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).

Reagents

- 4-Methylumbelliferyl β-D-glucopyranoside, Glycosynth/Sigma-Aldrich, Cat. #44059/ 18997-57-4
- Conduritol-b-epoxide, EMD Millipore Cat. #234599
- AMP-Deoxynojirimycin, SCB Cat #216758-20-2

- 1%Triton Base Buffer:

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:

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_3VO_4	2 mM	50 µL
0.1 M PMSF	0.5 mM	25 µL

- McIlvaine Buffer

pH	**0.2 M NaHPO4 (mL)**	**0.1 M citric acid (mL)**
6.0	12.63	7.37

Procedure

Step 0 : Sample Lysis

- Suspend samples in 50µl of 1% Triton extraction buffer
- Homogenize with a Dounce homogenizer for 25 strokes.
- Rotate samples for 30’ at 4°C
- Centrifuge at 13500 g, 4°C for 15’
- Collect supernatants

Step 1 : Substrate preparation

- Add 20.30mg 4-Methylumbelliferyl-β-D-glucopyranoside for 10mL ddH2O of substrate (6mM)
- Incubate at 55°C and vortex every 5’ until dissolved (approx. 30’ min)
- Store at 4°C until needed

Step 2 : Sample preparation

(for each sample)

- Add the equivalent of 10µg total protein in ddH2O to reach a final 45µl volume.
- Add to each 25 µl McIlave Buffer pH 6.0 and mix it.
  (for GBA2 inhibition, 5nM AMP-Deoxynojirimycin)
- Divide the overall 70 µl volume into two tubes (35 µl each).
  - Incubate one tube with 5ul CBE 1mM at RT for 30’.
  - Incubate the other one with 5ul ddH2O at RT for 30’.

Step 3 : Enzimatic reaction

- Add 25uL substrate to each reaction tube
- Incubate at 37 °C for 2h

Step 4: Measurement

- Take 10uL of each reaction tube into a 96-well plate (in triplicate)
- Add 90µL 0.2M glycine pH 10.2 to each well to stop the reaction
- Measure fluorescence: Excitation 355nm, Emission 460nm

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

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