Amplite® Colorimetric Lipase Activity Assay Kit

Catalog number: 11315
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 11315)
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® Colorimetric Lipase Activity Assay Kit offers a straightforward and efficient protocol for quantifying lipase activity. This kit utilizes a coupled enzymatic reaction, which converts the substrate into a colored product (586 nm) proportional to the lipase activity present. It is suitable for detecting lipase activity in a variety of biological samples, including cells, supernatant, tissue extracts, and serum. 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, thereby playing a crucial role in lipid metabolism and cell signaling. Lipases are produced by various organs and tissues, including the pancreas, liver, intestine, and adipose tissue. Monitoring 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. Measure the absorbance at 586 nm.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	586 nm
Recommended plate	Black or white 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/11315

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 absorbance at 586 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The absorbance reading in blank wells (containing only assay buffer) serves as a control and is subtracted from the readings of wells with the lipase standard and test samples. The standard curve for lipase is displayed 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.

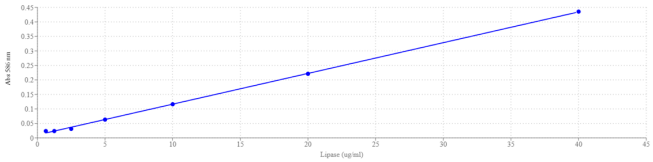

Figure 1. Lipase dose response was measured with the Amplite® Colorimetric Lipase Activity Assay Kit in a 96-well white/clear bottom plate using a SpectraMax microplate reader (Molecular devices).

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