

Amplite® Choline Quantitation Kit

 Catalog number: 40007
 Unit size: 200 Tests

Component	Storage	Amount (Cat No. 40007)
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 fluorescence intensity at Ex/Em = 540/590 nm

Important Note

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

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

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.

Choline standard stock solution (50 mM)

Add 400 µL of ddH₂O 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 SOLUTIONS

For convenience, use the Serial Dilution Planner:

<https://www.aatbio.com/tools/serial-dilution/40007>

Choline standard

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

well	Volume	Reagent
CS1 - CS7	50 µL	Serial Dilutions (0.03 to 30 µM)

BL	50 μ L	Assay Buffer (Component D)
TS	50 μ L	test sample

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 μ L 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 μ L/well. *Note:* For a 384-well plate, add 25 μ L of sample and 25 μ L of assay reaction mixture into each well.
3. Incubate the reaction for 15 to 60 minutes at room temperature, 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

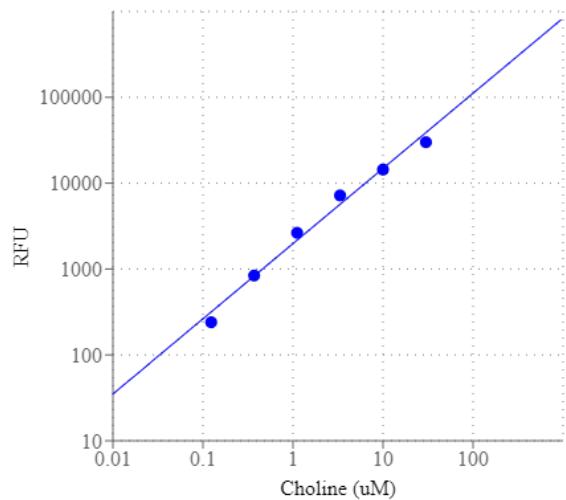


Figure 1. Choline dose response was obtained with Amplate® Choline Quantitation Kit in a 96-well solid black plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 100 nM (10 picomole/well) of choline can be detected with 30 minutes incubation time (n=3).

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.