aCella - AChE™*

Bioluminescence Assay for Monitoring Acetylcholinesterase Activity

*Patent Pending

PROTOCOL

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Introduction

Acetylcholinesterase (AChE) is one of the most important enzymes involved in nerve transmission. The enzyme is bound to cellular membranes of excitable tissue (synaptic junction, endoplasmic reticulum, etc) [1-3]. Acute toxicity to humans and animals through inhibition of AChE by both nerve gases and an important class of pesticides has long been a field of intensive scientific investigation [4,5]. AChE inhibitors have also been used clinically as Alzheimer's treatments (*e.g.*, tacrine (tetrahydroaminoacridine)) [6] and are the subject of increasing interest in various disease processes and treatment strategies [7,8]. However, both environmental detection of AChE inhibitors and development of modulators of AChE enzymatic activity as drugs have been hampered by the difficulty and complexity of the current assay methods.

Assay Principle

We have developed a highly sensitive, very rapid, extremely simple assay for AChE activity, using the natural substrate, acetylcholine. As shown in Figure 1, a series of coupled enzyme reactions quickly translates the presence of active AChE into a change in the luminance of the reaction. First (reaction I), acetylcholine is hydrolyzed by the AChE to yield acetate and choline. The acetate and choline then enter a coupled enzyme reaction (reaction II) that results in consumption of ATP, and finally the ATP concentration is measured by the well-established luciferase method (reaction III). These reactions can occur simultaneously, and the result is generally obtained in five minutes or less. Inhibitors of AChE are readily detected by an increase in luminance due to reduced consumption of ATP.

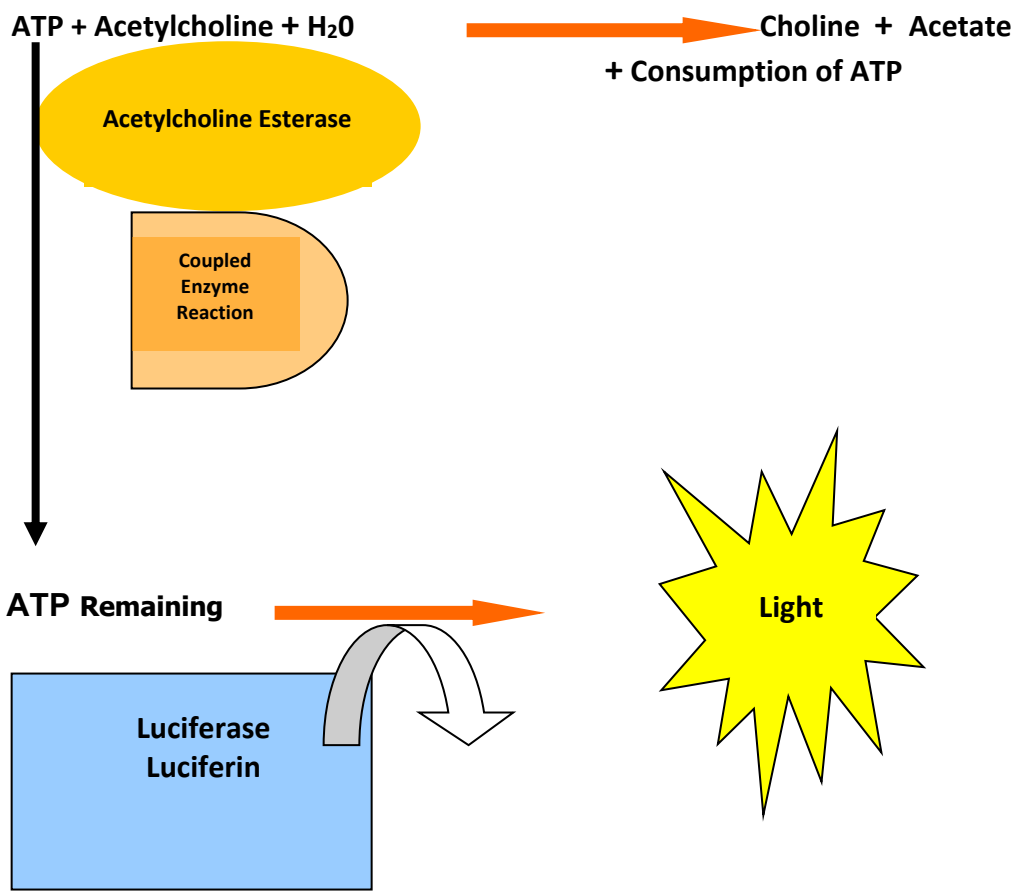
The following reaction illustrates the sequence of events if AChE inhibitors are present:

Reaction I: AChE + Inhibitor ⟶ **No Acetate and Choline.**

Reaction II: Coupled Enzyme Reaction + ATP ⟶ **Reaction does not proceed**

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

Figure 1


ATP + Acetylcholine + H_20
Choline + Acetate
+ Consumption of ATP
Acetylcholine Esterase
Coupled Enzyme Reaction
ATP Remaining
Light
Luciferase
Luciferin

I: Kit Contents:

1. **Component A**: 5.5mL..... Part# 3023
   Contains Acetylcholinesterase
2. **Component B**: 5.5mL.....Part# 3024
   Contains Detection reagent, acetylcholine and coupled enzyme reaction.
3. **Component C**: 5.5mL.....Part# 3025
   Control to measure maximum Luminescence.

II: Materials and Equipment Needed:

1. White 96 well plates for luminometer.
2. Luminometer.
3. Tacrine, Sigma Catalog# A3773 (for inhibition studies)

III: Reagent set up:

1. Component A: Frozen liquid, thaw and aliquot into single-use vials. Freeze at –70^{0}C.
2. **Component B**: Frozen liquid, thaw and aliquot into single-use vials. Freeze at –70^{0}C.
3. **Component C**: Frozen liquid, thaw and aliquot into single-use vials. Freeze at –70^{0}C.
Cautionary Notes:
1. Avoid repeated freeze thaw cycles.

