3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Lipase Activity Assay Kit III

Catalog Number **MAK048**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
The lipase family of enzymes catalyzes the cleavage of the ester bonds of lipids. In mammals, this family includes many critical members including pancreatic, hepatic, endothelial, and lipoprotein lipase. Lipases, such as pancreatic lipase, are critical for the metabolism of lipids. Lipases also play key roles in processes such as cell signaling and inflammation. Measurements of lipase activity are commonly used to screen for pancreatic injury or disease, and to monitor diseases such as cystic fibrosis, celiac disease, and Crohn's disease.

The Lipase Activity Assay Kit III provides a simple and direct procedure for measuring lipase activity in a variety of samples. Lipase activity is determined using a coupled enzyme reaction, which results in the generation of methylresorufin (λ_{ex} = 529/λ_{em} = 600 nm) proportional to the enzymatic activity present. One unit of lipase is the amount of enzyme that will generate 1.0 µmole of methylresorufin from the substrate per minute at 37 °C.

Components
The kit is sufficient for 100 assays in 96 well plates.

Component	Amount
Lipase Assay Buffer Catalog Number MAK048A	25 mL
Lipase Substrate Catalog Number MAK048B	0.2 mL
Methylresorufin Standard, 0.1 mM Catalog Number MAK048C	40 µL
Lipase Positive Control Catalog Number MAK048D	1 vl

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Lipase Assay Buffer – Allow buffer to come to room temperature before use.

Lipase Positive Control – Reconstitute with 100 µL of Lipase Assay Buffer. Mix well by pipetting (Do Not Vortex), then aliquot and store, protected from light at –20 °C. Use within 2 months of reconstitution.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Methylresorufin Standards for Fluorometric Detection
Dilute 10 µL of the 0.1 mM Methylresorufin Standard with 90 µl of the Lipase Assay Buffer to prepare a 10 µM standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 10 µM standard solution into a 96 well plate, generating 0 (blank), 20, 40, 60, 80, and 100 pmole/well standards. Add Lipase Assay Buffer to each well to bring the volume to 100 µL.

Sample Preparation

Tissue (50 mg) or cells (1×10^6) can be homogenized in 200 μL of ice-cold Lipase Assay Buffer. Centrifuge the samples at $13,000 \times g$ for 10 minutes to remove insoluble material.

Serum samples can be directly added to wells.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Bring samples to a final volume of 50 μL with Lipase Assay Buffer.

For the positive control (optional), add 2 μL of the Lipase positive control solution to 998 μL of buffer. Add 2 μL of the diluted Positive Control into the wells and adjust to 50 μL with the Lipase Assay Buffer. Discard the remaining diluted Positive Control.

Prepare a background control (Sample Blank) by adding 50 μL of Lipase Assay Buffer to a well.

Assay Reaction

1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 μL of the Master Reaction Mix is required for each sample and control well.

Table 1.
Master Reaction Mix

Reagent	Volume
Lipase Assay Buffer	48 μL
Lipase Substrate	2 μL

2. Add 50 μL of the Master Reaction Mix to each of the sample and control wells. Mix well using a horizontal shaker or by pipeting.

3. Incubate the plate at 37 °C. After 2–3 minutes, take the initial measurement ($T_{initial}$). Measure the fluorescence intensity ($FLU_{initial}$, λ_{ex} = 529/ λ_{em} = 600 nm).
   Note: It is essential $(FLU)_{initial}$ is in the linear range of the standard curve.

4. Continue to incubate the plate at 37 °C taking measurements (FLU) every 5 minutes. Protect the plate from light during the incubation.

5. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (10 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.

6. The final measurement [(FLU_{final}] for calculating the enzyme activity would be penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve, see step 5. The time of the penultimate reading is T_{final}.
   Note: It is essential the final measurement falls within the linear range of the standard curve.

Results

Calculations

Correct for the background by subtracting the final measurement [(FLU_{final}] obtained for the 0 (blank) methylresorufin standard from the final measurement [FLU_{final}] of the standards and samples. Background values can be significant and must be subtracted from all readings. Plot the standard curve.

Note: A new standard curve must be set up each time the assay is run.

Calculate the change in measurement from $T_{initial}$ to T_{final} for the samples.

$$\Delta FLU = FLU_{final} - FLU_{initial}$$

Also, subtract the Sample Blank Δmeasurement value from the sample Δmeasurement values. Compare the ΔFLU of each sample to the standard curve to determine the amount of methylresorufin generated by the lipase assay between $T_{initial}$ and T_{final} (B).

The Lipase activity of a sample may be determined by the following equation:

$$\text{Lipase Activity} = \frac{B \times \text{Sample Dilution Factor}}{(\text{Reaction Time}) \times V}$$

B = Amount (nmole) of methylresorufin generated between $T_{initial}$ and T_{final}.

Reaction Time = $T_{final} - T_{initial}$ (minutes)

V = sample volume (mL) added to well

Lipase activity is reported as:

nmole/min/mL = milliunit/mL

One unit of Lipase is the amount of enzyme that will generate 1.0 μmole of methylresorufin from the substrate per minute at 37 °C.

Example:

Methylresorufin amount (B) = 5.84 nmole

First reading ($T_{initial}$) = 3 minute

Second reading (T_{final}) = 32 minutes

Sample volume (V) = 0.05 mL

Sample dilution is 1

Lipase activity is:

$$\frac{5.84 \times 1}{(32-3) \times 0.05} = 4.02 \text{ milliunits/mL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For Fluorometric assays, use black plates with clear bottoms
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly 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	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	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 plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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