Acetylcholinesterase Inhibition Assay
Catalog Number: EZ2018

Kit Contents:

Component	Amount	Storage Condition
Substrate Solution	10 mL	2 - 8°C
Chromogen Solution	2 mL	2 - 8°C
Reaction Buffer	15 mL	2 - 8°C
AChE Solution	0.2 mL	2 - 8°C
Positive Control	100 µL	2 - 8°C
Negative Control	100 µL	2 - 8°C
96-well Plate	1 each	RT

Required Materials not provided:

- Micro-pipettes with disposable plastic tips (25-1000 µL)
- Multi-channel pipette (50-250 µL) or stepper pipette (50-250 µL), or electronic repeating pipette with disposable plastic tips
- Microtiter plate reader (wavelength 405-450 nm)
- Timer

AChE Test Method:

Acetylcholinesterase, also known as AChE, is an enzyme that degrades (through its hydrolytic activity) the neurotransmitter acetylcholine, producing choline and an acetate group. It is mainly found at neuromuscular junctions and cholinergic synapses in the central nervous system, where its activity serves to terminate synaptic transmission. AChE has a very high catalytic activity- each molecule of AChE degrades about 5000 molecules of acetylcholine per second. Acetylcholinesterase is also found on the red blood cell membranes. Acetylcholinesterase exists in multiple molecular forms, which possess similar catalytic properties, but differ in their oligomeric assembly and mode of attachment to the cell surface. The Attogene Acetylcholinesterase Assay Kit provides a convenient method for the detecting AChE activity and screening for inhibitors. The kit uses DTNB to quantify the thiocholine produced from the hydrolysis of acetylthiocholine by AChE. The absorption intensity of DTNB adduct is proportional to the formation of thiocholine, thus the AChE activity. Inhibition of the AchE leads to reduction in thiocholine and reduced signal. Inhibition of AchE are important target for the management of Alzheimer's disease and the most common drugs used for its management. In addition to Alzheimer's disease, AChE inhibitors have been useful in the diagnosis or treatment of diseases such as glaucoma, myasthenia gravis, bladder distention, and more.

Assay Protocol (NOTE: Order of addition is important):

1. Add 50 µL of sample, positive control or negative control into duplicate wells of the 96-well plate.
2. Add 100 µL of Reaction Buffer.
3. Add 2 µL of AChE solution (NOTE: can be replaced with Reaction Buffer for no enzyme control).
4. Incubate 5 minutes.

5. Add 100 µL of Substrate.
6. Add 20 µL of Chromogen.
7. Incubate the plate at room temperature for 10 minutes.
8. Review color change by reading the absorbance at 412 nm.
9. Analyze % inhibition.

If quantitative results are required, it is possible to set up a set of standards at known concentrations of specific pesticides or inhibitory compounds can be used to extrapolate the concentration in the sample being analyzed, loading into a 96 well plate and reading the samples at 412± 5 nm.

The reading (Absorbance) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Acetylcholinesterase in the known or unknow inhibitors. We recommend using the linear regression analysis.