GCase Activity Assay with 4-Mu

1. Mix 20 mL of 0.2M Na_2HPO_4 (0.852 g in 30 ml water) with 14 mL of 0.1M citrate (0.576 g in 30 ml water) and adjust pH to 5.4~5.5 by adding additional volume of 0.1M citrate.
2. Dissolve one protease inhibitor tablet (Roche cOmplet mini) in 10ml M-buffer, then add Triton X-100 solution to 0.25 % (e.g. 25 ul in 10 ml) and 0.2% of sodium taurocholate to make active GCase buffer. The fresh GCase buffer should be prepared on the daily basis.
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 mM CBE by diluting 10 mM CBE in DMSO (5 mg CBE in 3086 ul DMSO) with the GCase buffer (GCase buffer : CBE in DMSO = 92 : 8)
5. Prepare CBE-free carrier solution with the same volume of GCase buffer/DMSO (GCase buffer : DMSO = 92:8) as a CBE negative control.
6. Pipette 10 µL of protein lysate diluted with the GCase buffer (protein concentration: 0.7~1.2 mg/ml) into wells of a 384- well plate. Four replication sets are recommended. Protein concentration should be adjusted to be similar between control and experiment groups using GCase buffer.
7. Add 5 µL of 0.8 mM CBE solution to the CBE-positive wells or the sample volume of CBE-free carrier solution to the CBE-negative wells.
8. Cover the plate with aluminum sealing tape and briefly centrifuge. Incubate for 15 minutes at 37°C shaking at 600 rpm.
9. Prepare 4-Methylumbelliferyl-B-D-glucoside (4-Mu) by diluting 1M 4-MU (338 mg per 1ml DMF) with GCase buffer to a final concentration of 2.5 mM (1:400 dilution).
10. After the CBE incubation, spin down the plate and add 15 µL of assay buffer with 2.5 mM 4-MU to reach a total volume of 30 µL in each well
11. Cover the plate and incubate for 60 min at 37°C, shaking at 450 rpm.
12. Prepare 1M glycine stop solution (pH 10.5) by adding 1.877 g glycine up to 20~21 mL in water and add necessary volume of 5M NaOH (about 4 ml) and water to make total 25 ml solution (pH adjustment is not necessary).
13. After incubation, spin down the plate again and add 30 µL of 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.

$$\frac{fluorescence\ of\ CBE\text{–}free\ sample\ -\ fluorescence\ of\ sample\ with\ CBE}{Protein\ concetration\ (measured\ by\ BCA)}$$