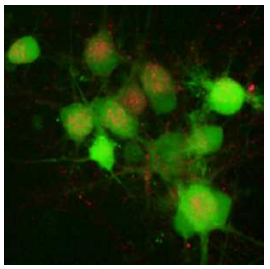
May 28, 2025

Live-Imaging of GCase Activity in iPSC-Derived Dopaminergic Neurons Using the PFB-FDGlu Probe



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We use this protocol and it's working

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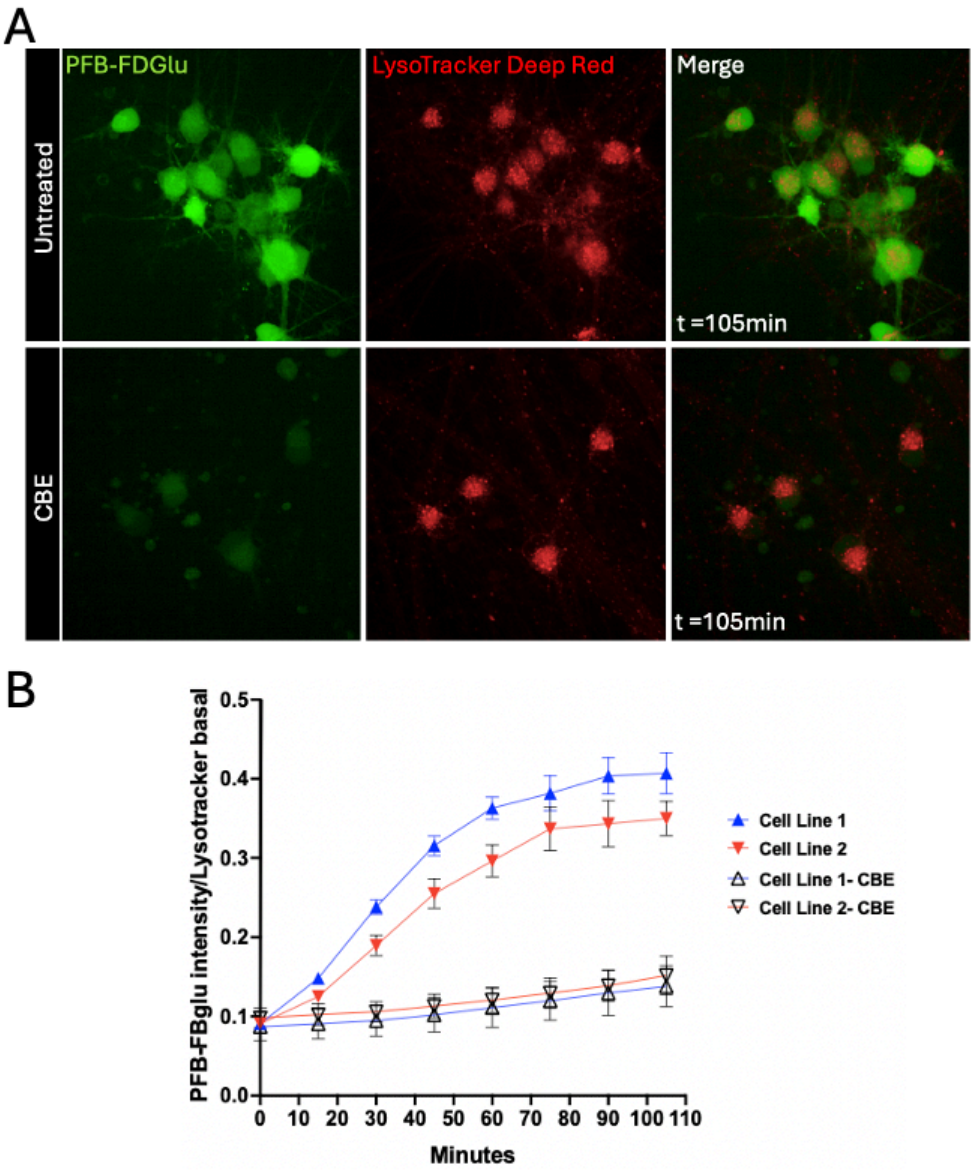
Abstract

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

PFB-FDGlu is a cell-permeable compound that localizes to endosomal and lysosomal compartments upon cellular uptake, without compromising cell viability. When cleaved by active GCase enzymes, it releases a green-fluorescent dye (PFB-F), enabling specific, single-cell analysis of GCase activity through fluorescence detection.



(A) Representative images of Cell Line 1 from the GCase activity assay in CBE-treated and untreated iPSC-derived dopaminergic neurons differentiated for 6 weeks. **(B)** Corresponding GCase activity curves.

Materials

Reagents	Company	Cat. Number
LysoTracker Deep Red	ThermoFisher Scientific	L12492
PFB-FDGlu (5-(Pentafluorobenzoylamino)Fluorescein Di-β-D-Glucopyranoside)	ThermoFisher Scientific	P11947
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

Before start

iPSC-derived dopaminergic neurons are cultured on 96-well plates for up to 4 to 6 weeks of differentiation. We use black Costar 96-well assay plates (Corning cat. no. 3904), which are compatible with imaging using the Opera Phenix high-content screening system.

One hour prior to imaging, turn on the Opera Phenix high-content screening system. Set the incubation chamber temperature to 37 °C and adjust the CO_2 concentration to 5%.

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 GCase substrate stock solution

3 While the cells are incubating, prepare the GCase substrate (PFB-FDGlu) stock solution. Reconstitute one 5 mg vial of PFB-FDGlu in 154 µL of DMSO for a [M] 37.5 millimolar (mM) stock, then aliquot into 10 µL single-use stock vials. Store aliquots at -20 °C , protected from light, and avoid repeated freeze-thaw cycles.

Addition of the GCase substrate

4 1. Prepare the PFB-FDGlu working solution by diluting the stock 1:200 in FluoroBrite DMEM.
2. Carefully remove the LysoTracker Deep Red solution from the wells and gently add 50 µL of the PFB-FDGlu 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 2h 30m

total duration of 02:30:00 . Maintain the plate under standard cell culture conditions (37 °C , 5% CO_2) throughout the imaging session.

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

7

- 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.