Acetylcholinesterase Assay Kit (Red Fluorescence)

Catalog Number KA4132

200 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

The Acetylcholinesterase Assay Kit (Red Fluorescence) provides one of the most sensitive methods for detecting AChE activity or screening AChE inhibitors in red florescence window.

Background

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.

Principle of the Assay

The kit uses Amplit 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.

✓ 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.

General Information

Materials Supplied

List of component

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

Storage Instruction

Keep in freezer. Avoid exposure to light.

Precautions for Use

- ✓ This kit is For Research Use Only.
- ✓ Avoid exposure to light.

Assay Protocol

Reagent Preparation

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

✓ Prepare stock solutions:

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.
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.
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.

✓ Prepare acetylcholinesterase assay mixture:

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 (from Step 3 of Prepare stock solutions) into the bottle of Acetylcholinesterase Probe mixture (from Step 1 of Prepare acetylcholinesterase assay mixture) and mix well.
3. Add 20 µL of 250X Amplite Red stock solution (from Step 1 of Prepare stock solutions) into the bottle of Acetylcholinesterase Probe mixture (from Step 2 of Prepare acetylcholinesterase assay mixture) 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.

Sample Preparation

✓ Prepare serially diluted acetylcholinesterase standards (0 to 100 mU/mL):

1. Add 20 µL of 50 units/mL acetylcholinesterase standard stock solution (from Step 2 of Prepare stock solutions) 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.

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

Table 1. 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*

Assay Procedure

✓ Run acetylcholinesterase assay:

1. Add 50 µL of acetylcholinesterase assay mixture (from Step 3 of Prepare acetylcholinesterase assay mixture) into each well of acetylcholinesterase standard, blank control, and test samples (see Step 3 of Prepare serially diluted acetylcholinesterase standards) 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.
2. Incubate the reaction at room temperature for 10 to 30 minutes, protected from light.
3. Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 540/590 nm.

✓ Summary

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

Data Analysis

Calculation of Results

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.

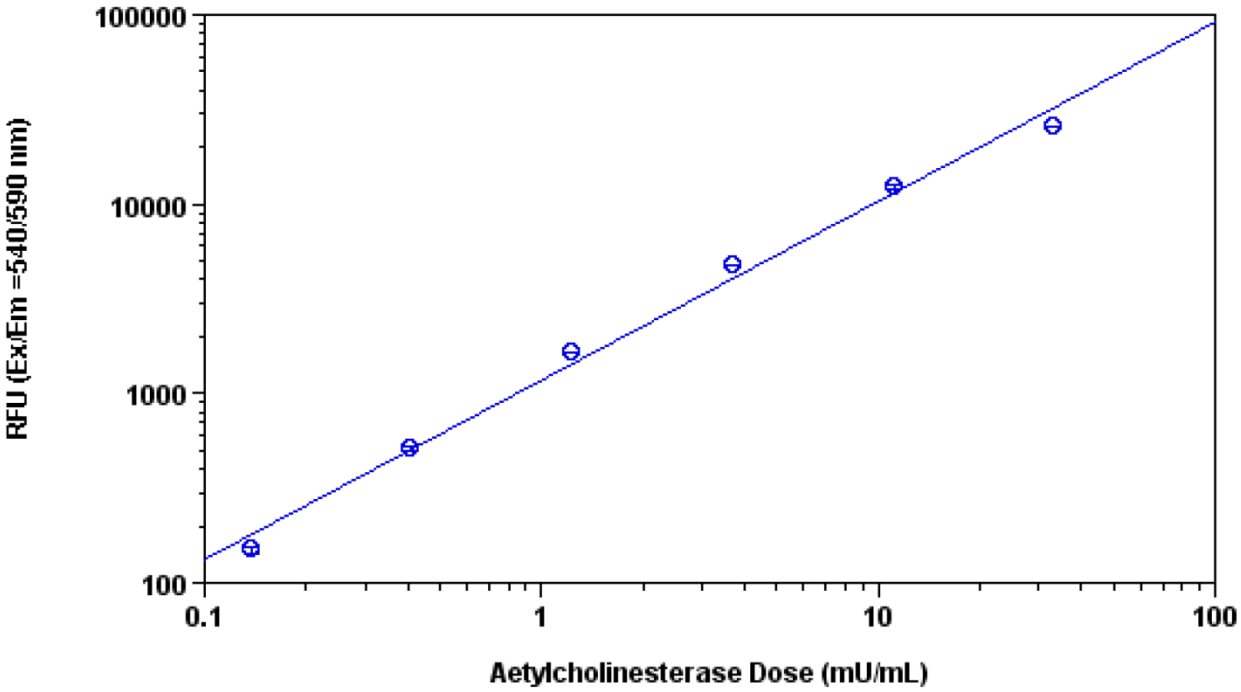

Figure 1. Acetylcholinesterase dose response was measured in a solid black 96-well plate with the Acetylcholinesterase Assay Kit (Red Fluorescence) using a Gemini fluorescence microplate reader (Molecular devices). As low as 0.01 mU/well (0.1 mU/mL) acetylcholinesterase can be detected with 20 minutes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

Resources

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.

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4	TS							
3	TS							
2	BL	AS1	AS2	AS3	AS4	AS5	AS6	AS7
1	BL	AS1	AS2	AS3	AS4	AS5	AS6	AS7
	A	B	C	D	E	F	G	H

AS= Acetylcholinesterase Standards

BL=Blank Control

TS=Test Samples