 100 µm², Splitting Sensitivity: 0.70, Common Threshold: 0.55). Save this population as "**Cells**".
4. **Select Cell Population**
   Apply Common Filters to the "Cells" population to refine it, creating a new population named "**Cells Selected**".
5. **Calculate Morphological Properties**
   For the "**Cells Selected**" population, use the Standard Method to calculate the **Area**.
6. **Calculate Intensity Properties**

- For the **Alexa 647** channel, use the Standard Method to calculate the **mean intensity** for the "Cells Selected" population.
- For the **Alexa 488** channel, use the Standard Method to calculate the **mean intensity** for the "Cells Selected" population.

7. **Define Results Output**

- Output: **Cells Selected** - **Intensity Cell Alexa 647 Mean**
- Output: **Cells Selected** - **Intensity Cell Alexa 488 Mean**

8. **Run the Analysis**
9. **Export Data**
   Download the results as a **CSV file**.

Assess GCase activity

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- From the table generated by Harmony, calculate the ratio of the PFB-FDGlu mean fluorescence intensity to the LysoTracker Deep Red (647) baseline reading (at t=0) for each time point.
- Enter these calculated values into Prism to generate the GCase activity curves.