Amplite™ Colorimetric Acetylcholinesterase Assay Kit

Catalog number: 11400
Unit size: 200 Tests

Component	Storage	Amount
Component A: DTNB	Freeze (<-15 °C), Dessicated, Avoid Light	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (25 mL)
Component C: Acetylthiocholine	Freeze (<-15 °C), Dessicated, Avoid Light	1 vial
Component D: Acetylcholinesterase Standard	Freeze (<-15 °C), Dessicated, Avoid Light	1 vial (5 units)

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™ Colorimetric Acetylcholinesterase Assay Kit provides a convenient method for the detecting AChE activity. The kit uses DTNB to quantify the thiolcholine produced from the hydrolysis of acetylthiolcholine by AChE. The absoprtion intensity of DTNB adduct is proportional to the formation of thiolcholine, 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 absorbance at 410 ± 5 nm

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

KEY PARAMETERS

Instrument:	Absorbance microplate reader
Absorbance:	410 ± 5 nm
Recommended plate:	Clear bottom

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. DTNB stock solution (20X):
Add 0.6 mL of Assay Buffer (Component B) into the vial of DTNB (Component A) to make 20X DTNB stock solution. Keep from light.

Note DNTB is not easy to dissolve, it is normal to see the cloudiness of the solution. One can use either the supernatant or the mixture for the experiment.

2. Acetylthiocholine stock solution (20X):
Add 0.6 mL of ddH_2O into the vial of Acetylthiocholine (Component C) to make 20X Acetylthiocholine stock solution.

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

PREPARATION OF STANDARD SOLUTION

Acetylcholinesterase standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11400

Add 20 µL of 50 U/mL Acetylcholinesterase standard solution to 980 µL of Assay Buffer (Component B) to generate 1000 mU/mL Acetylcholinesterase standard solution (AS7). Then take 1000 mU/mL Acetylcholinesterase standard solution (AS7) and perform 1:3 serial dilutions in Assay Buffer (Component B) to get 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 250 µL of 20X DTNB stock solution and 250 µL of 20X Acetylthiocholine stock solution into 4.5 mL of Assay Buffer (Component B) to make a total volume of 5 mL AChE working solution. Keep from light.

PREPARATION OF CELL SAMPLES

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

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Acetylcholinesterase standards and test samples in a white/clear bottom 96-well microplate. AS=Acetylcholinesterase Standards (AS1 - AS7, 1 to 1000 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 (1 to 1000 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.

   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 for 10 to 30 minutes at room temperature, protected from light.

4. Monitor the absorbance increase with an absorbance microplate reader at 410 ± 5 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance) 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

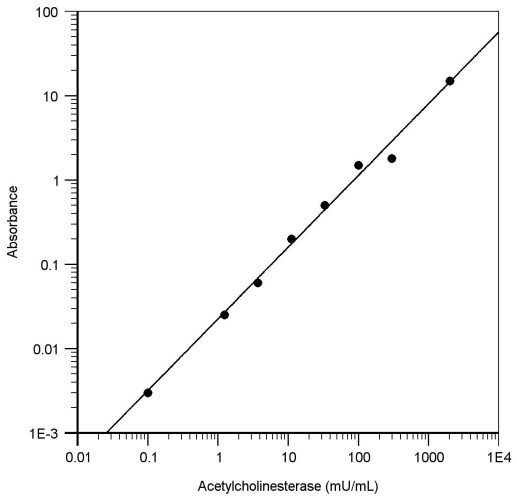

Figure 1. Acetylcholinesterase dose response was measured in a white/clear bottom 96-well plate with Amplite™ Colorimetric Acetylcholinesterase Assay Kit using a SpectraMax microplate reader (Molecular devices). As low as 0.1 mU/well of Acetylcholinesterase can be detected with 30 minutes incubation(n=3).

DISCLAIMER