Measurement of GCase activity in lysosomes in live cells

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We use this protocol and it's working

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ABSTRACT

Assay uses the substrate PFB-FDGluc (5-(Pentafluorobenzoylamino)Fluorescein Di-β-D-Glucopyranoside)from
ThermoScientific (Cat# P11947). Substrate is supposed to be taken up by late endosomes and lysosomes only and fluoresces green when cleaved by lysosomal GCase.
Described by Mazzulli et al., J
Neurosci. 2016 Jul
20;36(29):7693-706.

MATERIALS

PFB-FDGlu (5-(Pentafluorobenzoylamino)Fluorescein Di-β-D-Glucopyranoside)
Catalog number: P11947

Opti-MEM™ I Reduced Serum Medium
Catalog number: 31985062

1 Resuspend 5mg of substrate in 100 ul methanol to get 50mg/mL stock. Do not expose to light. Store at -20C

2 Culture cells in 48 well plate. Should be over 70% confluent to ensure a robust signal. If too confluent activity will plateau rapidly. 1d

3 For control wells add 10uM CBE or 100uM Bafilomycin overnight.

4 Wash cells once carefully with prewarmed PBS. 5m

5 Working substrate solution is 400 mg/ml (I don't get signal with lower concentrations). Prepare in OPTIMEM prewarmed to 37 °C: 125 ml/well. Therefore 1 ul of 50mg/ml stock substrate per 125 ml.

6 Add 125ul substrate (400ug/ml substrate (400 mg/ml in OPTIMEM) per well. Put in 37 °C incubator for 1 h. 1h

7 Aspirate substrate. Wash cells three times with 250 ml PBS (37 °C). 10m

8 ADD 125UL ml OPTIMEM (37 °C). Measure t=0 minutes on plate reader: Ex, 488nm, Em, 520 nm. Return cells to incubator.

9 Measure fluorescence every 20-30 minutes in green channel (excitation/emission maxima ~492/516 nm) for up to 3 hours. Keep checking cells are alive with microscope. 3h

10 Optional: At end of experiment carefully aspirate OPTIMEM. Lyse cells in wells with 200 ml 1% TX-100 in PBS on ice for 15 minutes. Pellet debris at 17,000 xg, 10 minutes, 4°C. Measure protein concentration in supernatant with BCA assay.

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