A Veritas™ Microplate Luminometer Method for aCella™ - AChE

1. INTRODUCTION

The Turner BioSystems' Veritas™ Microplate Luminometer in combination with Cell Technology's aCella™ – AChE kit provides a convenient, rapid, sensitive, and simple assay for detecting Acetylcholinesterase activity (AChE). Acetylcholine participates in the transmission of a nerve impulse and must be degraded before the arrival of the next nerve impulse. This critical degradation is performed by AChE. Inhibition of AChE by nerve toxins and pesticides has long been a field of interest for many scientists due to their acute toxicity to humans and animals.[1,2] In addition, AChE inhibitors are also being investigated for their possible function in disease treatments, such as Alzheimer's.[3]

Cell Technology's assay for AChE activity uses acetylcholine, in a series of coupled enzyme reactions, to quickly translate the presence of active AChE into a change in the luminescence of the reaction. The presence of an AChE inhibitor causes an increase in the level of ATP in the sample, which can be readily detected by an increase in luminescence.

Reaction I: AChE + Inhibitor → No Acetate, No Choline

Reaction II: Acetate kinase + ATP → Reaction does not proceed.

Reaction III: ATP(remaining) + Luciferase/Luciferin → LIGHT

The Veritas Microplate Luminometer employs a unique system for light detection to maximize the sensitivity and range of the aCella – AChE assay kit. Samples/inhibitors may be diluted in PBS, reagent-grade DI water or TRIS buffers. Samples may be serially diluted to determine potency of the inhibitor. All tests were conducted using the aCella – AChE assay kit (Cell Technology, Inc. Catalog # CLACHE 100-2).

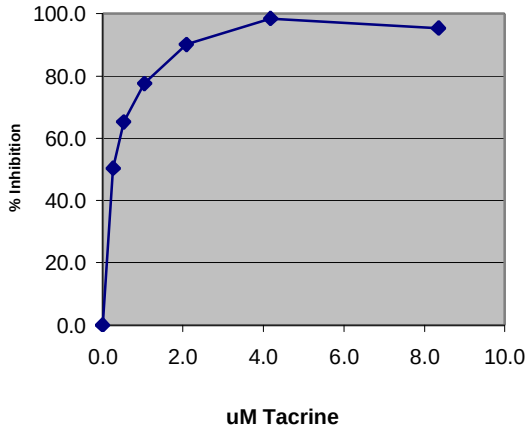

Figure 1. Tacrine (a mixed-mode inhibitor of AChE) was serially diluted in DI water. Next 10 µL of the diluted Tacrine (x axis labeling represents µM final concentration of Tacrine) was added to a white opaque 96 well microplate along with 50 µL of component A (AChE enzyme). The samples were incubated for 5 minutes after which 50 µL of component B was added to all the wells. Data were collected using a luminometer. Data shown represent T=2 minutes after the addition of component B.

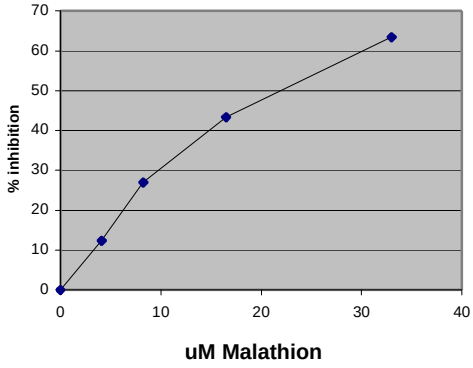

Figure 2. Malathion, a common pesticide, was first diluted in DMSO and subsequently serially diluted in Di water. 10µL of the diluted Malathion (x axis represents µM final concentration of Malathion) was added to a white opaque 96 well microplate followed by 50 µL of component A (AChE enzyme). The mixture was incubated for 15 minutes, after which 50 µL of component B was added to all the wells. Data were collected using a luminometer. Data shown is at T= 5 minutes. Data shown represents T= 2.5 minutes after the addition of component B.

