Amplite™ Cholesterol Quantitation Kit

Catalog number: 40006
Unit size: 200 Tests

Component	Storage	Amount
Component A: Amplite™ Red (light sensitive)	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (20 mL)
Component C: Cholesterol Enzyme Mix (lyophilized)	Freeze (< -15 °C), Minimize light exposure	2 bottles
Component D: Cholesterol Standard	Freeze (< -15 °C), Minimize light exposure	1vial (2 mM, 100 µL)
Component E: DMSO	Freeze (< -15 °C)	1 vial (200 µL)

OVERVIEW

Cholesterol is required to build and maintain cell membranes. It modulates membrane fluidity over the range of physiological temperatures. Within cells, cholesterol is the precursor molecule in several biochemical pathways. Cholesterol is also an important precursor molecule for the synthesis of Vitamin D and the steroid hormones, including the adrenal gland hormones cortisol and aldosterone as well as the sex hormones progesterone, estrogens, together with testosterone and their derivatives. This Amplite™ Cholesterol Quantitation Assay Kit provides one of the most sensitive methods for quantifying cholesterol. The kit uses Amplite™ Red to quantify the concentration of cholesterol, which is related to the production of hydrogen peroxide in the cholesterol oxidase-mediated enzyme coupling reactions in the presence of cholesterol. The amount of cholesterol 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 concentration of hydrogen peroxide that is converted to the concentration of cholesterol. The assay can be readily read with a fluorescence microplate reader.

AT A GLANCE

Protocol Summary

1. Prepare Cholesterol Assay working solution (50 µL)
2. Add cholesterol standards and/or test samples (50 µL)
3. Incubate at 37°C for 30 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

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. Cholesterol standard stock solution (20 µM)
Add 10 µL of Cholesterol Standard (Component D) into 990 µL of Assay Buffer (Component B) and mix well.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/40006

Cholesterol standard
Prepare a cholesterol standard (20 µM). Then perform 1:3 serial dilutions in Assay Buffer (Component B) to get approximately 10, 3, 1, 0.3, 0.1, 0.03 and 0.01 µM serially diluted cholesterol standards. A non-cholesterol buffer control is included as blank control.

PREPARATION OF WORKING SOLUTION

Cholesterol Assay working solution
Add 5 mL of Assay Buffer (Component B) into the bottle of Cholesterol Enzyme Mix (Component C), and mix them well. Add 20 µL of Amplite Red™ stock solution (250X) into the Cholesterol Enzyme Mix bottle.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Cholesterol standards and test samples in a solid black 96-well microplate. CS = Cholesterol 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 (0.01 to 10 µM)
BL	50 µL	Assay Buffer (Component B)
TS	50 µL	test sample

Cholesterol assay

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

2. Add 50 µL of Cholesterol Assay working solution into each well of cholesterol standard, blank control, and test samples (Table 2) to make the total cholesterol 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 30 minutes at 37 ° 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). *Note:* The contents of the plate can also be transferred to a white clear bottom plate and read by an absorbance microplate reader at the wavelength of 576±5 nm. The absorption detection has lower sensitivity compared to the fluorescence reading.

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 Cholesterol 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

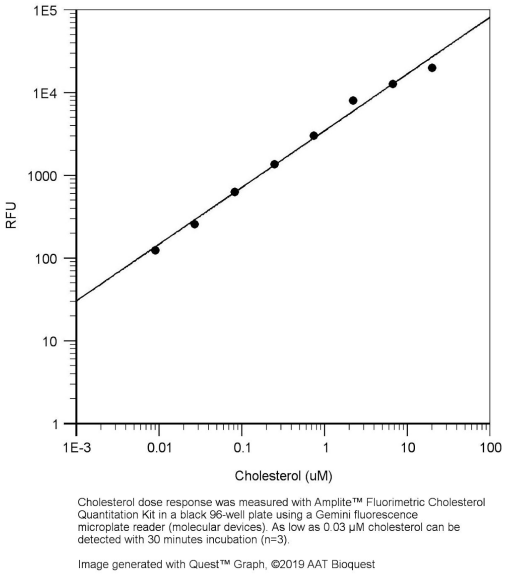

Figure 1. Cholesterol dose response was measured with Amplite™ Fluorimetric Cholesterol Quantitation Kit in a black 96-well plate using a Gemini fluorescence microplate reader (molecular devices). As low as 0.03 µM cholesterol can be detected with 30 minutes incubation (n=3).

DISCLAIMER

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