Lipase Activity Fluorometric Assay Kit III

(Catalog #BN00943; 100 assays; Store kit at -20°C)

I. Introduction:

Lipase is a subclass of the esterases that catalyze the hydrolysis of ester bonds of lipid substrates. Lipases perform essential roles in the digestion, transport and processing of dietary lipids (e.g. triglycerides, fats, oils) in most, if not all, living organisms. In humans, pancreatic lipase is the key enzyme responsible for breaking down fats in the digestive system by converting triglycerides to monoglycerides and free fatty acids. During the damage of the pancreas, lipase levels can rise 5- to 10-fold within 24 to 48 hours. In Assay Genie's Lipase Assay Kit III, Lipase hydrolyzes a specific substrate to generate the methylresorufin, which can be detected fluorometrically at Ex/Em = 529/600 nm. The kit provides a rapid, simple, more sensitive, and reliable test suitable for high throughput assay of Lipase activity. This kit can be used to detect Lipase as low as 0.1 µU/well.

II. Kit Contents:

Components	100 assays	Cap Code	Part Number
Lipase Assay Buffer	25 ml	WM	BN00943-1
Lipase Substrate	200 µl	Red	BN00943-2
Methylresorufin Standard (0.1 mM)	40 µl	Yellow	BN00943-3
Lipase Positive Control (lyophilized)	1 vial	Purple	BN00943-4

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:

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°C. 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 = 529/600nm.

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.
48 µl Assay Buffer
2 µl Lipase substrate
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.

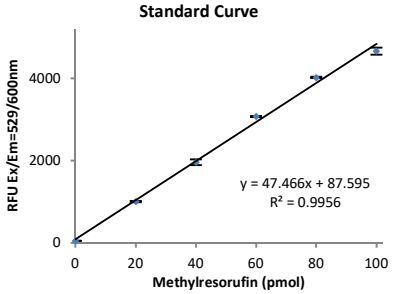
4. **Measurement:** Read Ex/Em = 529/600nm R_1 for sample and R_{1B} for background control at T_1. Read R_2 for sample and R_{2B} for background control again at T_2 after incubating the reaction at 37°C for 30 - 60 min (or incubate longer time if the Lipase activity is low), protect from light. The fluorescence generated by the hydrolysis of the Lipase substrate is $\Delta RFU = (R_2 - R_{2B}) - (R_1 - R_{1B})$. It is recommended to read the fluorescence kinetically to choose the R_1 and R_2 within the linear range of the standard curve.

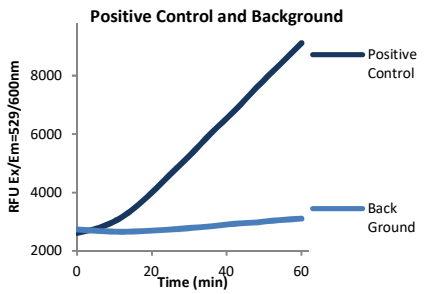
5. **Calculation:** Subtract the 0 Standard from all Standard readings. Plot the Standard Curve. Apply the ΔRFU to the standard curve to get B nmol of methylresorufin (amount of methylresorufin generated between T_1 and T_2 in the reaction wells):

Lipase Activity = $\frac{B}{(T2-T1)\times V}$ **×Sample Dilution Factor = nmol/min/ml = mU/ml**

Where: B is the methylresorufin amount from Standard Curve (in nmol).
T_1 is the time of the first reading (R_1) (in min).
T_2 is the time of the second reading (R_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 enzyme that hydrolyzes the substrate to yield 1.0 µmol of methylresorufin per minute at 37°C.





FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• 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.		