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

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Quantifying Acetylcholinesterase activity in striatal brain tissue with DTNB assay

Stefania Vietti-Michelina[1,2,3], Stephanie J Cragg[1,2,3]

[1]Department of Physiology, Anatomy and Genetics, University of Oxford, OX1 3PT, UK;
[2]Oxford Parkinson's Disease Centre, University of Oxford, Oxford, United Kingdom;
[3]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815

sophie.oakley

ABSTRACT

This protocol describes the steps to measure acetylcholinesterase (AChE) activity in mouse striatal brain tissue, including how to store tissue samples, extract AChE and obtain Michaelis-Menten like plots to measure the rate of AChE activity. This protocol can be used to quantify AChE activity across different brain regions or genotypes. Reagents and materials from Abcam kit ab138871 are used. This kit uses DTNB to measure the thiocholine (TCh) resulting from the hydrolysis of the substrate acetylthiocholine (ATCh) by AChE.

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MATERIALS

Reagent

- Ab138871 colorimetric AChE assay kit
- Bovine Serum Albumin Standard Ampules, 2 mg/ml (Thermo Fisher, Catalog #23209,) .
- IGEPAL CA-630 (Sigma-Aldrich)

Equipment:

- VT1200S Vibrating blade microtome (Leica)
- 96 well plates
- PHERAstar FSX microplate reader (BMG Labtech)
- Sonicator
- Refrigerated Centrifuge

Preparation of atrifical cerebral spinalfluid (aCSF):

- NaCl, 127 mM
- NaHCO3, 25 mM
- D-glucose, 25 mM
- KCl, 2.5 mM
- NaH2PO4, 1.25 mM
- MgSO4, 5 mM
- CaCl2, 1.6 mM

Software:

- MARSdata analysis software (BMG Labtech)
- Microsoft Excel

Preparation of *ex vivo* mouse brain slices

1 Harvest dorsal and ventral striatal tissue punches (1.2 mm diameter) were from 300 µm thick acute coronal striatal tissue slices, prepared with a vibratome.

2 Equilibrate slices at room temperature for at least 1-hour prior collection of the punches in bicarbonate-buffer based artificial cerebrospinal fluid (aCSF) (See **Materials.**)

3 Dry single tissue punches and place in 1.5 mL tubes. Snap freeze samples at -80°C and store at -80°C until needed for the assay.

Preparation of the DTNB Assay

4 Thaw all the kit components at RT before starting and prepare stock solutions as instructed in assay kit manual.

> Note
>
> Kit components need to be stored at -20°C

5 After preparing all stock solutions, divide into single use aliquots and store at -20°C

> Note
>
> DTNB stock needs to be stored in the dark. We advise preparing 50 uL aliquots for ATCh and DTNB and 10 uL aliquots for AChE

6 Prepare the AChE standards. From the AChE stock solution (50 U/mL) prepare the 1000 mU/mL solution and then use it to perform serial dilutions of 300, 100, 30, 10, 3, 1, 0.1 mU/mL.

> Note
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> Note that AChE standards are unstable and **need to be used within 4 hours of preparation**.

7 Prepare the lysis buffer, 0.5% IGEPAL CA-630 in 1x PBS.

> Note
>
> This reduces protein denaturation.

8 Prepare the tissue. Incubate each tissue punch in lysis buffer using 200 uL of lysis buffer for each sample. Incubate for 10 minutes on ice.

9 Sonicate for 10 - 15 seconds, 2-3 times with a hand-held sonicator.

10 Centrifuge each sample tube at 650 rcf for 5 minutes at 4°C. Then, collect the supernatant into a new tube ensuring no pellet is transferred.

Preparation of reaction mix

11 Add 20x DTNB stock and 20x ATCh stock into assay buffer.

Note

For obtaining a Michaelis-Menten like plot of AChE activity different levels of substrate (ATCh) can be delivered (for example, 100%-50%-10%-0%).

12 Prepare two different reaction mix, one with the substrate ATCh and one without, with final volumes informed by the number of wells in use.For the ATCh mix add 20x DTNB stock and 20x ATCh stock to assay buffer, while for the substrate-free mix add 20x DTNB stock into assay buffer

DTNB Assay

13 Put 50 uL of AChE standards in the standard wells (300-0-1 mU/mL in duplicates). Add 50 uL of assay buffer in the blank control wells (also in duplicates).

14 Add 50 uL of samples in each sample well (in duplicates).

Note

For endpoint activity add 50 uL of reaction mix to each well.

For the measurement of AChE activity in with differing substrate concentrations add either the ATCh or the substrate-free reaction mix to each appropriate well.

15 Incubate for 15 minutes at RT in the dark.

16 Measure changes in absorbance in a microplate reader at OD=410 nm

Analysis

17 Subtract the blank values and average all duplicates.

18 Calculate AChE activity as mU/mL for each sample from the standard curve.

19 When comparing genotypes or brain regions normalise by tissue volume.