

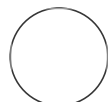


AUG 01, 2023

In-vitro GCase Activity Assay

Tae-Un Han¹, sidranse¹

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



Tae-Un Han

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 Na₂HPO₄
- 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

OPEN  ACCESS



DOI:
dx.doi.org/10.17504/protocols.io.dm6gp3b7dvzp/v1

Protocol Citation: Tae-Un Han, sidranse 2023. In-vitro GCase Activity Assay.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.dm6gp3b7dvzp/v1>




















License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Aug 01, 2023

Last Modified: Aug 01, 2023

Keywords: ASAPCRN,
GCase, GCase activity

- 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 ( 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  0.25 % (v/v) (e.g.  25 µL in  10 mL) and  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  0.8 millimolar (mM) CBE by diluting  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:  0.7 mg/mL ~  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 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 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.

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

