Amplite® Fluorimetric Lipase Activity Assay Kit

Catalog number: 11322
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 11322)
Component A: Lipase Assay Buffer	Freeze (< -15 °C)	25 mL
Component B: Lipase Substrate Buffer	Freeze (< -15 °C)	10 mL
Component C: Lipase Substrate	Freeze (< -15 °C), Minimize light exposure	0.25 mL
Component D: Lipase Standard	Freeze (< -15 °C), Minimize light exposure	1 Vial

OVERVIEW

The Amplite® Fluorimetric Lipase Activity Assay Kit provides a simple and quick protocol for measuring lipase activity. This assay leverages a coupled enzymatic reaction that converts a substrate into a fluorescent product (Ex/Em=540/610 nm), with fluorescence intensity proportional to the lipase activity present in the sample. This kit is suitable for detecting lipases from various biological samples like cells, supernatants, tissue extracts, or serum, and is adaptable for high-throughput screening applications. Lipases belong to a class of enzymes that catalyze the hydrolysis of ester bonds in lipids, such as triglycerides, phospholipids, and cholesterol esters. These enzymes are essential for the digestion, absorption, and metabolism of dietary fats in the body, playing crucial a role in lipid metabolism and cell signaling. Lipases are produced by various organs and tissues, including the pancreas, liver, intestine, and adipose tissue. Accurate measurement of lipase activity is critical in screening and diagnosis of diseases like pancreatitis, cystic fibrosis, celiac disease, Crohn's disease, and hyperlipidemia.

AT A GLANCE

Protocol Summary

1. Prepare test samples and the lipase standards (50 µL).
2. Add the lipase working solution (50 µL).
3. Incubate at 37 °C for 10-30 minutes.
4. Monitor the fluorescence intensity at Ex/Em = 540/610 nm (Cutoff = 570 nm).

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	570 nm
Emission	610 nm
Excitation	540 nm
Recommended plate	Solid black, or black plate with clear bottom

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

Lipase Standard Stock Solution

1. Reconstitute Lipase Standard (Component D) by adding 100 µL of ddH2O to achieve a concentration of 1 mg/mL. Mix thoroughly by pipetting up and down several times.

   Note: The Lipase Standard Stock Solution can be stored at -20 °C, protected from light and should be used within 1 month after reconstitution.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11322

Lipase Standard
Add 20 µL of Lipase Standard Stock Solution to 480 µL of Lipase Assay Buffer (Component A) to create a 40 µg/mL lipase standard solution (STD7). Take 250 µL of the STD7 solution and perform 2X serial dilutions in Lipase Assay Buffer (Component A) to produce a series of diluted lipase standards, labeled STD6 to STD1.

PREPARATION OF WORKING SOLUTION

Lipase Working Solution

1. Add 200 µL of Lipase Substrate (Component C) to 5 mL of Lipase Substrate Buffer (Component B). This 5 mL solution is sufficient for 100 tests. Prepare the required amount of Lipase Working Solution proportionally based on the number of tests you need.

Test Samples

1. Tissues and cells can be homogenized in the Lipase Assay Buffer (Component A). To remove insoluble material, centrifuge the sample at 13,000xg for 10 minutes. Serum samples can be added directly to the wells. Adjust the samples to a final volume of 50 µL using Lipase Assay Buffer.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of lipase standards and test samples in a 96-well clear bottom microplate. (STD = Lipase Standards (STD1-STD7, 0.625~40 µg/ml), BL= Blank Control, TS=Test Samples)

BL	BL	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 2. Reagent composition for each well.

Well	Volume	Reagent
Lipase STD 1- STD 7	50 µL	Serial Dilutions (0.625-40 µg/mL)
BL	50 µL	Assay Buffer
TS	50 µL	Test Sample

1. Prepare lipase standards (STD1-7), blank controls (BL), and test samples (TS) as outlined in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well.

2. Add 50 µL of Lipase Working Solution to each well containing the blank control, Lipase Standards, and test samples (TS). If using a 384-well plate, add 25 µL of Lipase Working Solution to each well instead.

3. Incubate at 37 °C for 10-30 minutes, protected from light.

4. Monitor the fluorescence intensity at Ex/Em = 540/610 nm (Cutoff = 570 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The fluorescence readings in blank wells (containing only assay buffer) serve as controls and are subtracted from the values obtained from wells containing the lipase standard and test samples. The standard curve for lipase is presented in Figure 1 To calculate the amount of lipase generated using this standard curve, we recommend using the Online Linear Regression Calculator available at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator.

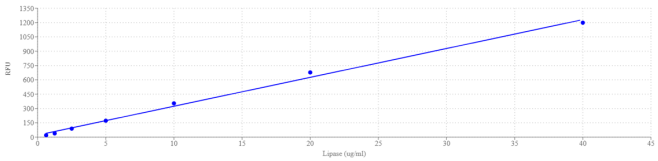

Figure 1. Lipase dose response was measured with the Amplite® Fluorimetric Lipase Activity Assay Kit on a 96-well clear bottom black solid microplate using a fluorescence microplate reader (Ex/Em = 540/610, Cutoff = 570 nm).

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