2. MATERIALS REQUIRED

- Veritas Microplate Luminometer (P/N 9100-000)
- 96-well plates, opaque white (E&K Scientific EK-25075)
- Cell Technology aCella-AChE assay kit (Cell Technology, CLACHE 100-2)
- p20, p200 pipette and pipette tips

3. EXPERIMENT PROTOCOL

3.1 Reagent Preparation

Component A: Use as supplied. Contains the AChE enzyme.

Component B: Use as supplied. Contains Detection reagent, acetylcholine and kinase enzymes.

Component C: Use as supplied. Control to measure maximum luminescence.

- Thaw all three components in the dark and let come to room temperature (RT) before starting assay. Aliquot into single-use vials and store at –70°C. Avoid repeated freeze thaw cycles.

3.2 Instrument Setup

3.2.1 Double click on the Veritas icon to start the software.

3.2.2 Click on "Create New Protocol" from the "Welcome to Veritas" dialog box.

3.2.3 Select "0 injectors" when prompted on the Create New Protocol Wizard.

3.2.4 Modify the delay before starting first run, number of runs, or select a delay time between runs.
For “Integration time” select 1 second.

3.2.5 Next, select the wells to be read. You may “Save protocol as” for future use, or select “Finish” to run the protocol without saving.

3.2.6 Enter your information into the "Experiment", "Operator", "Plate No.", and "Notes" fields in the "Main Dialog Box".

3.3 Sample Analysis

3.3.1 Add 10-50 µL of samples or known inhibitors, in triplicate, to each respective well in the 96-well plate.

3.3.2 Controls.

A. For maximum signal control, add 50 µL of component C, in triplicate, to individual wells plus 10 µL of the diluent used in the sample

B. For the No Inhibitor control, add 50 µL of component A, in triplicate, to individual wells plus 10 µL of the diluent used in the sample/inhibitor preparations.

3.3.3. Add 50 µL of component A to each well. You may pre-incubate this mixture at room temperature before proceeding onto step

3.3.4 Add 50 µL of component B to the plate.

3.3.5 **NOTE:** When running the protocol for the first time it is usually desirable to take readings at 30-second intervals to optimize readout of your assay. Once the time dependence of your reaction is established, it is acceptable to use a single time-point for analysis.

To take readings at 10 second intervals on the Veritas, select “Advanced Protocols” from the “Welcome Dialog Box”. Select the Kinetics folder and then the “NoInjectionKinetics” protocol. Then, click on “Options” from the “Main Dialog Box” to select the wells to be read on the “Plate Setup” screen. You can also modify the delay time before measuring, integration time, and data point frequency (1/10s in this case) in the “Other Options” tab. Once you have made your choices, click the “Apply Changes” button to accept changes, or the “Save Protocol as” button to save the protocol.

3.3.6 Insert sample plate into the Veritas™ Microplate Luminometer and click "Start" to begin assay. RLU values measured by the Veritas will appear in the Excel spreadsheet

after all the selected wells in each row have been read. If you encounter an error message, refer to the troubleshooting guide for more information.

Note: Opening another Excel Spreadsheet while the Veritas reads your sample plate is strongly discouraged.

3.3.7 Once the measurements are complete you can access Excel to analyze your data.

3.3.8 Remove sample plate after measurements.

4. REFERENCES

1. HA Berman and MM Decker**.** Kinetic, equilibrium, and spectroscopic studies on dealkylation ("aging") of alkyl organophosphonyl acetylcholinesterase. Electrostatic control of enzyme topography. J. Biol. Chem., Aug 1986; 261: 10646-10652

2. Arie Ordentlich *et al.* The Architecture of Human Acetylcholinesterase Active Center Probed by Interactions with Selected Organophosphate Inhibitors. J. Biol. Chem., May 1996; 271: 11953-11962.

3. Levy R. Tetrahydroaminoacridine and Alzheimer's disease. Lancet, 1987 Feb 7;1(8528):322.

5. ABOUT CELL TECHNOLOGY, INC

aCella™ – AChE is a trademark of Cell Technology, Inc. Orders for Cell Technology's products may be placed by:

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* Special thanks to Cell Technology for the use of their data.

6. ABOUT TURNER BIOSYSTEMS, INC.

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