Amplite™ Colorimetric Acetylcholinesterase Assay Kit

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 11400 (200 assays)	Keep in freezer and protect from light	Absorbance 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 the 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. This Amplite™ Colorimetric Acetylcholinesterase Assay Kit provides a convenient method for the detection of AChE activity. The kit uses DTNB to quantify the thiocholine produced from the hydrolysis of acetylthiocholine by AChE in blood, in cell extracts, and in other solutions. The absorption intensity of DTNB adduct 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™ Colorimetric Acetylcholinesterase Assay Kit provides a colorimetric one-step assay to detect as little as 0.1mU AChE in a 100 µL assay volume (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 an absorbance microplate reader at 410 ± 5 nm.

Kit Key Features

Broad Application:	Can be used to quantify acetylcholinesterase in solutions and in cell extracts.
Sensitive:	Detect as low as 0.1 mU 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: DTNB	1 vial
Component B: Assay Buffer	1 bottle (25 mL)
Component C: Acetylthiocholine	1 vial
Component D: Acetylcholinesterase Standard	1 vial (5 units)

Assay Protocol for One 96-well Plate

Brief Summary

Prepare AChE reaction mixture (50 µL) → Add AChE standards or AChE test samples (50 µL) → Incubate at room temperature for 10-30 minutes → Read absorbance at 410 ± 5 nm

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

1. Prepare stock solutions:

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Technical Support: support@aatbio.com; 408-733-1055

1.1 DTNB stock solution (20X): Add 0.6 mL of Assay Buffer (Component B) into the vial of DTNB (Component A) to make 20X stock solution.
Note: The unused DTNB stock solution should be divided into single use aliquots. Store at -20 °C and keep from light.

1.2 Acetylthiocholine stock solution (20X): Add 0.6 mL of ddH_2O into the vial of acetylthiocholine (Component C).
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)	4.5 mL
DTNB Stock Solution (20X, from Step 1.1)	250 µL
Acetylthiocholine Stock solution (20X, from Step 1.2)	250 µL
Total volume	5 mL

3. Prepare serial Acetylcholinesterase (0 to1000 mU/mL) solutions:

3.1 Add 20 µL of 50 units/mL acetylcholinesterase standard stock solution (from Step 1.3) to 980 µL of assay buffer (Component B) 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 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1 and 0 mU/mL standard acetylcholinesterase solutions.

3.3 Add acetylcholinesterase standards and acetylcholinesterase-containing test samples into a 96-well white/clear bottom 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 white/clear 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 1 to1000 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 in each well.

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

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

Data Analysis

The absorbance 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 absorbance background increases with time, thus it is important to subtract the absorbance intensity value of the blank wells for each data point.

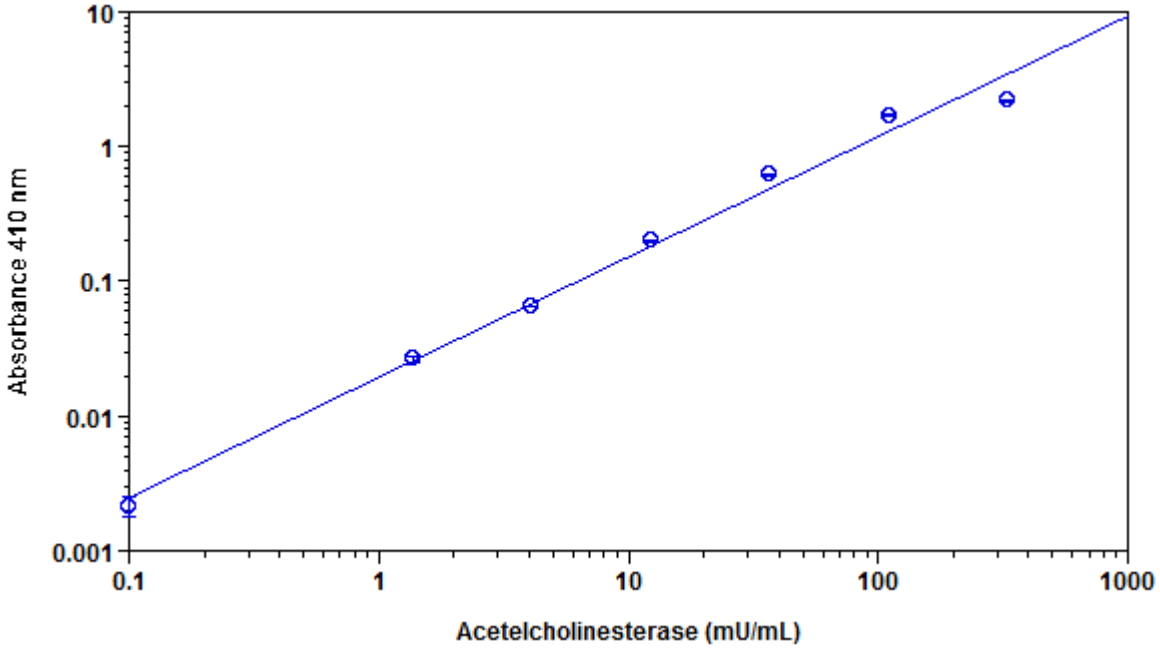

Figure 1. Acetylcholinesterase dose response was measured in a 96-well clear plate with Amplite™ Colorimetric Acetylcholinesterase Assay Kit using a SpectraMax microplate reader (Molecular devices). As low as 0.1 mU/well of acetylcholinesterase can be detected with 30 minutes incubation time (n=3).

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.