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 inhibitors are among the key drugs approved for Alzheimer's disease (AD) and myasthenia gravis.

Our Amplite™ Colorimetric Acetylcholinesterase Assay Kit provides a convenient method for the detection of AChE activity. It 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 provides a colorimetric one-step assay to detect as little as 0.1 mU AChE in a 100 µL assay volume (1 mU/mL) as shown in Figure 1. Its signal can be easily read by an absorbance microplate reader at ~410 nm. The kit is robust and can be used for continuously monitoring AChE activities.

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 → Monitor absorbance at 410 ± 5 nm

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

1. Prepare stock solutions:

1.1 ~~20X DTNB stock solution:~~ Add 0.6 mL of Assay Buffer (Component B) into the vial of DTNB (Component A) to make 20X DTNB 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 20X Acetylthiocholine stock solution: Add 0.6 mL of ddH_2O into the vial of acetylthiocholine (Component C).
Note: The unused 20X 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 vial of acetylcholinesterase standard (Component D) to make a 50 units/mL acetylcholinesterase 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 Table 1 and keep from light.

Table 1 Acetylthiocholine reaction mixture for one 96-well plate

Components	**Volume**
Assay Buffer (Component B)	4.5 mL
20X DTNB Stock Solution (from Step 1.1)	250 µL
20X Acetylthiocholine Stock solution (from Step 1.2)	250 µL
Total volume	5 mL

3. Prepare serial dilutions of acetylcholinesterase standard (0 to 1000 mU/mL):

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

3.2 Take 200 µL of 1000 mU/mL acetylcholinesterase standard to perform 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1 and 0 mU/mL serial dilutions of acetylcholinesterase standard.

3.3 Add serial dilutions of acetylcholinesterase standard and acetylcholinesterase-containing test samples into a white/clear bottom 96-well microplate as described in Tables 2 and 3.
Note: Treat cells or tissue samples as desired.

Table 2. Layout of acetylcholinesterase standards and test samples in a white/clear bottom 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 3. Reagent composition for each well

Acetylcholinesterase Standard	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	Assay Buffer: 50 µL	50 µL

**Note: Add the serial dilutions of acetylcholinesterase standard 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 with an absorbance microplate reader at 410 ± 5 nm.

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.*

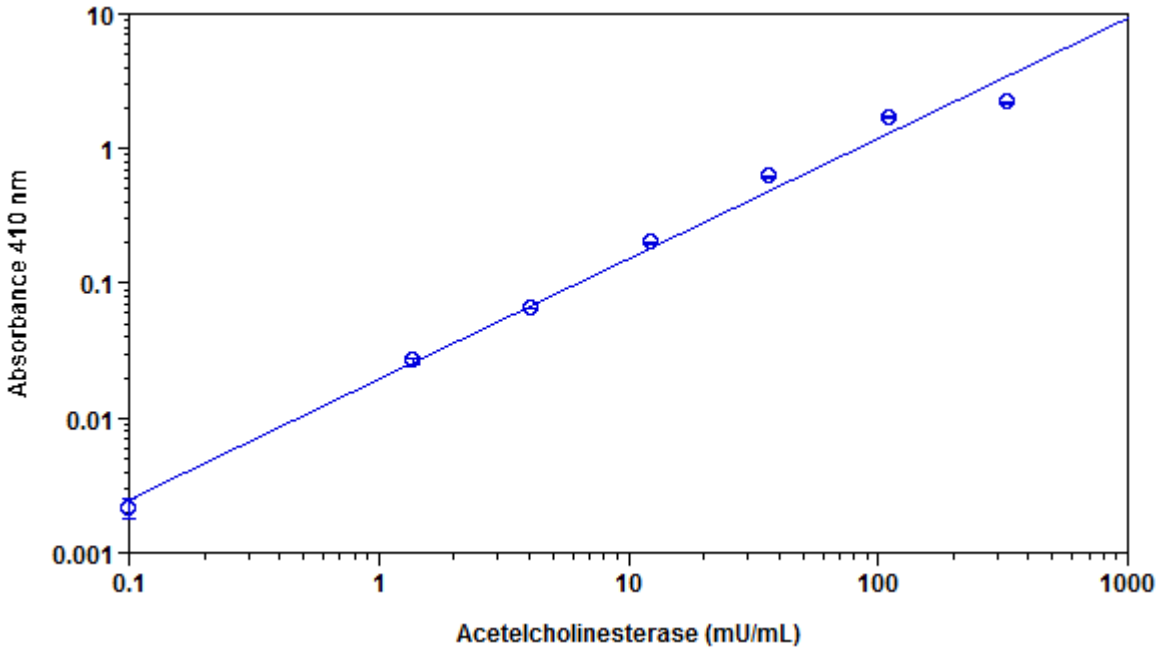

Figure 1. Acetylcholinesterase dose response was measured in a white/clear bottom 96-well plate with Amplite™ Colorimetric Acetylcholinesterase Assay Kit using a SpectraMax microplate reader (Molecular devices). As low as 0.1 mU/well of acetylcholinesterase can be detected with 30 minutes incubation(n=3).

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.