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GCase activity assay

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We use this protocol and it's working

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Abstract

This protocol describes a commonly used approach to measure GCase activity by utilising blue fluorogenic substrate 4-methylumbelliferyl-β-D-glucopyranoside (4-MUG). In order to simulate the lysosome's low pH, this method uses GCase protein that has been isolated from a cellular model and diluted in an acidic enzyme assay buffer. The inclusion of a lipid or detergent to keep the enzyme in an active confirmation is a crucial part of the assay. Taurocholate, a regularly utilised bile salt, is used in this protocol.

Materials

Assay Buffer:

	A	B
	Citric acid	50 mM
	K2HPO4	176 mM
	Sodium Taurocholate (Sigma Aldrich REF 86339-5G)	10 mM
	Tween 20 (Sigma Aldrich REF P9416-50ML)	0.01%
	dH2O	

NOTE: The pH should adjusted down to make it closer to pH 5.8

Complete Lysis Buffer:

	A	B
	Cold Assay Buffer	10 ml
	20% Triton X (Sigma Aldrich‰ PCode: 1002733102)	100 µl
	Protease Cocktail Inhibitor (Thermob Scientific REF A32965)	100 µl

STOP solution:

	NaOH	0.5 M
	Glycine	0.5 M

NOTE: The solution need to be adjusted to 1000ml with distilled H2O (ie addition of 650ml) and the solution also needs to be adjusted to a pH of 10 approximately. The solution could be stored at room temperature.

Substrate:

[M] 1 millimolar (mM) 4-methylumbelliferyl β-D glucopyranosidase (4-MUG) (Sigma Aldrich® REF M3633-250MG)

Note

Dilute 4MUG in Assay Buffer.

Sodium taurocholate hydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**86339

TWEEN® 20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P9416

Pierce™ Protease Inhibitor Tablets, EDTA-free **Thermo Fisher Catalog #**A32965

4-Methylumbelliferyl β-D-glucopyranoside **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M3633

Phosphate buffered saline (PBS) without Ca/Mg **Thermo Fisher Scientific Catalog #**14190144

GCase activity assay

1d 1h 20m

1 Seed the total of $3*10^4$ cells in each well of a 96-well plate (Corning® Assay Plate REF 3340) and left to incubate for 24:00:00 . 1d

2 Remove the cell medium and wash each well 2 times with 1X PBS (Gibco® REF 14190-144) at Room temperature

3 Lyse the cells using 50 µL cold Complete Lysis Buffer for 00:10:00 at 4 °C in a shaker. 10m

4 Afterwards, wash the cells two times in 200 µL of sterile 1X PBS.

5 Subsequently, supplement 50 µL of Assay Buffer with [M] 1 millimolar (mM) 4-MUG that are added to each well and left the samples to incubate Overnight at Room temperature and protected from light. 10m

6 Stop the reaction by adding 100 µL of STOP solution to each well, and then is left to incubate for 01:00:00 at Room temperature . 1h

7 Analyse the plates using CytationTM® plate reader using endpoint protocol with wavelength ex/em set up at 360/460nm.

8 Normalise the obtained data to control and expresses as a fraction.