Amplite™ Fluorimetric Acetylcholinesterase Assay Kit

Green Fluorescence

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 11401 (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 synaptic transmission. AChE is also found on the red blood cell membranes, where it constitutes the Yt blood group antigen. AChE inhibitors are among the key drugs approved by the FDA for the management of Alzheimer's disease (AD) and myasthenia gravis. Our Amplite™ Fluorimetric Acetylcholinesterase Assay Kit provides the most sensitive method for the detection of AChE activity. The kit uses our outstanding Thiolite Green™ to quantify the thiocholine produced from the hydrolysis of acetylthiocholine by AChE in blood, in cell extracts, and in other solutions. The fluorescence intensity of Thiolite Green™ is used to measure the amount of thiocholine formed, which is proportional to the AChE activity. The kit is an optimized "mix and read" assay that is compatible with HTS liquid handling instruments.

The Amplite™ Fluorimetric Acetylcholinesterase Assay Kit provides an ultrasensitive fluorometric one-step assay to detect as little as 0.01mU AChE in a 100 µL assay volume (0.1 mU/mL) as shown in Figure1. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. Its signal can be easily read by a fluorescence microplate reader at Ex/Em = 490/520 nm.

Kit Key Features

Broad Application:	Can be used for quantifying acetylcholinesterase in solutions, and in cell extracts.
Sensitive:	Detect as low as 0.01mU of acetylcholinesterase in solution.
Continuous:	Easily adapted to automation without a separation step.
Convenient:	Formulated to have minimal hands-on time.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	Amount
Component A: Thiolite Green™	1 vial
Component B: Assay Buffer	1 bottle (25 mL)
Component C: Acetylthiocholine	1 vial
Component D: Acetylcholinesterase Standard	1 vial (5 units)
Component E: DMSO	1 vial (100 µL)

Assay Protocol for One 96-well Plate

Brief Summary

Prepare ACh reaction mixture (50 µL) → Add AChE standards or AChE test samples (50 µL) → Incubate at room temperature for 10-30 min → Read fluorescence intensity at Ex/Em = 490/520 nm

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

1. Prepare stock solutions:

1.1 Thiolite Green™ stock solution (400X): Add 25 µL of DMSO (Component E) into the vial of Thiolite Green™ (Component A) to make 400X stock solution.
Note: The unused Thiolite Green™ stock solution should be divided into single use aliquots. Store at -20 °C and avoid exposure to light.

1.2 Acetylthiocholine stock solution (500X): Add 0.6 mL of ddH2O into the vial of acetylthiocholine (Component B).
Note: The unused acetylthiocholine stock solution should be divided into single use aliquots and stored at -20 °C.

1.3 Acetylcholinesterase stock solution: Add 100 µL of ddH_2O with 0.1% BSA into the acetylcholinesterase standard vial (Component D) to make a 50 units/mL stock solution.
Note: The unused acetylcholinesterase stock solution should be divided into single use aliquots and stored at -20 °C.

2. Prepare acetylthiocholine reaction mixture:

Prepare the acetylthiocholine reaction mixture according to the following table and keep from light.

Table 1. Acetylthiocholine reaction mixture for one 96-well plate

Components	**Volume**
Assay buffer (Component B)	5 mL
Thiolite Green™ stock solution (400X, from Step 1.1)	12.5 µL
Acetylthiocholine stock solution (500X, from Step 1.2)	10 µL
Total volume	5.02 mL

3. Prepare serial Acetylcholinesterase (0 to100 mU/mL) solutions:

3.1 Add 20 µL of 50 units/mL acetylcholinesterase standard stock solution (from Step 1.3) to 980 µL assay buffer (Component C) to generate 1000 mU/mL standard.
Note: Diluted acetylcholinesterase standard solution is unstable and should be used within 4 hours.

3.2 Take 200 µL of 1000 mU/mL standard to perform 1:10 and 1:3 serial dilutions to get 100, 10, 1, 0.3, 0.1, 0.03, 0.01 and 0 mU/mL standard acetylcholinesterase solutions.

3.3 Add acetylcholinesterase standards and acetylcholinesterase-containing test samples into a 96-well solid black 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 Standard	**Blank Control**	**Test Sample**
Serial dilutions* (50 µL)	Assay 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.*

4. Run acetylcholinesterase assay:

4.1 Add 50 µL of acetylthiocholine reaction mixture (from Step 2.1) to each well of the 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 at Ex/Em = 490/520 nm by using a fluorescence microplate reader.

Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and 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.

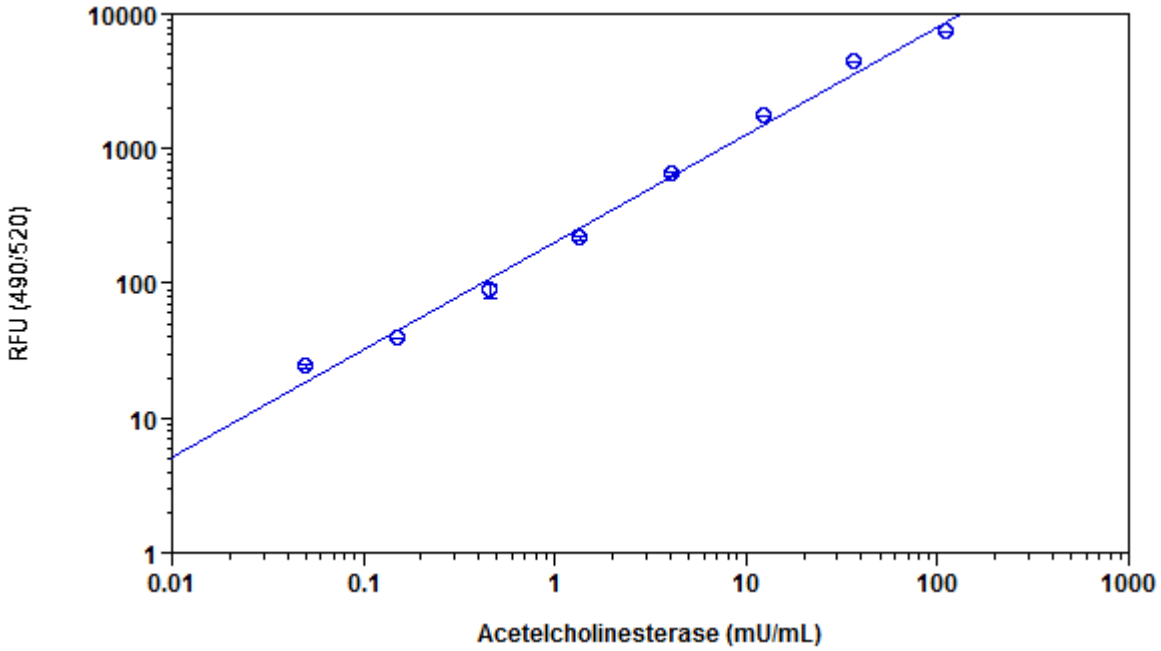

Figure 1. Acetylcholinesterase dose response was measured in a 96-well clear plate with Amplite™ Fluorimetric Acetylcholinesterase Assay Kit using a Gemini fluorescence microplate reader (Molecular devices). As low as 0.01 mU/well of acetylcholinesterase can be detected with 20 minutes incubation time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