Amplite™ Fluorimetric Acetylcholinesterase Assay Kit *Red Fluorescence*

Catalog number: 11402
Unit size: 200 Tests

Component	Storage	Amount
Component A: Amplite™ Red	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Acetylcholinesterase Probe	Freeze (<-15 °C), Minimize light exposure	2 bottles (lyophilized powder)
Component C: Acetylcholine	Freeze (<-15 °C), Minimize light exposure	1 vial
Component D: Acetylcholinesterase Standard	Freeze (<-15 °C), Minimize light exposure	1 vial (5 units)
Component E: Assay Buffer	Freeze (<-15 °C)	1 bottle (10 mL)
Component F: Dilution Buffer	Freeze (<-15 °C), Minimize light exposure	1 bottle (10 mL)
Component G: DMSO	Freeze (<-15 °C)	1 vial (100 µL)

OVERVIEW

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, where it constitutes the Yt blood group antigen. 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. This Amplite™ Fluorimetric Acetylcholinesterase Assay Kit provides one of the most sensitive methods for the detecting AChE activity. The kit uses Amplite™ Red to quantify the choline produced from the hydrolysis of acetylcholine by AChE through choline oxidase-mediated enzyme coupling reactions. The fluorescence intensity of Amplite Red™ is proportional to the formation of choline, thus the AChE activity.

AT A GLANCE

Protocol summary

1. Prepare AChE working solution (50 µL)
2. Add AChE standards or AChE test samples (50 µL)
3. Incubate at room temperature for 10 - 30 minutes
4. Monitor fluorescence intensity at Ex/Em =540/590 nm (Cutoff = 570 nm)

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Amplite™ Red stock solution (250X):
Add 40 µL of DMSO (Component G) into the vial of Amplite™ Red (Component A) to make 250X Amplite™ Red stock solution.

Note Avoid exposure to light.

2. Acetylcholinesterase standard solution (50 units/mL):
Add 100 µL of Assay Buffer (Component E) into the vial of Acetylcholinesterase Standard (Component D) to make 50 Units/mL Acetylcholinesterase standard solution.

3. Acetylcholine stock solution (1000X):
Add 100 µL of Assay Buffer (Component E) into the vial of Acetylcholine (Component C) to make a 1000X Acetylcholine stock solution.

PREPARATION OF STANDARD SOLUTION

Acetylcholinesterase standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11402

Add 20 µL of 50 Units/mL Acetylcholinesterase standard solution to 980 µL of Dilution Buffer (Component F) to generate 1000 mU/mL Acetylcholinesterase standard solution. Take 1000 mU/mL Acetylcholinesterase standard and perform 1:10 in Dilution Buffer (Component F) to get 100 mU/mL Acetylcholinesterase standard (AS7). Then take 100 mU/mL Acetylcholinesterase standard (AS7) and perform 1:3 serial dilutions to get serially diluted Acetylcholinesterase standard (AS6 - AS1) with Dilution Buffer (Component F).

Note Diluted Acetylcholinesterase standard solution is unstable and should be used within 4 hours.

PREPARATION OF WORKING SOLUTION

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

2. Add 5 µL of 1000X Acetylcholine stock solution into the bottle of Acetylcholinesterase Probe mixture and mix well.

3. Add 20 µL of 250X Amplite™ Red stock into this bottle of Acetylcholinesterase Probe mixture to make Acetylcholinesterase (AChE) working solution.

Note This Acetylcholinesterase (AChE) working solution should be used promptly and kept from light. The assay background would increase with longer storage time.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Acetylcholinesterase standards and test samples in a solid black 96-well microplate. AS= Acetylcholinesterase Standards (AS1 - AS7, 0.14 to 100 mU/mL); BL=Blank Control; TS=Test Samples.

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

Table 2. Reagent composition for each well.

Well	Volume	Reagent
AS1 - AS7	50 µL	Serial Dilutions (0.14 to 100 mU/mL)
BL	50 µL	Dilution Buffer (Component F)
TS	50 µL	test sample

1. Prepare Acetylcholinesterase standards (AS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

   Note Treat cells or tissue samples as desired.

2. Add 50 µL of AChE working solution to each well of Acetylcholinesterase standard, blank control, and test samples to make the total Acetylcholinesterase assay volume of 100 µL/well. For a 384-well plate, add 25 µL of AChE working solution into each well instead, for a total volume of 50 µL/well.

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

4. Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 540/590 nm (Cutoff = 570 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) 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 samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

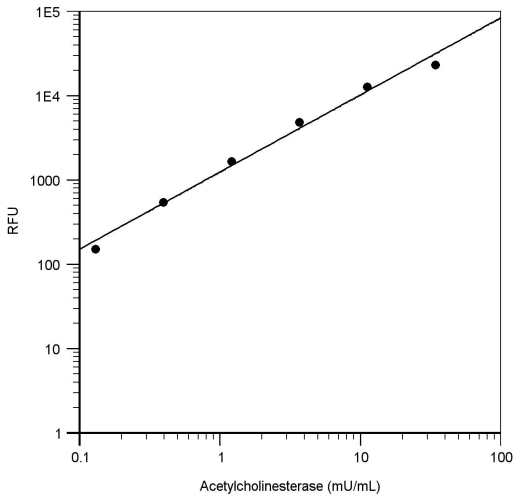

Figure 1. Acetylcholinesterase dose response was measured in a solid black 96-well plate with Amplite™ Fluorimetric Acetylcholinesterase Assay Kit using a Gemini fluorescence microplate reader (Molecular devices).

DISCLAIMER