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GCase activity in mouse brain samples

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol is designed for assessing the beta-glucocerebrosidase activity in fresh collected mouse brain samples

Materials

Reagent Buffer (pH 4.10):

Citrate phosphate buffer (0.15M) (*Sigma C0909-500g H2O free MW258,06g/mol*)

ddH₂O

Stopping buffer (pH 10.4):

Glycine buffer (0.25M) (*Roth 3908.2 MW 75,07g/mol*)

ddH₂O

Substrate :buffer:

Sodium taurocholate (*Sigma 86339, 150mM*)

4-Methylumbelliferyl β-D-glucopyranoside (MUG, *Sigma M3633, 10mM*)

ddH₂O

Standard:

4-Methylumbelliferone (*M1381, Sigma*)

Methanol



Samples homogenization

10m

- 1 Homogenize fresh collected mouse brain samples in lysis buffer (50 mM Tris-HCl, pH 7.4 and 750 mM NaCl, 5 mM EDTA and 10% Triton X-100).
- 2 Centrifuge samples at 5000 g ( 00:10:00 at  4 °C)
- 3 Collect the supernatant
- 4 Measure protein concentration with the Pierce BCA Protein Assay Kit (Fisher Scientific, 23225).

10m

Assesment of GCase activity

1h

- 5 Make a serial dilution of 4-Methylumbelliferone (*M1381, Sigma*) dissolved in 1% Methanol ddH₂O
- 6 Put 10µL of each standard dilution and of each sample in a pre-prepared black 1.5 ml tube (in duplicate or triplicate).
- 7 Add 25µL of Reaction Buffer and 65µL of Substrate buffer in each tube
- 8 Incubate  01:00:00 at  37 °C
- 9 Remove samples from the incubator and add 90µL of Stopping buffer to each tube
- 10 Load 150µL of all samples, standards and blank (35µL of Reaction Buffer + 65µL of Substrate buffer + 90µL of Stopping buffer) into a 96 well black plate
- 11 Measure fluorescence using plate reader (Excitation at 365 nm and emission at 450 nm)
- 12 Calculate the amount of 4-Methylumbelliferone generated in each sample based on the standard curve

1h



- 13 Normalize the data obtained based on the amount of protein (mg) that were in each reaction tube