MA-0149

Lipase Activity Assay I

(100 wells, Colorimetric, OD 450 nm or Fluorometric, Ex/Em = 535/587 nm, Store at -20°C)

Introduction:

Lipases play a pivotal role in the digestion, absorption, and metabolism of dietary fats in organisms. In the human digestive system, lipases break down triglycerides into fatty acids and glycerol, enabling efficient nutrient absorption in the intestine. Lipase is a key regulator of energy balance. By releasing fatty acids from adipose tissue during periods of energy demand, lipases enable the body to access stored energy reserves. This is particularly crucial during fasting or physical activity when glucose availability is limited. Lipase activity influences lipid-related disorders such as obesity, diabetes, and cardiovascular diseases. Dysregulation of lipase function can lead to abnormal lipid profiles and contribute to the development of these conditions. AkrivisBio's Lipase Activity Assay I is a simple sensitive way to measure triglyceride lipase activity in a variety of biological samples.

Assay Principle:

Triglycerides are hydrolyzed by lipase forming free fatty acids and glycerol.
Glycerol is phosphorylated by glycerol kinase, then the glycerol phosphate is oxidized by glycerol phosphate oxidase forming H_2O_2.
Peroxidase utilizes hydrogen peroxide to oxidize ADHP to resorufin with the development of color at 570 nm and fluorescence (535/587 nm).

Assay Components:

Assay Buffer	25 ml	WM	MA-0149A
ADHP Solution	200 µl	Red	MA-0149B
GK/GPO/HRP	lyoph	Green	MA-0149C
Triolein	400 µl	Blue	MA-0149D
Glycerol Standard (0.4 mM)	500 µl	Yellow	MA-0149E
Lipase Positive Control	lyoph	Purple	MA-0149F

Storage and Handling Considerations:

Store the unopened assay at -20°C. Allow components to come to room temperature before use. Centrifuge all vials for a few seconds before opening.
Assay buffer: Ready to use as supplied. Warm to 37°C before use. Store at 4°C.
ADHP Solution: Ready to use as supplied. Warm to room temperature before use. Store at -20°C.
GK/GPO/HRP: Reconstitute with 220 µl Assay Buffer. Aliquot to convenient portions and store at -20°C to avoid repeated freeze/thaw cycles.
Triolein: Freezing for storage may cause the triolein to separate into two phases. To redissolve, place vial in hot water (80 - 100°C) for 1-2 minutes until the solution gets cloudy. Vortex as it cools. Repeat. The solution should now be clear.
Lipase Positive Control: Reconstitute in 100 µl of Assay Buffer. Dilute 1:10 and use 1 – 5 µl as Positive Control. Store at -20°C

Assay Protocol:

1. Standard Curve:
Add 0 – 5 – 10 – 15 – 20 – 25 µl of the glycerol standard to a series of wells in a 96-well plate. Adjust all well volumes to 50 µl with Assay Buffer giving 0, 2, 4, 6, 8 and 10 nmol of glycerol.
2. Samples:
Homogenize tissue (10 mg) or cells (10^6) in 150 µl of Assay Buffer. Centrifuge at 16,000 X g, for 10 minutes to pellet insoluble material. Transfer the clear supernatant to a fresh tube avoiding any floating lipids. Serum and other liquid samples can be used directly in the assay. Transfer up to 50 µl of each sample to wells of a 96-well plate.
Note: Some Lipases require calcium. If your lipase requires calcium avoid EGTA in sample preparation and add calcium (1 - 5 mM) to the Lipase assay buffer before use. Glycerol in the sample causes a constant background which does not interfere with a constantly increasing lipase activity.
3. Reaction Mix: Each well will require 100 µl of Reaction Mix. Prepare sufficient material for the number of wells (Standards, Samples, Background Controls, Positive Controls) to be run, containing:

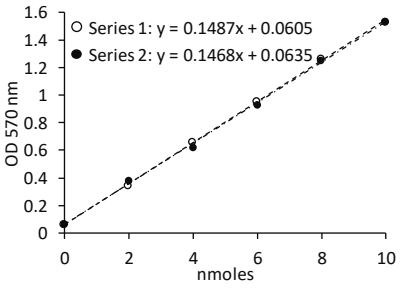
	Reaction Mix
Assay Buffer	93 µl
ADHP Solution	2 µl
GK/GPO/HRP	2 µl
Triolein	3 µl

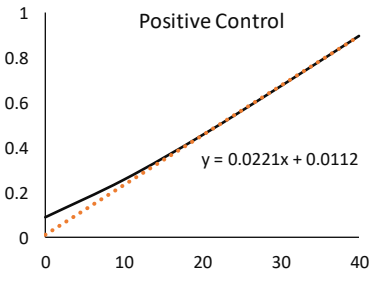
Add 100 µl of the Reaction Mix to each well.

4. Measurement: Monitor change in OD at 570 nm for 60 – 90 minutes.

5. Typical Results:

nmole Standard	Standard Raw Values OD		Background Corrected Values	
0	0.0631	0.0634	0	0
2	0.3445	0.3805	0.2814	0.3171
4	0.6610	0.6221	0.5979	0.5587
6	0.9553	0.9323	0.8922	0.8689
8	1.2662	1.2538	1.2031	1.1904
10	1.5324	1.5323	1.4693	1.4689





6. Calculations: Subtract the zero standard value from all standards and test samples. Plot the zero corrected Standard Curve. Determine the slope of the Standard Curve. This defines the sensitivity of the system. Observe the test samples and determine the time range of linear increase. There is typically a ~15 minute lag before the reaction rate is linear. If the rate is too fast, the lipase activity will deplete too much substrate and the rate will start to slow down. Take the linear portion of the rate curve and determine the slope in OD/minute. Divide the slope of the rate curve by the slope of the Standard Curve to get enzyme rate in nmol/minute (mUnits) in the well. To convert back to activity in the original sample:

A – Divide the mU in the well by the volume of sample added to the well in µl = mU/µl of sample.
B – Multiply the mU/µl of sample by the total volume of clear supernatant in step 2 above = Total mU of activity in the sample.
C – Divide the Total mU of activity in the sample by the mg of tissue used (or # of cells, etc.) = mU/mg of tissue (or /# of cells, etc.)

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