Amplite™ Fluorimetric Acetylcholinesterase Assay Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 11402 (200 assays)	Keep in freezer Avoid exposure to light	Fluorescence microplate readers

Introduction

Acetylcholinesterase (AChE) is one of the most crucial enzymes for nerve response and function. AChE degrades the neurotransmitter acetylcholine (ACh) into choline and acetic acid. It is mainly found at neuromuscular junctions and cholinergic synapses in the central nervous system, where its activity serves to terminate the synaptic transmission. AChE inhibitors are among the key drugs approved for Alzheimer's disease (AD) and myasthenia gravis.

Our Amplite™ Fluorimetric Acetylcholinesterase Assay Kit provides one of the most sensitive methods for detecting AChE activity or screening AChE inhibitors in red florescence window. The kit uses Amplite™ Red to quantify the choline produced from the hydrolysis of acetylcholine by AChE through choline oxidase-mediated enzyme coupling reactions. It can be used for monitoring and quantifying the AChE activity in blood, cell extracts or other solutions. The fluorescence intensity of Amplite™ Red is used to measure the amount of choline formed, which is proportional to the AChE activity. The kit is an optimized "mix and read" assay that provides a simple one-step fluorimetric assay to detect as little as 0.01 mU AChE in a 100 µL assay volume (0.1 mU/mL). Its signal can be easily read with a fluorescence microplate reader at Ex/Em = ~540/590 nm or an absorbance microplate reader at ~575 nm.

Kit Key Features

Broad Application:	Can be used for quantifying acetylcholinesterase in solutions and in cell extracts.
Sensitive:	Detect as low as 0.01 mU of acetylcholinesterase in solution.
Continuous:	Easily adapted to automation without a separation step.
Convenient:	Formulated to have minimal hands-on time.

Kit Components

Components	**Amount**
Component A: Amplite ™ Red	1 vial
Component B: Acetylcholinesterase Probe	2 bottles (lyophilized powder)
Component C: Acetylcholine	1 vial
Component D: Acetylcholinesterase Standard	1 vial (5 units)
Component E: Assay Buffer	1 bottle (10 mL)
Component F: Dilution Buffer	1 bottle (10 mL)
Component G: DMSO	1 vial (100µL)

Assay Protocol for One 96-well Plate

Brief Summary

Prepare AChE assay mixture (50 µL) → Add AChE standards and/or AChE test samples (50 µL) → Incubate at room temperature for 10-30 minutes → Monitor fluorescence intensity at Ex/Em = 540/590 nm

Note: Thaw all the kit components at room temperature before starting the experiment.

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1. Prepare stock solutions:

1.1 250X Amplite™ Red stock solution: Add 40 µL of DMSO (Component G) into the vial of Amplite™ Red (Component A) to make 250X Amplite™ Red stock solution.
Note: The unused Amplite™ Red stock solution should be divided into single use aliquots. Store at -20 °C and avoid exposure to light.

1.2 Acetylcholinesterase standard stock solution: Add 100 µL of Assay Buffer (Component E) into the vial of acetylcholinesterase standard (Component D) to make a 50 units/mL acetylcholinesterase standard stock solution.
Note: The unused acetylcholinesterase standard stock solution should be divided into single use aliquots and stored at -20 °C.

1.3 Acetylcholine stock solution: Add 100 µL of Assay Buffer (Component E) into the vial of Acetylcholine (Component C) to make 1000X acetylcholine stock solution.
Note: The unused acetylcholine standard stock solution should be divided into single use aliquots and stored at -20 °C.

2. Prepare acetylcholinesterase assay mixture:

2.1 Add 5 mL of Assay Buffer (Component E) to the bottle of Acetylcholinesterase Probe (Component B) and mix well.

2.2 Add 5 µL of 1000X acetylcholine stock solution (from Step 1.3) into the bottle of Acetylcholinesterase Probe mixture (from Step 2.1) and mix well.

2.3 Add 20 µL of 250X Amplite™ Red stock solution (from Step 1.1) into the bottle of Acetylcholinesterase Probe mixture (from Step 2.2) to make the acetylcholinesterase assay mixture before running the assay.
Note: The acetylcholinesterase assay mixture should be used promptly and kept from light. The assay background would increase with longer storage time.

3. Prepare serially diluted acetylcholinesterase standards (0 to100 mU/mL):

3.1 Add 20 µL of 50 units/mL acetylcholinesterase standard stock solution (from Step 1.2) to 980 µL Dilution Buffer (Component F) to generate 1000 mU/mL acetylcholinesterase standard solution.
Note: Diluted acetylcholinesterase standard solution is unstable and should be used within 4 hours.

