



Cell Technology, Inc

Fluoro AChE™ Fluorescence Assay for monitoring/detecting red blood cell acetylcholinesterase activity

Key Benefits

- Non Radioactive
- Can monitor multiple time points to follow kinetics.
- One-step, no wash assay.
- Adaptable for High Throughput format.
- Versatile: can detect acetylcholinesterase activity in RBC's, saliva & tissue lysates.

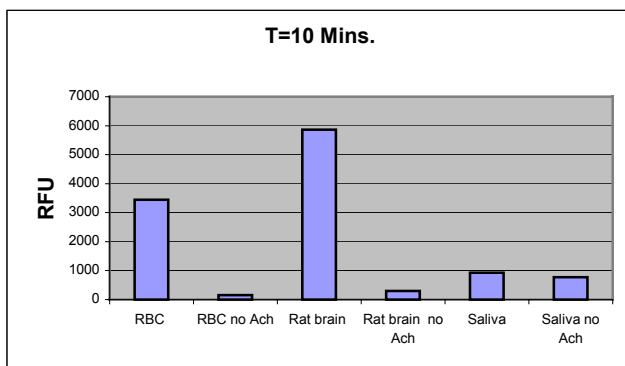
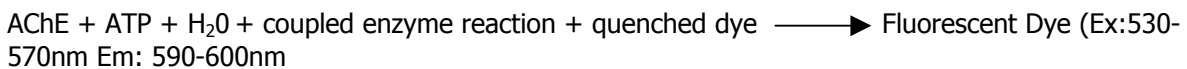
Introduction

Exposure to chemical nerve agents, pesticides and certain drugs (anesthetics, cocaine and therapeutic drugs) reduces the activity of red blood cell (RBC) acetylcholinesterase (AChE). The RBC-AChE can be used as a biomarker to monitor suppressed and or increased AChE function in the peripheral and central nervous system (9).

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.

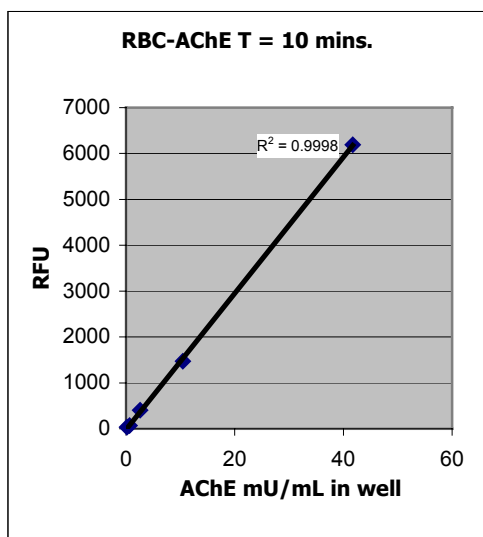
Assay Principle

We have developed a highly sensitive, very rapid, extremely simple assay to determine acetylcholinesterase activity in RBC's, using the natural substrate, acetylcholine. Additionally, by using specific inhibitors, the kit can be used to detect AChE activity in a variety of samples. A series of coupled enzyme reactions quickly translates the presence of active AChE into a change in the fluorescence of a quenched detection reagent.



Time=10 min	Dilution	Volume used	ug protein/well	RFU *
RBC	1:1000	10uL	5	3450
RBC no Ach	1:1000	10uL	5	152
Rat brain	1:500	10uL	20	5864
Rat brain no Ach	1:500	10uL	20	296
Saliva	neat	10uL	ND	925
Saliva no Ach	neat	10uL	ND	772

Fig. 1: Protein concentration for each sample was determined using the BCA Protein Assay Kit (Pierce). The lysates were diluted in 1X reaction buffer and the indicated volumes add to the Fluoro AChE reaction (component A+B). Samples were incubated at room temperature in the dark for 10 minutes. Fluorescence was measured in a 96 well plate reader Ex: 530nm Em: 590nm. (ND= Not determined). Acetylcholine: 1mM final.
* Control 2 (background –see protocol) subtracted from



RBC-AChE mU/mL in well	RFU	SD RFU
167.000	9999	0.00
41.750	6375	121.70
10.438	1659	9.00
2.609	590	9.81
0.652	256	7.23
0.163	222	8.72
0	186	8.08

Fig.2 Red Blood Cell AChE (RBC-AChE) was purified and protein concentration determined using the BCA Protein Assay Kit (Pierce). The RBC-AChE was titrated in 1X reaction buffer and activity determined using the Fluoro: AChE kit. Acetylcholine concentration = 1mM final. In the graph the background value has been subtracted (0 RBC-AChE) to generate standard curve.

Ordering Information

Catalog #	Size	Price (US\$)
ACHE 100-2	100	395
ACHE 100-3	500	1595

References:

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- (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 .
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- (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.
- (9) Nigg HN, Knaak JB. Blood cholinesterases as human biomarkers of organophosphorus pesticide exposure. *Rev. Environ. Contam. Toxicol.* 2000;163: p29-111.

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