Product Information

Lipase Activity Colorimetric Assay Kit

I. Kit Contents:

Components	K2227-100 100 assays	Cap Color	Part Number
Lipase Assay Buffer	25 ml	WM	K2227-C-1
OxiRed™ (in DMSO)	200 µl	Red	K2227-C-2
Enzyme Mix (lyophilized)	1 vial	Green	K2227-C-3
Lipase Substrate	400 µl	Blue	K2227-C-4
Glycerol Standard (100 mM)	200 µl	Yellow	B7776
Lipase Positive Control (lyophilized)	1 vial	Purple	K2227-C-5

II. Introduction:

Lipase is an enzyme that catalyzes fats (lipids) hydrolysis and is a subclass of the esterases. Lipase plays important roles in the digestion, transport and processing of dietary lipids (e.g. oils, triglycerides, fats) in most living organisms. In human digestive system, pancreatic lipase is the key enzyme that converts triglycerides to monoglycerides and free fatty acids. In the damaged pancreas, lipase levels can rise 5 to 10-fold within 24 to 48 hours.

The Lipase Activity Colorimetric Assay Kit provides a sensitive, easy and fast way for detection of lipase activity in various samples based on colorimetric method. In the assay, lipase in the sample hydrolyzes a triglyceride substrate to produce glycerol, which is quantified enzymatically through monitoring a linked change in the OxiRed probe absorbance (λ = 570 nm). The kit is suited for high throughput screening assay of lipase activity and can detect lipase activity as low as 10 mU/well.

III.Storage and Handling:

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.

IV. 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 redissolve 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, vortex for 30 seconds. The substrate should be clear. Repeat heat and vortex one 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.

V. Lipase Assay Protocol:

1. Standard Curve Preparation:

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

2. Sample Preparations:

Tissues (40 mg) or cells (2 x 10^6) can be homogenized in 4 volumes of Assay Buffer. Centrifuge to remove insoluble material at 13,000 x g, 10 min.

Serum samples 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 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 will interfere with the result. It is corrected for by using a (substrate deficient) control for the sample.

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

	Sample	Control
Assay Buffer	93 µl	96 µl
OxiRed Probe	2 µl	2 µl
Enzyme Mix	2 µl	2 µl
Lipase substrate	3 µl	---

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

4. Incubate: Measure OD 570 nm at T1 to read A1, measure OD 570 nm again at T2 after incubating the reaction at 37°C for 60 - 90 min (or incubate longer time if the Lipase activity is low) to read A2, protect from light.

5. Calculation: The OD generated by oxidation of glycerol is ΔA570 nm = A2 - A1.Subtracting the OD 570 nm value of control from the sample to avoid glycerol in the sample. Plot Glycerol Standard Curve, Apply the ΔA570 nm to the glycerol standard curve to get B nmol of glycerol (glycerol amount generated between T1 and T2 in the reaction wells).Glycerol generated in the test samples can then be calculated:

Lipase Activity = (B x Dilution factor)/[(T2 - T1) x V] = nmol/min/ml = mU/ml

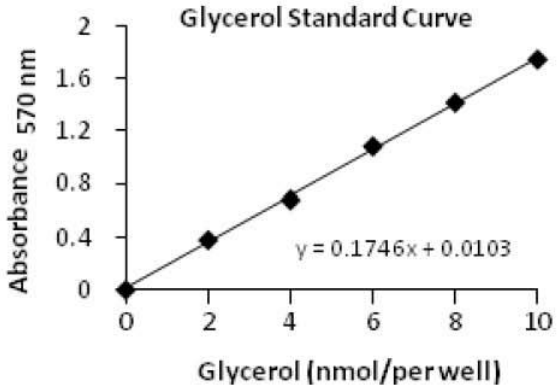
Where: B is the Glycerol amount from the Standard Curve (in nmol).

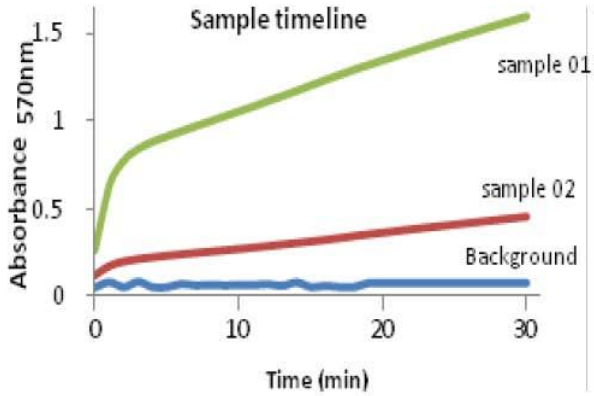
T1 is the time of the first reading (A1) (in min).

T2 is the time of the second reading (A2) (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 1.0µmol of glycerol per minute at 37°C





General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• 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 ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• 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	• 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, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• 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	• 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 data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• 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	• 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
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• 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.		

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