3.2 Take 200 µL of 1000 mU/mL acetylcholinesterase standard solution to perform 1:10 and 1:3 serial dilutions to get 100, 30, 10, 3, 1, 0.3, 0.1 and 0 mU/mL serially diluted acetylcholinesterase standards.

3.3 Add serially diluted acetylcholinesterase standards and/or acetylcholinesterase containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.
Note: Treat the cells or tissue samples as desired.

Table 1 Layout of acetylcholinesterase standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	….	….						
AS1	AS1	….	….	….	….						
AS2	AS2										
AS3	AS3										
AS4	AS4										
AS5	AS5										
AS6	AS6										
AS7	AS7										

Note: AS= Acetylcholinesterase Standards; BL=Blank Control; TS=Test Samples

Table 2 Reagent composition for each well

Acetylcholinesterase Standards	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	Dilution Buffer: 50 µL	50 µL

**Note: Add the serially diluted acetylcholinesterase standards from 0.01 to100 mU/mL into wells from AS1 to AS7 in duplicate.*

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4. Run acetylcholinesterase assay:

4.1 Add 50 µL of acetylcholinesterase assay mixture (from Step 2.3) into each well of acetylcholinesterase standard, blank control, and test samples (see Step 3.3) to make the total acetylcholinesterase assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of acetylthiocholine reaction mixture into each well.

4.2 Incubate the reaction at room temperature for 10 to 30 minutes, protected from light.

4.3 Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 540/590 nm.

Data Analysis

The fluorescence in blank wells (with the Dilution Buffer only) is used as a control, and is subtracted from the values for those wells with the acetylcholinesterase reactions. An acetylcholinesterase standard curve is shown in Figure 1. *Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.*

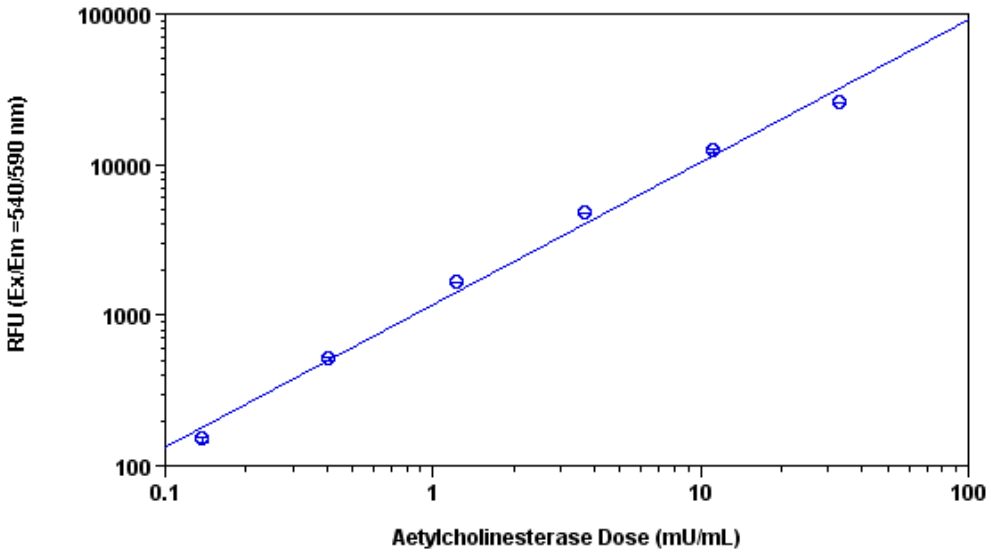

Figure 1. Acetylcholinesterase dose response was measured in a solid black 96-well plate with Amplite™ Fluorimetric Acetylcholinesterase Assay Kit using a Gemini fluorescence microplate reader (Molecular devices). As low as 0.01 mU/well (0.1 mU/mL) acetylcholinesterase can be detected with 20 minutes incubation (n=3).

References

1. Kovarik, Z et al. (2003). Acetylcholinesterase active centre and gorge conformations analysed by combinatorial mutations and enantiomeric phosphonates. Biochem. J. (2003) 373, 33–40.
2. Ordentlich, A. et al. (1996). The Architecture of Human Acetylcholinesterase Active Center Probed by Interactions withSelected Organophosphate Inhibitors. J. Biol. Chem. 271 (20):11953–11962.
3. Magnottl, RA. et al. (1987). Measurement of Acetylcholinesterase in Erythrocytes in the Field. Clin. Chem. 33/10, 1731-1 735.