




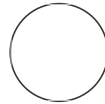
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In vitro GCase activity assay (total cell lysate)

 In 8 collections

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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

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

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57493

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 ₂	1.5 mM	75 µL
100% glycerol	10%	5 mL
Milli-Q H ₂ 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 ₃ VO ₄	2 mM	50 µL
0.1 M PMSF	0.5 mM	25 µL

■ **Mcllvaine Buffer:**

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

Sample Lysis

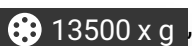
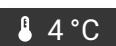
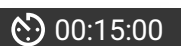
- 1 Suspend samples in  50 μL of 1% Triton extraction buffer.



- 2 Homogenize with a Dounce homogenizer for 25 strokes.

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

30m

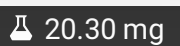


- 4 Centrifuge at  13500 x g,  4 $^{\circ}\text{C}$ for  00:15:00.



15m

- 5 Collect supernatants.

Substrate preparation

- 6 Add  20.30 mg 4-Methylumbelliferyl- β -D-glucopyranoside for  10 mL ddH₂O 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 ddH₂O 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 $5\ \mu\text{L}$ ddH₂O 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  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.