Amplite™ Cholesterol Quantitation Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 40006 (200 assays)	Keep at -20 °C and avoid exposure to light	Fluorescence microplate readers

Introduction

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™ Fluorimetric Cholesterol Quantitation 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 Ex/Em = ~540/590 nm.

Kit Components

Components	**Amount**
Component A: Amplite™ Red (light sensitive)	1 vial
Component B: Assay Buffer	20 mL
Component C: Cholesterol Enzyme Mix (lyophilized)	2 bottles
Component D: Cholesterol Standard	1vial (2 mM, 100 µL)
Component E: DMSO	1vial (200 µL)

Protocol for One 96-Well Plate

Brief Summary

Prepare assay reaction mixture (50 µL) → Add cholesterol standards or test samples (50 µL) → Incubate at room temperature or 37 °C for 30 minutes → Monitor fluorescence increase at Ex/Em = 540/590 nm

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

1. Prepare Amplite™ Red stock solution (250X):

Add 40 µL of DMSO (Component E) into the vial of Amplite™ Red (Component A). The stock solution should be used promptly. Any remaining solution needs to be aliquoted and refrozen at -20 °C.
Note 1: Avoid repeated freeze-thaw cycles.
Note 2: The Amplite™ Red 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 is also unstable at high pH (> 8.5). The reactions should be performed at pH 7–8. The provided assay buffer, pH 7.4, is recommended.

2. Prepare serially diluted cholesterol standards (0 to 10 µM):

2.1 Add 10 µL of 2 mM Cholesterol Standard (Component D) into 990 µL of Assay Buffer (Component B) to generate 20 µM cholesterol standard.
2.2 Take 300 µL of 20 µM cholesterol standard to perform 1:3 serial dilutions to get 10, 3, 1, 0.3, 0.1, 0.03, 0.01, and 0 µM serially diluted cholesterol standards.
2.3 Add serially diluted cholesterol standards and/or cholesterol containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.
Note: Treat the samples as desired.

Table 1. Layout of cholesterol standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	….	….						
CHL 1	CHL 1	….	….	….	….						
CHL 2	CHL 2										
CHL 3	CHL 3										
CHL 4	CHL 4										
CHL 5	CHL 5										
CHL 6	CHL 6										
CHL 7	CHL 7										

Note: CHL= Cholesterol Standards, BL=Blank Control, TS=Test Samples

Table 2. Reagent composition for each well

Cholesterol Standards	**Blank Control**	**Test Sample**
Serial dilutions*: 50 µL	Assay Buffer: 50 µL	50 µL

**Note: Add the serially diluted cholesterol standards from 0 to 10 µM into wells from CHL1 to CHL7 in duplicate.*

3. Prepare assay reaction mixture:

3.1 Add 5 mL of Assay Buffer (Component B) into the bottle of Cholesterol Enzyme Mix (Component C), and mix them well.

3.2 Add 20 µL of Amplite Red™ stock solution (250X, from Step 1) into the Cholesterol Enzyme Mix bottle (from Step 3.1).

4. Run cholesterol assay:

4.1 Add 50 µL of assay reaction mixture (from Step 3.2) into each well of cholesterol standard, blank control, and test samples (see Step 2.3, Table 1) 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.

4.2 Incubate the reaction at room temperature or 37 °C for 30 minutes, protected from light.

4.3 Monitor the fluorescence increase at Ex/Em = 530-570 nm /590-600 nm (optimal at Ex/Em = 540/590 nm) using a fluorescence plate reader.
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.

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

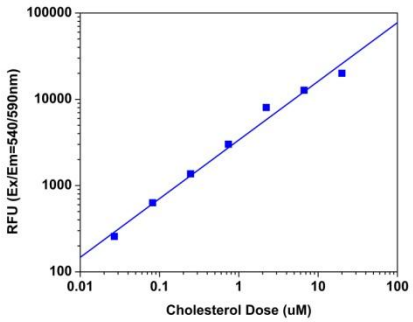

Figure 1 Cholesterol dose response was measured with Amplite™ Fluorimetric Cholesterol Quantitation Kit in a black 96-well plate using a Gemini fluorescence microplate reader (molecular devices). As low as 0.03 µM cholesterol can be detected with 30 minutes incubation (n=3).