




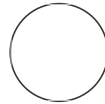
MAR 30, 2023

# In vitro GCase activity assay (total cell lysate)

 In 1 collection

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## 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

## OPEN ACCESS

**DOI:**  
[dx.doi.org/10.17504/protocols.io.261ge3767l47/v1](https://dx.doi.org/10.17504/protocols.io.261ge3767l47/v1)

**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.261ge3767l47/v1>

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

**Created:** Mar 28, 2023

**Last Modified:** Mar 30, 2023

**PROTOCOL integer ID:**  
 79530

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

A	B	C
1 M HEPES pH 7.4	20 mM	1 mL
0.5 M EDTA	1 mM	100 µL
1 M MgCl <sub>2</sub>	1.5 mM	75 µL
100% glycerol	10%	5 mL
Milli-Q H <sub>2</sub> O	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 Na <sub>3</sub> VO <sub>4</sub>	2 mM	50 µL
0.1 M PMSF	0.5 mM	25 µL

■ **Mcllvaine Buffer:**

A	B	C
pH	0.2 M NaHPO <sub>4</sub> (mL)	0.1 M citric acid (mL)
6.0	12.63	7.37

## Sample Lysis


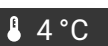
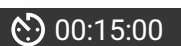
1 Suspend samples in  50  $\mu\text{L}$  of 1% Triton extraction buffer.



2 Homogenize with a Dounce homogenizer for 25 strokes.

3 Rotate samples for  00:30:00 at  4  $^{\circ}\text{C}$  .

30m

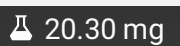


4 Centrifuge at  13500 x g ,  4  $^{\circ}\text{C}$  for  00:15:00 .

15m



5 Collect supernatants.

## Substrate preparation

6 Add  20.30 mg 4-Methylumbelliferyl- $\beta$ -D-glucopyranoside for  10 mL ddH<sub>2</sub>O of substrate ( 6 millimolar (mM) ).



7 Incubate at  55  $^{\circ}\text{C}$  and vortex every  00:05:00 until dissolved (approx.  00:30:00 ).

35m



8 Store at  4  $^{\circ}\text{C}$  until needed.

## Sample preparation

9



Add the equivalent of  $10\ \mu\text{g}$  total protein in ddH<sub>2</sub>O to reach a final  $45\ \mu\text{L}$  volume.

### Note

For each sample

10



Add to each  $25\ \mu\text{L}$  Mcllave Buffer  $\text{pH } 6$  and mix it.

### Note

For GBA2 inhibition,  $5\ \text{nM}$  AMP-Deoxynojirimycin

11



Divide the overall  $70\ \mu\text{L}$  volume into two tubes ( $35\ \mu\text{L}$  each).

11.1



Incubate one tube with  $5\ \mu\text{L}$  CBE  $1\ \text{mM}$  at Room temperature for  $00:30:00$ .

30m


11.2



Incubate the other one with  $5\ \mu\text{L}$  ddH<sub>2</sub>O at Room temperature for  $00:30:00$ .

30m

## Enzymatic reaction

12 Add  25  $\mu\text{L}$  substrate to each reaction tube.



13 Incubate at  37 °C for  02:00:00 .



2h

## Measurement

14 Take  10  $\mu\text{L}$  of each reaction tube into a 96-well plate (in triplicate).



15 Add  90  $\mu\text{L}$   0.2 Molarity (M) glycine  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.