

National Center for Advancing Translational Sciences

Abstract

Acetylcholinesterase (AChE) is an enzyme responsible for the metabolism of acetylcholine, a neurotransmitter associated with muscle movement, cognition, and other neurobiological processes. Inhibition of AChE can help treat Alzheimer's disease, glaucoma, and myasthenia gravis, but can also lead to toxicological effects such as gastrointestinal upset, vomiting, and muscular paralysis. While a variety of pesticides, phytochemicals, and drugs can inhibit AChE, there are no available methods for the identification of AChE inhibitors that is compatible with higherthroughput screening platforms. Recently, the Amplex Red assay was developed, in which AChE activity was determined by measuring fluorescence of the resorufin produced from coupled enzyme reactions. In our study, this assay has been optimized and miniaturized into a 1536-well format and validated against the LOPAC library. Overall, this assay will aid in the identification of chemical compounds that inhibit the activity of AChE, a key metabolic enzyme involved in neurotoxicity.

Assay Technology

The Amplex[®] Red Acetylcholinesterase Assay Kit (A12217) was bought from Thermo Fisher. A library of pharmacologically active compounds (LOPAC), containing 1280 compounds with known pharmacological actives, as well as the other compounds used in this study were purchased from Sigma-Aldrich (St. Louis, MO).

The Development of a High-Throughput Assay for Acetylcholinesterase

Samuel Solomon, Shuaizhang Li, Ruili Huang, Menghang Xia



tep	Parameter	Value	Description	
1	Plate enzyme	4uL	Addition using a BioRAPTR	
2	Incubation	18 hr	Incubation at 37 °C	
3	Compound	23nL	Pintool transfer of control and compounds	
4	Incubation	60 min	Incubate at room temperature	
5	Reagent	4 uL	Addition using a BioRAPTR	
6	Incubation	40 min, 60 min	Incubate at room temperature	
7	Readout	ViewLux	Fluorescence (540/598), light energy: 1000 0.5", fast, high, 1x	

AChE	40 minutes		
CV (%)	4.86 ± 1.27		
Z'	0.83 ± 0.04		
B/I	7.87 ± 0.28		



Assay	Active match	Inactive Match	Inconclusive	Mismatch	AC50 Fold Change	Score
40 min	16.67%	79.97%	3.36%	0.00%	1.16	109.94
60 min	15.86%	81.94%	2.19%	0.00%	1.15	111.48

Table 1. Reproducibility of the LOPAC online validation assay: The LOPAC library was tested in triplicate. Active Match, Inactive Match, Inconclusive, and Mismatch – how AC50 differences for the same compound tested in the three runs. Score – assay performance (a score > 80 is considered good)

