Product Information

Lipase Activity Fluorometric Assay Kit III

I. Kit Contents:

Components	K2095-100 100 assays	Cap Color	Part Number
Lipase Assay Buffer	25 ml	WM	K2095-C-1
Lipase Substrate	0.2 ml	Red	K2095-C-2
Methylresorufin Standard (0.1 mM)	40 µl	Yellow	K2095-C-3
Lipase Positive Control (lyophilized)	1 Vial	Purple	K2095-C-4

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 Fluorometric Assay Kit III provides a highly sensitive, easy and fast way for detection of lipase activity in various samples based on fluorometric method. In the assay, lipase in the sample hydrolyzes a specific substrate to produce the methylresorufin, which can be detected at Em/Ex = 529/600 nm by fluorometric method. The kit is suited for high throughput screening assay of lipase activity and can measure lipase activity as low as 0.1 mU/well.

III. Storage and Handling:

Store the kit at -20℃, 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:

Lipase Positive Control: Reconstitute with 100 µl assay buffer. Mix 2 µl Positive Control with 998 µl Lipase Assay Buffer; add 2 µl of the diluted Positive Control into a well and adjust the volume to 50 µl/well with Lipase Assay Buffer. Discard the remaining diluted Positive Control after each use. Aliquot and store the reconstituted Positive Control solution at -20℃. Use within two months.

V. Lipase Assay Protocol:

1. Standard Curve Preparation: Add 10 µl of the 0.1mM Methylresorufin Standard to 90 µl Lipase Assay Buffer to generate a 10 µM standard solution. Add 0, 2, 4, 6, 8, 10 µl to each well individually. Adjust the volume to 100 µl/well with Lipase Assay Buffer to generate 0, 20, 40, 60, 80, 100 pmol/well of Methylresorufin Standard. Read fluorometrically at Ex/Em = 529/600 nm.

2. Sample Preparations: Tissues (50 mg) or cells (1×10^6) can be homogenized in ~ 200 µl ice-cold Lipase Assay Buffer then centrifuged to remove insoluble material at 13,000 x g, 10 min. Serum sample can be directly diluted in the Lipase Assay Buffer. Prepare test samples of up to 50 µl/well with Lipase Assay Buffer in a 96-well plate. We suggest testing several doses of your sample to make sure the readings are within the standard curve range.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix.

Assay Buffer 48 µl

Lipase substrate 2 µl

Add 50 µl of the Reaction Mixes to each well containing the samples and positive controls. Mix well. Include a reagent background control by adding 50 µl assay buffer to 50 µl reaction mix into a well.

4. Measurement: Read Ex/Em = 529/600nm R1 for sample and R1B for background control at T1. Read R2 for sample and R2B for background control again at T2 after incubating the reaction at 37℃ for 30 - 60 min (or incubate longer time if the Lipase activity is low), protect from light. The fluorescence generated by the hydrolysis of the Lipase substrate is ΔRFU = (R2 - R2B) - (R1 -R1B). It is recommended to read the fluorescence kinetically to choose the R1 and R2 within the linear range of the standard curve.

5. Calculation: Subtract the 0 Standard from all Standard readings.

Plot the Standard Curve.

Apply the ΔRFU to the standard curve to get B nmol of methylresorufin (amount of methylresorufin generated between T1 and T2 in the reaction wells):

Lipase Activity = B/[(T2-T1)× V]×Sample Dilution Factor = nmol/min/ml = mU/ml

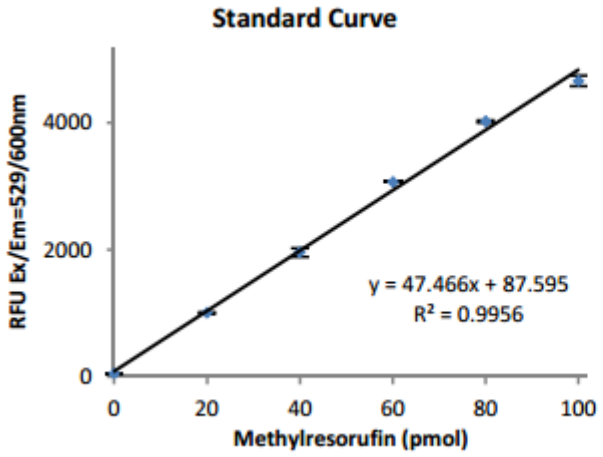
Where: B is the methylresorufin amount from Standard Curve (in nmol).

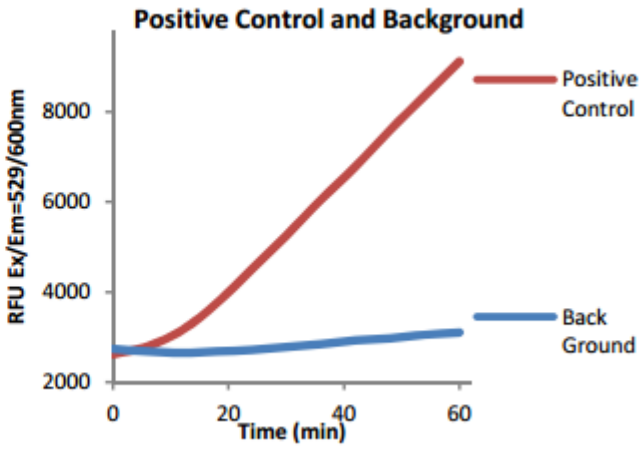
T1 is the time of the first reading (R1) (in min).

T2 is the time of the second reading (R2) (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 enzyme that hydrolyzes the substrate to yield 1.0 µmol of methylresorufin per minute at 37℃.





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	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet

	• 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	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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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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