



Lipase Assay Kit

Catalog Number KA0877

100 assays

Version: 06

Intended for research use only

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Introduction

Background

Lipases perform essential roles in the digestion, transport and processing of dietary lipids (e.g. fats and oils) in living organisms. In humans, pancreatic lipase is the key enzyme responsible for breaking down fats in the digestive system by converting triglyceride to monoglyceride and free fatty acid. Pancreatic lipase monitoring is also used to help diagnose Crohn's disease, cystic fibrosis and celiac disease. Damage to the pancreas can exhibit a 5 - 10 fold increase of serum lipase levels within 24 to 48 hours. In Lipase Assay Kit, lipase hydrolyzes a triglyceride substrate to form glycerol which is quantified enzymatically by via monitoring a linked change in the OxiRed probe absorbance ($\lambda = 570\text{nm}$). This assay is rapid, simple, sensitive, and reliable, as well as, suitable for high throughput activity screening of lipase. This kit detects lipase activity as low as 0.02 mU per well.

General Information

Materials Supplied

List of component

Component	Amount
Lipases Assay Buffer	25 ml
OxiRed (in DMSO)	0.2 ml
Enzyme Mix (lyophilized)	1 vial
Lipase Substrate	0.4 ml
Glycerol Standard (100 mM)	0.2 ml
Lipase positive control (lyophilized)	1 vial

Storage Instruction

Store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

Assay Protocol

Reagent Preparation

- ✓ Probe: Ready to use as supplied. Warm to room temperature to melt frozen DMSO before use. Store at -20°C, protect from light and moisture.
- ✓ Enzyme Mix: Dissolve in 220 µl Assay Buffer. Partition into aliquots in vials, and store at -20°C. Use within two months.
- ✓ Lipase Substrate: Freezing for storage may cause the substrate to separate from the aqueous phase. To re-dissolve the substrate, keep the cap tightly closed, thaw, then place in a hot water bath (80 - 100°C) for 1 minute until the substrate looks cloudy, then vortex for 30 seconds. The substrate should be clear. Repeat the heat and vortex on more time. The substrate is now completely in solution, and ready for use.
- ✓ Lipase positive control: Dissolve the positive control in 100 µl Assay Buffer. Add 5 µl and adjust the volume to 50 µl/well with Assay Buffer as positive control. Store at -20°C.

Assay Procedure

1. Standard Curve Preparation

Add 10 µl of the glycerol standard to 990 µl of Assay Buffer to generate 1 mM glycerol standard, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of glycerol Standard.

2. Sample Preparations

Tissue (40 mg) or cells (2×10^6) can be homogenized in 4 volumes of Assay Buffer. Centrifuge to remove insoluble material at 13,000 x g for 10 minutes. Serum sample can be directly diluted in the Assay Buffer. Prepare test samples of up to 50 µl/well with Assay Buffer in a 96-well plate. We suggest testing several doses of your sample to make sure readings are within the standard curve. Glycerol in the sample will interfere with the result. It is corrected for by using a (substrate deficient) control for the sample.

Note: some Lipase 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 will interfere with the result. It is corrected for by using a (substrate deficient) control for the sample.

3. Reaction Mix

Mix enough reagents for the number of assays to be performed. For each well, prepare a total 100 µl Reaction Mix.

Component	Sample	Control
Assay Buffer	93 μ l	96 μ l
OxiRed Probe	2 μ l	2 μ l
Enzyme Mix	2 μ l	2 μ l
Lipase substrate	3 μ l	--

Add 100 μ l of the Sample Reaction Mix to each well containing the Glycerol Standards, Lipase positive controls, and test samples. Add 100 μ l Control Reaction Mix to each well containing the sample controls. Mix well.

4. Incubate:

Measure O.D.570 nm at T_1 to read A_1 , measure O.D.570 nm again at T_2 after incubating the reaction at 37°C for 60 - 90 min (or incubate longer time if the Lipase activity is low) to read A_2 . Protect from light.

Data Analysis

Calculation of Results

The OD generated by oxidation of glycerol is $\Delta A_{570\text{ nm}} = A_2 - A_1$. Subtracting the OD 570 nm value of control from the sample to avoid glycerol in the sample. Plot Glycerol Standard Curve, Apply the $\Delta A_{570\text{ nm}}$ to the glycerol standard curve to get B nmol of glycerol (glycerol amount generated between T_1 and T_2 in the reaction wells). Glycerol generated in the test samples can then be calculated:

$$\text{Lipase Activity} = \left[\frac{(B \times \text{Dilution factor})}{(T_2 - T_1) \times V} \right] = \text{nmol/min/ml} = \text{mU/ml}$$

Where:

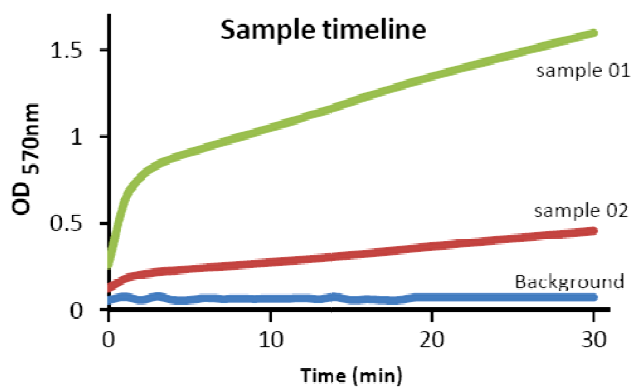
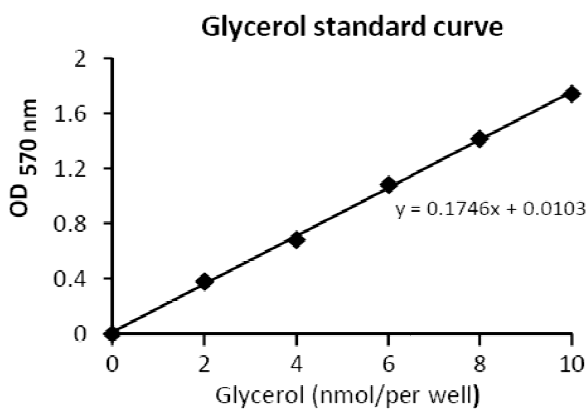
B is the Glycerol Amount from the Standard Curve (in nmol).

T_1 is the time of the first reading (A_1) (in min).

T_2 is the time of the second reading (A_2) (in min).

V is the pretreated sample volume added into the reaction well (in ml).

Unit Definition: One unit is defined as the amount of lipase that hydrolyzes triglyceride to yield 1.0 μmol of glycerol per minute at 37°C.



Resources

Troubleshooting

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed • Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly

<p>Readings do not follow a linear pattern for Standard curve</p>	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
<p>Unanticipated results</p>	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range

Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.