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GCase activity in mouse brain samples

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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*)
ddH_2O

Stopping buffer (pH 10.4):
Glycine buffer (0.25M) (*Roth 3908.2 MW 75,07g/mol*)
ddH_2O

Substrate :buffer:
Sodium taurocholate (*Sigma 86339, 150mM)*
4-Methylumbelliferyl β-D-glucopyranoside (MUG, Sigma M3633, 10mM)
ddH_2O

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) 10m

3 Collect the supernatant

4 Measure protein concentration with the Pierce BCA Protein Assay Kit (Fisher Scientific, 23225).

Assesment of GCase activity

1h

5 Make a serial dilution of 4-Methylumbelliferone (*M1381, Sigma*) dissolved in 1% Methanol dd$H_2$0

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 1h

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

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