Amplite™ Fluorimetric Acetylcholine Assay Kit

Red Fluorescence

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 11403 (200 assays)	Keep in freezer Avoid exposure to light	Fluorescence microplate readers

Introduction

Acetylcholine and its metabolites are needed for three main physiological purposes: structural integrity and signaling roles for cell membranes, cholinergic neurotransmission (acetylcholine synthesis), and as a major source for methyl groups via its metabolite, trimethylglycine (betaine) that participates in the S-adenosylmethionine synthesis pathways. Acetylcholine (ACh) is a neurotransmitter in both the central and peripheral nervous systems. It is one of many neurotransmitters in the autonomic nervous system (ANS) and the only neurotransmitter used in the motor division of the somatic nervous system. It is involved in a number of biological events that have been linked to Myasthenia gravis, diabetic vasculopathy, hypertension, coronary heart disease and Alzheimer's disease. Our Amplite™ Fluorimetric Acetylholine Assay Kit provides one of the most sensitive methods for quantifying acetylcholine. The kit uses Amplite Red™ to quantify acetylcholine through the choline oxidase-mediated enzyme coupling reactions. The fluorescence intensity of Amplite Red™ is proportional to acetylcholine. The kit is an optimized "mix and read" assay that is compatible with HTS liquid handling instruments.

The Amplite™ Fluorimetric Acetylcholine Assay Kit provides an ultrasensitive one-step fluorimetric assay to detect as little as 0.01 nmoles ACh in a 100 µL assay volume (0.1µM) 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 with a fluorescence microplate reader at Ex/Em = 540±10/590 ±10nm (maximum Ex/Em = 540/590 nm) or an absorbance microplate reader at 576±5 nm.

Kit Key Features

Broad Application:	Can be used for quantifying acetylcholine in solutions and in cell extracts.
Sensitive:	Detect as low as 0.01 nmoles of acetylcholine 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: Amplite Red™	1vial
Component B: Acetylcholine Probe	2 bottles (lyophilized powder)
Component C: Acetylcholine Standard	1vial
Component D: Assay Buffer	1 bottle (25 mL)
Component E: DMSO	1vial (100µL)

Assay Protocol for One 96-well Plate

Brief Summary

Prepare ACh Assay mixture (50 µL) → Add ACh standards or ACh test samples (50 µL) → Incubate at room temperature for 10-30 min → Read fluorescence intensity at Ex/Em = 540/590 nm

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

1. Prepare stock solutions:

1.1 Amplite Red™ stock solution (250X): Add 40 µL of DMSO (Component E) into the vial of Amplite Red™ (Component A) to make a 250X 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.

1.2 Acetylcholine stock solution: Add 200µL of ddH_2O into the vial of Acetylcholine Standard (Component C) to make 50 mM acetylcholine stock solution.
Note: The unused acetylcholine stock solution should be divided into single use aliquots and stored at -20 °C.

2. Prepare acetylcholine assay mixture:

2.1 Add 5 mL of Assay Buffer (Component D) to the bottle of Acetylcholine Probe (Component B) and mix well.

2.2 Add 20 µL of Amplite Red™ stock solution (250X, from Step 1.1) into the Acetylcholine Probe bottle (from Step 2.1) to make the acetylcholine assay mixture.
Note: The Assay mixture should be used promptly and kept from light. The assay background would increase with longer storage time.

3. Prepare serial dilutions of acetylcholine (0 to100µM) solutions:

3.1 Add 20 µL of 50 mM acetylcholine standard stock solution (from Step 1.2) to 980 µL Assay Buffer (Component D) to generate 1000 µM standard.
Note: Diluted acetylcholine standard solution is unstable, and should be used within 4 hours.

3.2 Take 200 µL of 1000 µM 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 µM standard acetylcholine solutions.

3.3 Add acetylcholine standards and acetylcholine 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 acetylcholine 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= Acetylcholine Standards; BL=Blank Control; TS=Test Samples

Table 2. Reagent composition for each well

Acetylcholine Standard	**Blank Control**	**Test Sample**
Serial dilutions* (50 µL)	Assay buffer: 50 µL	50 µL

**Note: Add the serially diluted acetylcholine standards from 0.01 to100 µM into wells from AS1 to AS7 in duplicate.*

4. Run acetylcholine assay:

4.1 Add 50 µL of acetylcholine assay mixture (from Step 2.2) to each well of the acetylcholine standard, blank control, and test samples (see Step 3.3) to make the total acetylcholine assay volume of 100 µL/well. *Note: For a 384-well plate, add 25 µL sample and 25 µL of acetylcholine assay mixture per 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 = 540/590 nm with a fluorescence microplate reader.

Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the acetylcholine reactions. An acetylcholine 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.*

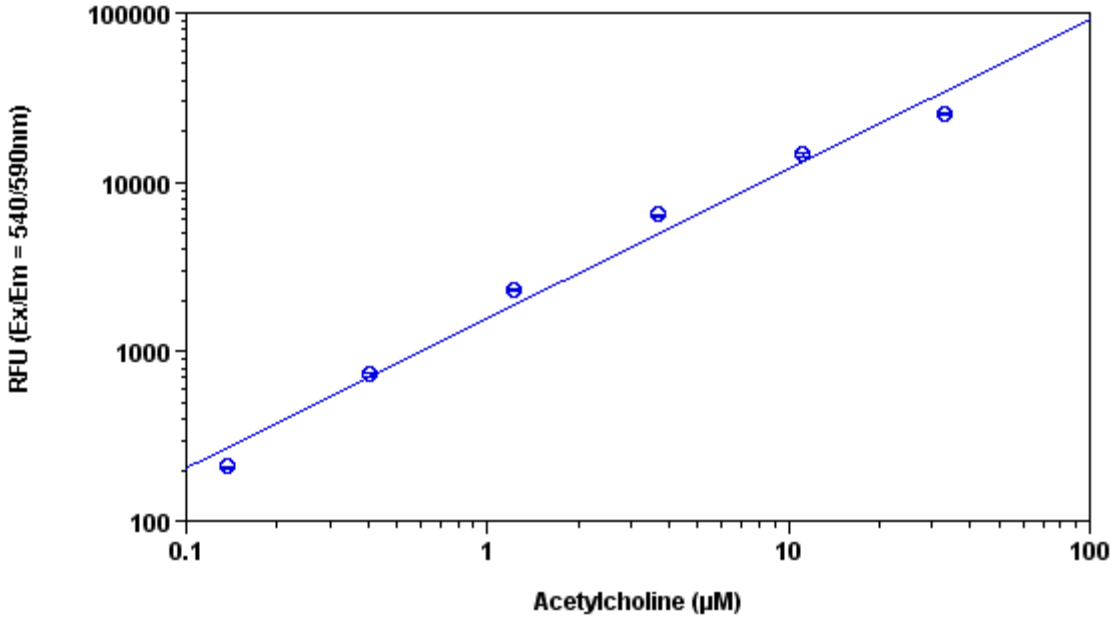

Figure 1. Acetylcholine dose response was measured in a 96-well black solid plate with Amplite™ Fluorimetric Acetylcholine Assay Kit (Cat. # 11403) using a Gemini fluorescence microplate reader (Molecular devices). As low as 0.01 nmoles/well (0.1µM) of acetylcholine can be detected with 10 minutes incubation 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.