Amplite™ Choline Quantitation Kit

Catalog number: 40007
Unit size: 200 Tests

Component	Storage	Amount
Component A: Amplite™ Red	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Choline Probe	Freeze (<-15 °C), Minimize light exposure	2 bottles (lyophilized powder)
Component C: Choline Standard	Freeze (<-15 °C), Minimize light exposure	1 vial (2.8 mg)
Component D: Assay Buffer	Freeze (<-15 °C)	1 bottle (25 mL)
Component E: DMSO	Freeze (<-15 °C)	1 vial (100 µL)

OVERVIEW

Choline and its metabolites play an important role in the structural integrity and signaling of cell membranes and cholinergic neurotransmission (acetylcholine synthesis). It is a major source for methyl groups via its metabolite, trimethylglycine that participates in the S-adenosylmethionine synthesis pathways. Choline deficiency may cause liver disease, atherosclerosis and possibly neurological disorders. Despite its importance in the central nervous system as a precursor for acetylcholine and membrane phosphatidylcholine, the role of choline in mental illness has been little studied. This Amplite™ Choline Quantitation Kit provides one of the most sensitive methods for quantifying choline. The kit uses Amplite™ Red to quantify the concentration of choline, which is related to the production of hydrogen peroxide in the choline oxidase-mediated enzyme coupling reactions. The amount of choline is proportional to the concentration of hydrogen peroxide formed in the enzyme coupling reaction cycle. In the presence of peroxidase, the fluorescence intensity of Amplite™ Red is proportional to the formation of hydrogen peroxide that is converted to the concentration of choline. The assay can be readily read with a fluorescence microplate reader. Alternatively the assay can also be read at ~570 nm with an absorption microplate reader.

AT A GLANCE

Protocol summary

1. Prepare and add choline standards and/or test samples (50 µL)
2. Prepare and add Choline Assay working solution (50 µL)
3. Incubate at room temperature for 15-60 minutes
4. Monitor fluroscence intensity at Ex/Em = 540/590 nm

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

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 substrate (Component A). The stock solution should be used promptly. Any remaining solution should be aliquoted and refrozen at -20 °C.

Note Avoid repeated freeze-thaw cycles.

Note The Amplite™ Red substrate is unstable in the presence of thiols such as dithiothreitol (DTT) and 2-mercaptoethanol. The final concentration of DTT or 2-mercaptoethanol in the reaction should be no higher than 10 µM. The Amplite™ Red substrate is also unstable at high pH (> 8.5). Therefore, the reaction should be performed at pH 7–8. The provided Assay Buffer (pH 7.4) is recommended.

2. Choline standard stock solution (50 mM):
Add 400 µL of ddH_2O into the vial of Choline Standard (Component C) and mix well.

Note The unused choline stock solution should be divided into single use aliquotes and stored at -20°C.

PREPARATION OF STANDARD SOLUTION

Choline standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/40007

Add 20 µL of Choline standard stock solution (50mM) to 980 µL Assay Buffer (Component D) to generate 1000 µM standard solution.

Note Diluted choline standard solution is unstable, and should be used within 4 hours. Take 30 µL of 1000 µM standard to 970 µL Assay Buffer (Component D) to generate 30 µM choline standard solution, and then perform 1:3 serial dilutions to get 10, 3, 1, 0.3, 0.1, 0.03, and 0 µM choline standard. (Refer link in the Preparation of Standard Solution)

PREPARATION OF WORKING SOLUTION

Choline Assay working solution:
Add 5 mL of Assay Buffer (Component D) into the bottle of Choline Probe (Component B), and mix them well. Add 20 µL of Amplite Red™ stock solution (250X) into the Choline Probe.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Choline standards and test samples in a solid black 96-well microplate. CS = Choline standard (CS1-CS7); BL = blank control; TS = test sample.

BL	BL	TS	TS
CS1	CS1	...	...
CS2	CS2	...	...
CS3	CS3		
CS4	CS4		
CS5	CS5		
CS6	CS6		
CS7	CS7		

Table 2. Reagent composition for each well

Choline Standard	Blank Control	Test Sample

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

Serial Dilutions: 50 µL	Assay Buffer (Compound B): 50 µL	50 µL

Choline assay

1. Add choline standards and choline containing test samples into a 96-well solid black microplate as described in Tables 1 and 2.

2. Add 50 µL of Choline Assay working solution into each well of choline standard, blank control, and test samples (Table 2) to make the total choline assay volume of 100 µL/well.

   Note For a 384-well plate, add 25 µL of sample and 25 µL of assay reaction mixture into each well.

3. Incubate the reaction for 10 to 30 minutes at 37 ^{o}C, protected from light.

4. Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em= 530-570 nm/590-600 nm (optimal Ex/Em = 540/590 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 Choline 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

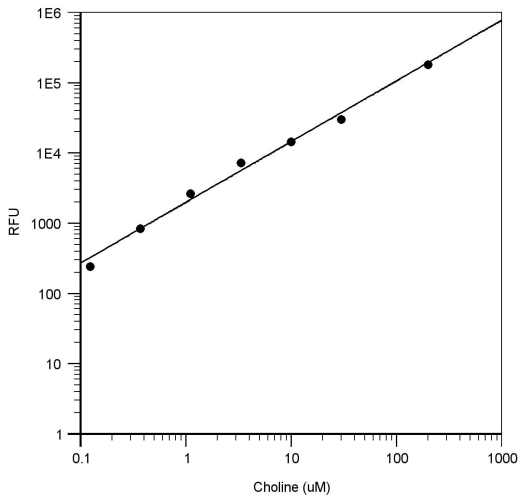

Figure 1. Choline dose response was obtained with Amplite™ Choline Quantitation Kit in a 96-well solid black plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 100 nM (10 picomole/well) of choline can be detected with 30 minutes incubation time (n=3).

DISCLAIMER