Amplite™ Choline Quantitation Kit

Red Fluorescence

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 40007 (200 assays)	Keep in freezer Avoid light	Fluorescence microplate readers

Introduction

Choline is an essential nutrient. Choline and its metabolites play an important role in the structural integrity and signaling of cell membranes and cholinergic neurotransmission (choline synthesis). It is a major source of methyl group 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.

Our 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. The kit is an optimized "mix and read" assay that is compatible with HTS liquid handling instruments. It detects as little as 10 picomole choline in 100 µL assay volume (100 nM) as shown in Figure 1. 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 = ~540/590 nm. Alternatively the assay can also be read at ~576±5 nm with an absorbance microplate reader.

Kit Key Features

Broad Application:	Can be used for quantifying choline in solutions and in cell extracts.
Sensitive:	Detect as low as 10 picomole of choline in solution.
Continuous:	Easily adapted to automation without a separation step.
Convenient:	Formulated to have minimal hands-on time.

Kit Components

Components	Amount
Component A: Amplite™ Red	1vial
Component B: Choline Probe	2 bottles (lyophilized powder)
Component C: Choline Standard	1vial (2.8 mg)
Component D: Assay Buffer	1 bottle (25 mL)
Component E: DMSO	1vial (100µL)

Assay Protocol for One 96-well Plate

Brief Summary

Prepare choline assay mixture (50 µL) → Add choline standards or choline test samples (50 µL) → Incubate at RT for 15-60 min → Read fluorescence intensity at Ex/Em = 540/590 nm

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

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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 Choline stock solution: Add 400 µL of ddH_2O into the vial of Choline Standard (Component C) to make 50 mM choline stock solution.
Note: The unused choline stock solution should be divided into single use aliquots and stored at -20 °C.

2. Prepare choline assay mixture:

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

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

3. Prepare serial dilutions of choline standard (0 to 30 µM):

3.1 Add 20 µL of 50 mM choline standard stock solution (from Step 1.2) 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.

3.2 Take 30 µL of 1000 µM standard (from Step 3.1) 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.

3.3 Add choline standards and choline 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 choline standards and test samples in a solid black 96-well microplate*

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

**Note: CS= Choline Standards; BL=Blank Control; TS=Test Samples*

Table 2. Reagent composition for each well*

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

**Note: Add the serially dilutions of choline standards from 0.03 to30 µM into wells from CS1 to CS7 in duplicate.*

4. Run choline assay:

4.1 Add 50 µL of choline assay mixture (from Step 2.2) to each well of the choline standard, blank control, and test samples (see Step 3.3) to make the total choline assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL sample and 25 µL of choline 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 with a fluorescence microplate reader at Ex/Em = 540/590 nm (cut off at 570 nm).

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 choline reactions. A choline standard curve is shown in Figure 1. The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

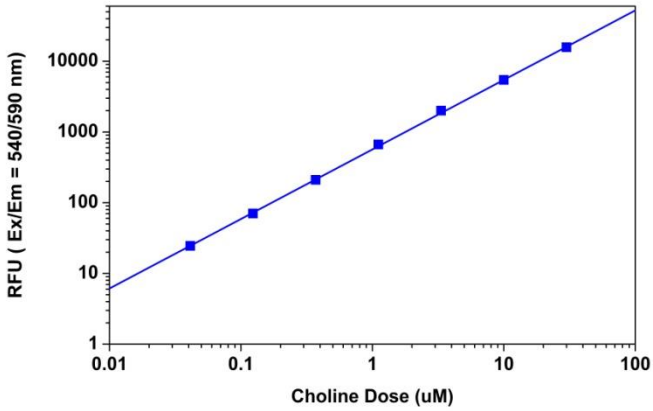

Figure 1. Choline dose response was obtained with Amplite™ Choline Quantitation Kit in a 96-well solid black plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 100 nM (10 picomole/well) of choline can be detected with 30 minutes incubation time (n=3).

References

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5. Panfili G, Manzi P, Compagnone D, Scarciglia L, Palleschi G. (2000) Rapid assay of choline in foods using microwave hydrolysis and a choline biosensor. J Agric Food Chem, 48, 3403.