In-vitro GCase Activity Assay

Tae-Un Han[1], sidranse[1]

[1]Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, USA.

Tae-Un Han

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ABSTRACT

This is version 2 of in-vitro GCase activity assay.
We optimized the assay condition to enhance sensitivity and specificity.
Both cell lysates and protein lysates prepared in M-buffer containing protease inhibitor and 0.25% TritonX can use this protocol.

ATTACHMENTS

GCase Activity Assay.pdf

MATERIALS

- Cell lysates and protein lysates prepared in M-buffer containing protease inhibitor and 0.25% TritonX
- 0.2M Na_2HPO_4
- 0.1M citrate
- protease inhibitor tablet (Roche cOmplet mini)
- Triton X-100 solution
- 384 well plate (flat bottom, black)
- 10 mM CBE (5 mg CBE in 3086 ul DMSO)
- DMSO
- aluminum foil
- sodium taurocholate powder
- 1 M 4-Methylumbelliferyl-B-D-glucoside (4-MU-G, 338 mg per 1ml DMF)
- 5 M NaOH
- glycine
- microplate reader

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

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1 Mix 20 mL 0.2M Na2HPO4 (0.852 g in 30 mL water) with 14 mL 0.1M citrate (0.576 g in 30 mL water) to make M-buffer (pH 5.4 , no additional pH adjustment required).

2 Dissolve one protease inhibitor tablet (Roche cOmplet mini) in 10 mL M-buffer , then add Triton X-100 solution to [M] 0.25 % (v/v) (e.g. 25 µL in 10 mL) and [M] 0.2 Mass / % volume of sodium taurocholate to make active GCase buffer.

3 Set up the desired plate layout in a 384 well plate (flat bottom, black). There should be two sections, as each sample must be prepared and assayed both with and without CBE (CBE: GCase1 inhibitor).

4 Prepare [M] 0.8 millimolar (mM) CBE by diluting [M] 10 millimolar (mM) CBE in DMSO with the GCase buffer (GCase buffer:CBE = 92:8).

5 Prepare CBE-free carrier solution in the same volume of GCase buffer/DMSO (GCase buffer:DMSO = 92:8).

6 Pipette 10 µL protein lysate diluted with GCase buffer into wells of a 384- well plate. Four replication sets should be run (sample concentration: [M] 0.7 mg/mL ~ [M] 1.2 mg/mL). Protein concentration should be adjusted to be similar between control and experiment groups using GCase buffer.

7 Add 5 µL 0.8 mM CBE solution to the CBE-positive wells or the same volume of CBE-free carrier solution to the CBE-negative wells.

Cover the plate with aluminum foil and briefly centrifuge. Incubate shaking:

600 rpm, 37°C, 00:15:00 .

9 During incubation, prepare 4-Methylumbelliferyl-B-D-glucoside (4-MU) diluent by diluting 1 M 4-Mu (338 mg per 1ml DMF) with GCase buffer to a final concentration of [M] 2.5 millimolar (mM) (1:400 dilution).

10 After the CBE incubation, spin down the plate and add 15 µL assay buffer with 2.5 mM 4-MU to reach a total volume of 30 µL in each well.

11 Cover the plate with aluminum and briefly centrifuge, and Incubate shaking:

450 rpm, 37°C, 01:00:00 .

12 Prepare stop solution by adding 4 mL 5M NaOH and 1.877 g glycine up to 25 mL in water. Final concentration of glycine is [M] 1 Molarity (M) (pH 10.5)

13 After incubation, spin down the plate again and Add 30 µL stop solution to each well.

14 Read the 4-MU fluorescence with a microplate reader (Excitation: 365 nm; Emission: 449 nm; Cutoff: 435nm; 3 reads/well).

15 GCase activity in each protein lysate can be calculated as below.

$$\frac{fluorescence\:of\:CBE\:-free\:sample \quad- \quad fluorescence\:of\:sample\:with\:CBE}{protein\:concentration\:(measured\:by\:BCA)}$$