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Product Information

Lipase Activity Assay Kit

Catalog Number **MAK046**
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 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 a colorimetric (570 nm) product proportional to the enzymatic activity present. One unit of Lipase is the amount of enzyme that will generate 1.0 μmole of glycerol from triglycerides per minute at 37 °C.

Components

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

Lipase Assay Buffer Catalog Number MAK046A	25 mL
Peroxidase Substrate in DMSO Catalog Number MAK046B	0.2 mL
Enzyme Mix Catalog Number MAK046C	1 vl
Lipase Substrate Catalog Number MAK046D	0.4 mL
Glycerol Standard, 100 mM Catalog Number MAK046E	0.2 mL
Lipase Positive Control Catalog Number MAK046F	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the 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.

Peroxidase Substrate – Warm to room temperature before use. Mix well by pipetting, then aliquot and store, protected from light and moisture, at –20 °C.

Enzyme Mix – Reconstitute with 220 μL of Lipase Assay Buffer. Mix well by pipetting (don't vortex), then aliquot and store, protected from light at –20 °C. Use within 2 months of reconstitution.

Lipase Substrate – Substrate may separate during storage. To redissolve substrate, thaw tube and then place in a hot water bath (80–100 °C) for 1 minute until substrate looks cloudy. Vortex for 30 seconds. Repeat heat and vortex steps one more time. The substrate should look clear. The substrate is now ready for use. Aliquot and store the remainder at –20 °C.

Lipase Positive Control – Reconstitute with 100 μL of Lipase Assay Buffer. Mix well by pipetting (don't 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.

Glycerol Standards for Colorimetric Detection
Dilute 10 µL of the 100 mM Glycerol Standard with 990 µL of the Lipase Assay Buffer to prepare a 1 mM standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Lipase Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue (40 mg) or cells (2×10^6) can be homogenized in 4 volumes of ice-cold Lipase Assay Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material.

Serum samples can be directly added to wells.

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

For the positive control (optional), add 5 µL of the Lipase positive control solution to wells and adjust to 50 µL with the Lipase Assay Buffer.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 100 µL of the Reaction Mix is required for each reaction (well).
   Note: Glycerol in the samples will generate a background signal. To remove the effect of glycerol background, a Sample Blank may be set up for each sample by omitting the Lipase Substrate.

Table 1.
Reaction Mixes

Reagent	Standards and Samples	Sample Blank
Lipase Assay Buffer	93 µL	96 µL
Peroxidase Substrate	2 µL	2 µL
Enzyme Mix	2 µL	2 µL
Lipase Substrate	3 µL	–

Note: Some lipases are calcium dependent. To assay for calcium-dependent lipases, avoid the use of EGTA or EDTA in the sample preparation buffer and supplement the Lipase Assay Buffer with 1–5 mM of calcium chloride before use in Reaction Mixes.

2. Add 100 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate at 37 °C. After 2–3 minutes ($T_{initial}$), measure the absorbance at 570 nm $(A_{570})_{initial}$.
   Note: It is essential $(A_{570})_{initial}$ is in the linear range of the standard curve.

4. Continue to incubate the plate at 37 °C measuring the absorbance (A_{570}) after 60–90 minutes (or longer if activity is low). 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 absorbance measurement $[(A_{570})_{final}]$ for calculating the enzyme activity would be the 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 [$(A_{570})_{final}$] obtained for the 0 (blank) glycerol standard from the final measurement [$(A_{570})_{final}$] of the standards and samples. Background values can be significant and must be subtracted from all readings. Plot the glycerol standard curve.

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

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

$$\Delta A_{570} = (A_{570})_{final} - (A_{570})_{initial}$$

Also, subtract the Sample Blank Δmeasurement value from the sample Δmeasurement values. Compare the ΔA_{570} of each sample to the standard curve to determine the amount of glycerol (B) generated by the lipase between $T_{initial}$ and T_{final}.

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 glycerol 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 glycerol from triglycerides per minute at 37 °C.

Example:
Glycerol 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 colorimetric assays, use clear plates
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 used
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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