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In vitro GCase activity assay (total cell lysate)

In 2 collections

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

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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

PDF

ggmvbqjbx.pdf

615KB

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) **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
	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

	A	B	C
	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

Troubleshooting

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 ddH_2O of substrate ([M] 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 ddH_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

11 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 ddH_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.