Amplite™ Fluorimetric Acetylcholinesterase Assay Kit *Green Fluorescence*

Catalog number: 11401
Unit size: 200 Tests

Component	Storage	Amount
Component A: Thiolite™ Green	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (25 mL)
Component C: Acetylthiocholine	Freeze (< -15 °C), Minimize light exposure	1 vial
Component D: Acetylcholinesterase Standard	Freeze (< -15 °C), Minimize light exposure	1 vial (5 units)
Component E: 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 the most sensitive method for the detecting AChE activity. The kit uses our outstanding Thiolite Green™ to quantify the thiocholine produced from the hydrolysis of acetylthiocholine by AChE. The fluorescence intensity of Thiolite Green™ is proportional to the formation of thiolcholine, thus the AChE activity.

AT A GLANCE

Protocol Summary

1. Prepare AChE standards and/or AChE test samples (50 µL)
2. Add AChE working solution (50 µL)
3. Incubate at room temperature for 10 - 30 minutes
4. Monitor fluorescence intensity at Ex/Em = 490/525 nm

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

KEY PARAMETERS

Fluorescence microplate reader

Excitation	490 nm
Emission	525 nm
Cutoff	515 nm
Recommended plate	Solid black

CELL PREPARATION

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

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. Thiolite™ Green stock solution (200X)
Add 50 µL of DMSO (Component E) into the vial of Thiolite™ Green (Component A) to make 200X Thiolite™ Green stock solution.

2. Acetylthiocholine stock solution (500X)
Add 0.6 mL of ddH_2O into the vial of Acetylthiocholine (Component C).

3. Acetylcholinesterase standard stock solution
Add 100 µL of ddH_2O with 0.1% BSA into the vial of Acetylcholinesterase Standard (Component D) to make a 50 U/mL Acetylcholinesterase standard solution.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11401

Acetylcholinesterase standard
Add 20 µL of 50 U/mL Acetylcholinesterase standard solution to 980 µL Assay Buffer (Component C) to generate 1000 mU/mL Acetylcholinesterase standard solution. Take 1000 mU/mL Acetylcholinesterase standard solution to perform 1:10 to get 100 mU/mL Acetylcholinesterase standard solution(AS7). Then perform 1:3 serial dilution to obtain remaining serially diluted acetylcholinesterase standards (AS6 - AS1). Note: Diluted acetylcholinesterase standard solution is unstable and should be used within 4 hours.

PREPARATION OF WORKING SOLUTION

Add 10 µL of Acetylthiocholine stock solution (500X) and 25 µL of Thiolite™ Green stock solution (200X) into 5 mL of Assay Buffer (Component B) to make a total volume of 5.03 mL Acetylcholinesterase(AChE) working solution.

Note The AChE working solution is not stable and needs to be used within 30 minutes. Keep from light .

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of acetylcholinesterase standards and test samples in a solid black 96-well microplate. AS= Acetylcholinesterase Standards (AS1 - AS7, 0.01 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 Dilution (0.01 to 100 mU/mL)
BL	50 µL	Assay Buffer (Component B)
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.

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 = 490/525 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

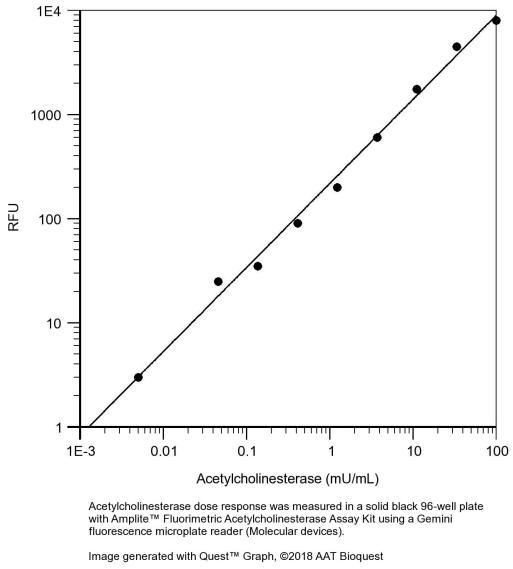

Figure 1. Acetylcholinesterase dose response was measured in a solid black 96-well plate with Amplite™ Fluorimetric Acetylcholinesterase Assay Kit using a Gemini fluorescence microplate reader (Molecular devices).

DISCLAIMER

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