IV: Assay Set-Up

1. Thaw Components A, B and C in the dark and let them come to room temperature before starting assay.

2. Sample preparation: samples/inhibitors maybe diluted in PBS, reagent grade Di-water or TRIS buffers. Samples may be serially diluted to determine potency of inhibitor.

3. Add 10-50□L of samples or known inhibitors in tripilicate to each respective well. Next add 50□□L of component A (contains the the acetylcholinesterase (AChE) enzyme) to these wells. You may pre-incubate the AChE and samples/inhibitors before proceeding to step 4.

4. Controls

A. Control 1: Maximum signal control. This is the maximum signal generated by the ATP in the reaction, which can represent 100% inhibition of AChE. The maximum signal control can be set up by adding 50□L of component C, in triplicate, to individuals wells + 10-50□L of the diluent used in the sample/ inhibitor preparations.

B. Control 2: No inhibitor control. This control measures ATP consumption over time via the fully active AChE reactions. The no-inhibition control can be set up by adding 50□L of component A, in triplicate, to individuals wells + 10-50□L of the diluent used in the sample/ inhibitor preparations. The following table presents an example of a controlled test for AChE-inhibitory activity in two samples:

	1	2	3
A	Sample 1	Sample 1	Sample 1
B	Sample 1 (1:10 dilution)	Sample 1 (1:10 dilution)	Sample 1 (1:10 dilution)
C	Sample 1 (1:20 dilution)	Sample 1 (1:20 dilution)	Sample 1 (1:20 dilution)
D	Sample 2	Sample 2	Sample 2
E	Sample 2 (1:10 dilution)	Sample 2 (1:10 dilution)	Sample 2 (1:10 dilution)
F	Sample 2 (1:20 dilution)	Sample 2 (1:20 dilution)	Sample 2 (1:20 dilution)
G	Control 1	Control 1	Control 1
H	Control 2	Control 2	Control 2

Table 1.

5. Next add 50□L of Component B and read the plate in a luminometer. When first using the protocol it is usually desirable to take several readings at 30-second intervals to optimize the readout of your assay. Once the time dependence of your reaction is established, it is generally possible to take a single time-point for analysis.

6. Calculations

To calculate % inhibition use the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Average RLU for sample/inhibitor}) - (\text{Average control 2}) \times 100}{(\text{Average control 1}) - (\text{Average control 2})}$$

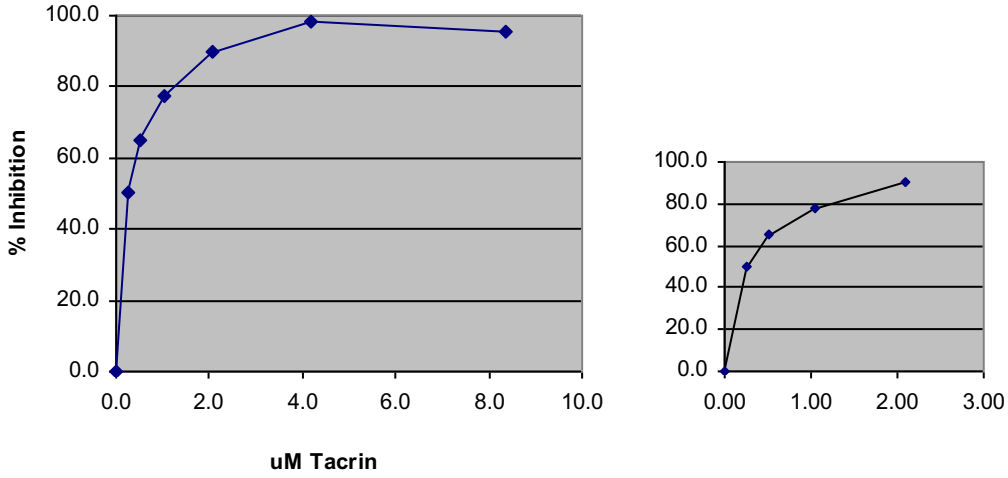


Figure 1. Tacrine (Sigma, catalog # A3773, a mixed-mode inhibitor of AChE) was serially diluted in DI water. Next 10□L of the diluted Tacrine (x axis labeling represents □M final concentration of Tacrine) was added to a white opaque 96 well microplate along with 50 □L of component A (AChE enzyme). The samples were incubated for 5 minutes after which 50□L 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.

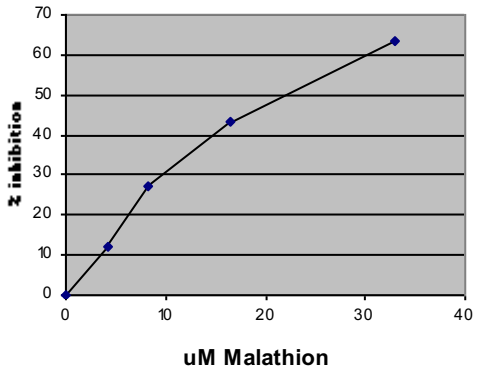


Figure 2. Malathion, a common pesticide, was first diluted in DMSO and subsequently serially diluted in Di water. 10□L of the diluted Malathion (x axis represents □M final concentration of Malathion) was added to a white opaque 96 well microplate followed by 50 □L of component A (AChE enzyme). The mixture was incubated for 15 minutes, after which 50 □L 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.

Notes: * Patent pending: this and other coupled luminescent assays of AChE activity.

References

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