Amplite® Fluorimetric Triglyceride Assay Kit *Red Fluorescence*

Catalog number: 40012
Unit size: 100 tests

Component	Storage	Amount (Cat No. 40012)
Component A: Amplite™ Red	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Lipase	Freeze (< -15 °C)	1 vial
Component C: Enzyme Mix	Freeze (< -15 °C)	1 bottle
Component D: Triglyceride Assay Buffer	Freeze (< -15 °C)	10 mL
Component E: Triglyceride Standard- 5 mM	Freeze (< -15 °C)	200 µL
Component F: DMSO	Freeze (< -15 °C)	100 µL

OVERVIEW

Amplite® Fluorimetric Triglyceride Assay Kit is a complete assay system designed for rapid and sensitive quantification of triglycerides in biological samples using a fluorescence-based detection format. The kit employs an enzymatic cascade that produces a stable fluorescent signal, which is easily measured at Ex/Em = 540/590 nm using a standard fluorescence plate reader.

This kit supports a wide range of sample types. With its straightforward workflow, strong signal stability, and high sensitivity, it is ideal for lipid metabolism research and drug development.

AT A GLANCE

1. Prepare test samples along with triglyceride standards (50 µL).
2. Add triglyceride working solution (50 µL).
3. Incubate at 30-60 minutes at RT.
4. Monitor fluorescence intensity at Ex/Em= 540 nm/590 nm (Cutoff = 570 nm).

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

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	570 nm
Emission	590 nm
Excitation	540 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

Amplite™ Red Stock Solution (100X):

Add 60 µL of DMSO (Component F) into Amplite™ Red (Component A) to make 100X Amplite™ Red stock solution.

Lipase Stock Solution (50X):

Add 110 µL ddH2O to Lipase vial (Component B) to make 50X Lipase stock solution.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/40012

Triglyceride Standard Dilution:
Add 80 µL 5mM Triglyceride standard (Components E) to 920 µL of PBS to make 400 µM of triglyceride standard (STD7). Take 500 µL (STD7) and perform 1:2 serial dilution in PBS to get 200, 100, 50, 25, 12.5, 6.25, and 0 µM serially diluted triglyceride standards (STD6 – STD1).

PREPARATION OF WORKING SOLUTION

Triglyceride Working Solution:

Add 5 mL Triglyceride Assay Buffer (Component D) to Enzyme Mix (Component C) and mix well. Add 100 µL of 50X Lipase Stock Solution and 50 µL of 100X Amplite™ Red Stock Solution to the bottle of Enzyme Mix.

Table 1: Preparation of 5 mL Triglyceride Working Solution

Assay Buffer (Component D)	5 mL
Enzyme Mix (Component C)	Whole bottle
50X Lipase Stock Solution	100 µL
100X Amplite™ Red Stock Solution	50 µL

Note 1: This Triglyceride working solution should be prepared freshly before the experiment, and kept away from light. 5 mL is sufficient for 100 tests.

Note 2: Alternatively, one can make a 12.5-25X of the Enzyme Mix stock solution by adding 400-200 µL of ddH2O into the bottle of Component C, and then prepare the working solution by mixing the stock solution with assay buffer, 50X lipase stock solution and Amplite™ Red stock solution proportionally. Aliquot and store the remaining stock solutions at -80°C.

SAMPLE EXPERIMENTAL PROTOCOL

Table 2. Layout of triglyceride standards and test samples in a black 96-well microplate with solid bottom. STD = Triglyceride Standards (STD1-STD7, 6.25 to 400 µM), BL=Blank Control, TS=Test Samples.

BL	BL	TS	TS
STD 1	STD 1	...	...
STD 2	STD 2	...	...
STD 3	STD 3		
STD 4	STD 4		
STD 5	STD 5		
STD 6	STD 6		
STD 7	STD 7		

Table 3. Reagent composition for each well.

Well	Volume	Reagent
STD1 - STD7	50 µL	Serial Dilutions (6.25 to 400 µM)
BL	50 µL	PBS
TS	50 µL	Test Sample

1. Prepare triglyceride standards (STD7 to STD1), blank controls (BL), and test samples (TS) according to the layout provided in Table 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 50 µL of Triglyceride working solution to each well of test sample, blank control, and triglyceride standards. For a 384-well plate, add 25 µL of Triglyceride working solution into each well instead.
3. Incubate at 30-60 minutes at RT, protected from light.
4. Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em = 540 nm/590 nm (Cutoff = 570 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

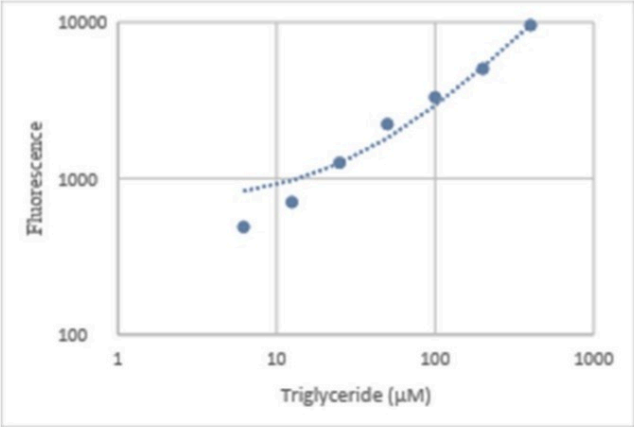

Figure 1. Dose response of triglyceride standard measured with Amplite® Fluorimetric Triglyceride Assay Kit on a 96-well solid black microplate using a Gemini microplate reader (Molecular Devices) at Ex/Em = 540 nm/590 nm (Cutoff = 570 nm).

DISCLAIMER

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