Amplite™ Fluorimetric Acetylcholine Assay Kit *Red Fluorescence*

Catalog number: 11403
Unit size: 200 Tests

Component	Storage	Amount
Component A: Amplite™ Red	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Acetylcholine Probe	Freeze (< -15 °C), Minimize light exposure	2 bottles (lyophilized powder)
Component C: Acetylcholine Standard	Freeze (< -15 °C), Minimize light exposure	1 vial
Component D: Assay Buffer	Freeze (< -15 °C)	1 bottle (25 mL)
Component E: DMSO	Freeze (< -15 °C)	1 vial (100 µL)

OVERVIEW

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. It plays an important role in the central nervous system as a precursor for acetylcholine and membrane phosphatidylcholine. This Amplite™ Fluorimetric Acetylcholine Assay Kit provides one of the most sensitive methods for the quantifying acetylcholine. The kit uses Amplite Red™ to quantify acetylcholine through choline oxidase-mediated enzyme coupling reactions. The fluorescence intensity of Amplite Red™ is proportional to acetylcholine.

AT A GLANCE

Protocol Summary

1. Prepare ACh standards or ACh test samples (50 µL)
2. Add ACh working solution (50 µL)
3. Incubate at room temperature for 10 - 30 minutes
4. Monitor fluorescence intensity at Ex/Em = 540/590 nm (Cutoff = 570 nm)

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

KEY PARAMETERS

Fluorescence microplate reader

Excitation	540 nm
Emission	590 nm
Cutoff	570 nm
Recommended plate	Solid black

CELL PREPARATION

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

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

2. Acetylcholine standard solution (50 mM)
Add 200 µL of ddH_2O into the vial of Acetylcholine Standard (Component C) to make 50 mM Acetylcholine standard solution.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11403

Acetylcholine standard
Add 20 µL of 50 mM Acetylcholine standard solution to 980 µL Assay Buffer (Component D) to generate 1000 µM Acetylcholine standard solution. Take 1000 µM Acetylcholine standard and perform 1:10 in Assay Buffer (Component D) to get 100 µM Acetylcholine standard (AS7). Take 100 µM Acetylcholine standard (AS7) and 1:3 serial dilutions to get serially dilited of acetylcholine standard (AS6 - AS1) with Assay Buffer (Component D). Note: Diluted Acetylcholine standard solution is unstable, and should be used within 4 hours.

PREPARATION OF WORKING SOLUTION

1. Add 5 mL of Assay Buffer (Component D) to the bottle of Acetylcholine Probe(Component B) and mix well.
2. Add 20 µL of 250X Amplite Red™ stock solution into the bottle of AcetylcholineProbe solution to make Acetylcholine (ACh) working solution.

   Note This Acetylcholine (ACh) working solution should be used promptly andkept from light. The assay background would increase with longer storage time.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Acetylcholine standards and test samples in a solid black 96-well microplate. AS= Acetylcholine Standards (AS1 - AS7, 0.14 to 100 µM); 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 (0.14 to 100 µM)
BL	50 µL	Assay Buffer
TS	50 µL	test sample

1. Prepare Acetylcholine 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 Acetylcholine (ACh) working solution to each well of Acetylcholine standard, blank control, and test samples to make the

total Acetylcholine assay volume of 100 µL/well. For a 384-well plate, add 25 µL of Acetylcholine (ACh) working solution into each well instead, for a total volume of 50 µL/well.

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

4. Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 540/590 nm (Cutoff = 570 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) 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 Acetylcholine 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

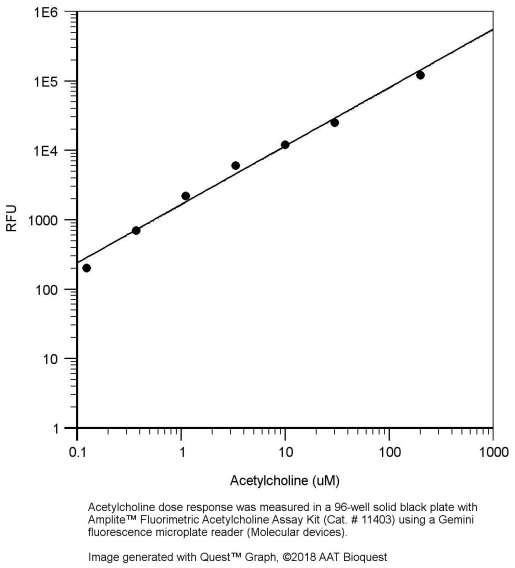

Figure 1. Acetylcholine dose response was measured in a 96-well solid black plate with Amplite™ Fluorimetric Acetylcholine Assay Kit (Cat. # 11403) using a Gemini fluorescence microplate reader (Molecular devices).

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