# In vitro GCase activity assay (total cell lysate)

In 8 collections

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MAR 17, 2023

OPEN ACCESS

**DOI:**
dx.doi.org/10.17504/protocols.io.b4dvqs66

**Protocol Citation:** Federico Bertoli, Michela Deleidi 2023. In vitro GCase activity assay (total cell lysate). **protocols.io** https://dx.doi.org/10.17504/protocols.io.b4dvqs66

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

**Created:** Jan 27, 2022

**Last Modified:** Mar 17, 2023

**PROTOCOL integer ID:** 57493

**Keywords:** In vitro GCase activity assay, total cell lysate

## ABSTRACT

Glucocerebrosidase is a lysosomal enzyme that catalyzes the hydrolysis of glucosylceramide (GlcCer), a membrane glyco-sphingolipid, to ceramide and glucose. This assay detects GBA activity by using a fluorogenic substrate that reacts with cell lysates previously treated with or without CBE (GBA1 inhibitor).

## ATTACHMENTS

ggmvbqjbx.pdf

## MATERIALS

### Reagents

- 4-Methylumbelliferyl β-D-glucopyranoside **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M3633**
- Conduritol-b-epoxide **Merck Millipore (EMD Millipore) Catalog #234599**
- AMP-Deoxynojirimycin (CAS 216758-20-2) **Contributed by users Catalog #sc-223780**

- **1%Triton Base Buffer:**

| | A | B | C |
|---|---|---|---|
| | 1% Triton Base Buffer | Final concentration | Amount |
| | Triton X-100 | 1% | 0.5 mL |
| | 5 M NaCl | 150 mM | 1.5 mL |

| | A | B | C |
|---|---|---|---|
| | 1 M HEPES pH 7.4 | 20 mM | 1 mL |
| | 0.5 M EDTA | 1 mM | 100 µL |
| | 1 M MgCl2 | 1.5 mM | 75 µL |
| | 100% glycerol | 10% | 5 mL |
| | Milli-Q H2O | n/a | 41.825 mL |

- **1% Triton extraction buffer:**

| | A | B | C |
|---|---|---|---|
| | 1% Triton Extraction Buffer | Final concentration | Amount |
| | 1% Triton Base Buffer | n/a | 4.425 mL |
| | PIC | n/a | ½ tablet |
| | 500 mM NaF | 50 mM | 500 µL |
| | 200 mM Na3VO4 | 2 mM | 50 µL |
| | 0.1 M PMSF | 0.5 mM | 25 µL |

- **McIlvaine Buffer:**

| | A | B | C |
|---|---|---|---|
| | pH | 0.2 M NaHPO4 (mL) | 0.1 M citric acid (mL) |
| | 6.0 | 12.63 | 7.37 |

## Sample Lysis

1 Suspend samples in 50 µL of 1% Triton extraction buffer.

2 Homogenize with a Dounce homogenizer for 25 strokes.

3 Rotate samples for 00:30:00 at 4 °C. 30m

4 Centrifuge at 13500 x g, 4 °C for 00:15:00. 15m

5 Collect supernatants.

## Substrate preparation

6 Add 20.30 mg 4-Methylumbelliferyl-β-D-glucopyranoside for 10 mL dd$H_2O$ of substrate (6 millimolar (mM)).

7 Incubate at 55 °C and vortex every 00:05:00 until dissolved (approx. 00:30:00). 35m

8 Store at 4 °C until needed.

## Sample preparation

9

Add the equivalent of 10 µg total protein in dd$H_2O$ to reach a final 45 µL volume.

> **Note**
>
> For each sample

10

Add to each 25 µL McIlave Buffer pH 6 and mix it.

> **Note**
>
> For GBA2 inhibition, [M] 5 nanomolar (nM) AMP-Deoxynojirimycin

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Divide the overall 70 µL volume into two tubes ( 35 µL each).

11.1

Incubate one tube with 5 µL CBE [M] 1 millimolar (mM) at Room temperature for 00:30:00 . 30m

11.2

Incubate the other one with 5 µL dd$H_2O$ at Room temperature for 00:30:00 . 30m

## Enzymatic reaction

12 Add 25 µL substrate to each reaction tube.

13 Incubate at 37 °C for 02:00:00 . 2h

## Measurement

14 Take 10 µL of each reaction tube into a 96-well plate (in triplicate).

15 Add 90 µL [M] 0.2 Molarity (M) glycine pH 10.2 to each well to stop the reaction.

16 Measure fluorescence: Excitation 355nm, Emission 460nm.

**Note**

GBA1 activity is obtained by subtracting the background and GBA2 activity from the total GCase activity.