



Cell Technology, Inc

aCella –AChE Bioluminescence assay for Monitoring Acetylcholinesterase Activity

Patent Pending*

Key Benefits

- **Safe** - Non Radioactive enzyme activity assay
- **FAST** - Results in 30 seconds - 5 minutes
- **Homogenous** - One-step, no wash assay.
- **Ultra Sensitive assay** to monitor AChE activity.
- **Versatile**: Nerve gas, pesticide monitoring; drug screening applications.
- Adaptable for **High Throughput format** for drug screening.

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

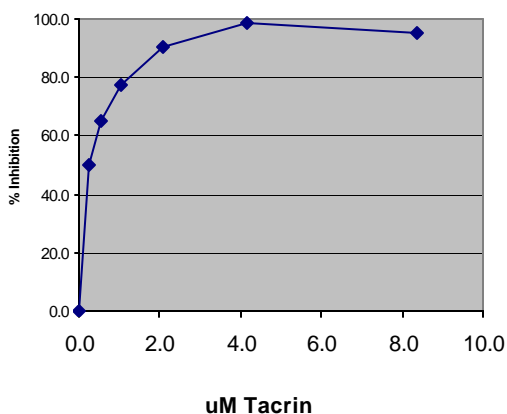


Figure 1. Tacrine (a mixed-mode inhibitor of AChE) was serially diluted in DI water. Next 10 μ L of the diluted Tacrine (x axis labeling represents μ M final concentration of Tacrine) was added to a white opaque 96 well microplate along with 50 μ L of component A (AChE enzyme). The samples were incubated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

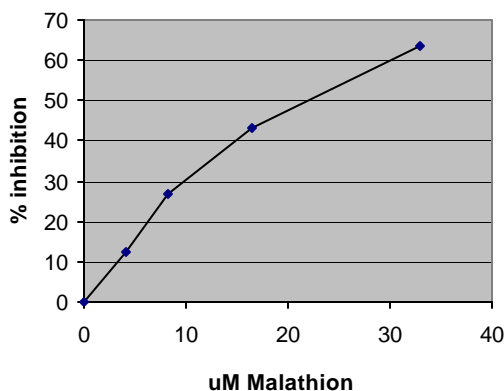


Figure 2. Malathion, a common pesticide, was first diluted in DMSO and subsequently serially diluted in DI water. 10 μ L of the diluted Malathion (x axis represents μ M final concentration of Malathion) was added to a white opaque 96 well microplate, followed by 50 μ L of component A (AChE enzyme). The mixture was incubated for 15 minutes, after which 50 μ L of component B was added to all the wells. Data was collected using a luminometer. Data shown represents T= 30 seconds after the addition of component B.

Kit Contents

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

Ordering Information

Catalog #	Size	Price (US\$)
CLACHE 100-2	100	395
CLACHE 100-3	500	1295
CLACHE 100-4	1000	2,195

References:

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- (2). Friedenber, R., and Seligman, A.: Acetylcholinesterase at the Myoneural Junction: Cytochemical Ultrastructure and Some Biochemical Considerations, *J Histochem Cytochem* 20, 771, 1972
- (3). Nachmansohn, D.: Proteins in Excitable Membranes, *Science* 168, 1059, 1970.
- (4) 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
- (5) 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.
- (6) Levy R. Tetrahydroaminoacridine and Alzheimer's disease. *Lancet*, 1987 Feb 7;1(8528):322.
- (7) Bolognesi ML *et al.* Propidium-based polyamine ligands as potent inhibitors of acetylcholinesterase and acetylcholinesterase-induced amyloid-beta aggregation. *J Med Chem.* 2005 Jan 13;48(1):24-7.
- (8) Schallreuter KU *et al.* Activation/deactivation of acetylcholinesterase by H2O2: more evidence for oxidative stress in vitiligo. *Biochem Biophys Res Commun.* 2004 Mar 5;315(2):502-8.

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