Technical Bulletin

LPL Activity Assay Kit

Catalog Number MAK109

Product Description

Lipoprotein lipase (LPL) hydrolyzes triglycerides associated with VLDL. The LPL Activity Assay Kit includes a non-fluorescent substrate emulsion that becomes intensely fluorescent upon interaction with LPL and a pre-hydrolyzed substrate for use as a standard to convert the fluorescence intensity to nanomoles of reactant formed. The assay is **not** specific for LPL and will also detect hepatic lipase activity.

Components

The kit is sufficient for 100 assays in 200 µL total assay volume.

- Substrate Emulsion 0.125 mL
  Catalog Number MAK109A
- LPL Standard, 75.7 mM 0.1 mL
  Catalog Number MAK109B
- LPL Assay Buffer 30 mL
  Catalog Number MAK109C

Reagents and Equipment Required but Not Provided

- 96-well U-bottom black plates for fluorescence assays
- 37 °C water bath incubator
- Fluorescent multiwell plate reader
- Orlistat (Catalog Number O4139), for assay validation

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light. DO NOT FREEZE.

Refer to the certificate of analysis for batch-specific expiration date.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Substrate Emulsion: Vortex before use.

Procedure

All samples and standards should be run in duplicate.

Standards for Fluorometric Detection

Note: Standards are not to be incubated with samples, use a separate plate for measuring the fluorescence intensity of the standard curve.

1. Disperse 1 µL of the LPL Standard into 2.5 mL of LPL Assay Buffer to generate a 30.3 µM standard solution. Make 8-12 additional 2-fold serial dilutions (use at least 0.1 mL of previous standard solution and 0.1 mL of LPL Assay Buffer), generating solutions with decreasing concentrations of LPL Standard.
2. Add 0.2 mL of each Standard dilution to a fluorescent compatible microplate. 0.2 mL of LPL Assay Buffer is used as the 0 (Blank) Standard (see Table 1.) Measure the fluorescence intensity (RFU) at λ_{Ex} = 370 nm/λ_{Em} = 450 nm).

Table 1.
Example Data of Fluorescence Readings of Standards

nmoles/ 0.2 ml	Average of Duplicate Standards (RFU)	Ave RFU minus 0 (Blank) Standard
6.06	240,407	240,370
3.03	133,697	133,660
1.515	67,513	67,476
0.758	33,385	33,347
0.379	17,221	17,184
0.189	8,669	8,631
0.095	4,351	4,314
0.047	2,180	2,142
0.024	1,123	1,086
0.012	556	518
0.006	286	248
0 (Blank) Standard	37.50	0

3. Plot the Standard curve (RFU to nmoles of label) and determine the slope (m) and intercept (b). This is the standard curve for calculating nmoles of reacted substrate from fluorescence intensity units (RFU).

Sample Preparation

1. Set up the Master Reaction Mix according to the scheme in Table 2. Add 195 µL of the Master Reaction Mix to each reaction (well).

Table 2.
Master Reaction Mix

Reagent	Volume
LPL Assay Buffer	194 µL
LPL Substrate Emulsion (see Note)	1 µL

Note: The LPL Substrate Emulsion is in a liquid crystalline state and difficult to pipette accurately in small volumes. It is recommended to pipette the substrate either by using a wide barrel pipette tip, or to cut the end off of a standard pipette tip for use.

2. Add 5 µL of desired LPL source (Sample) to the appropriate wells. Prepare a Reagent Blank by adding 5 µL of LPL Assay Buffer in place of LPL source (Sample).

   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.

   The amount of LPL to use in the assay and the incubation temperature will depend on the specific activity of the protein. For example, a purified sample of LPL with high specific activity may require dilution with LPL Assay Buffer and/or incubation at 25 °C in order for the assay to provide a linear response. It is recommended for first-time users to start with a lower reaction temperature, ~15 minute incubation time points, and multiple reads to optimize assay conditions.

Assay Reaction

1. Seal the plate and incubate for 15-60 minutes at 25–37 °C.
2. Measure the increase in fluorescence (RFU) of samples using a fluorometer (λ_{Ex} = 370 nm/ λ_{Em} = 450 nm). Determine the fluorescence intensity of the samples by subtracting the fluorescence intensity (RFU) of the Reagent Blank from each Sample.
3. Calculate nanomoles of label in the assay by subtracting the intercept (b) from (y) and then dividing by the slope (m) of the Standard curve.

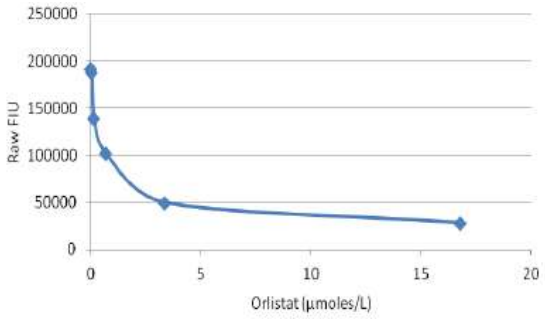
Assay Validation Procedure

The steps to validate the assay were adapted from a published procedure,[1] using Orlistat (Catalog Number O4139), also known as tetrahydrolipstatin (THL), to inhibit LPL activity.

Note: LPL as an ammonium sulfate suspension (e.g., Catalog Number L2254) will NOT be inhibited by orlistat. Other sources of LPL without ammonium sulfate, including bacterial LPL (e.g., Lipoprotein Lipase from *Pseudomonas* sp., Catalog Number 62335), work well to validate the assay with inhibitor.

1. Bacterial LPL (Catalog Number 62335, 3.3 mg/mL) was dissolved in 10 mM Tris HCL, pH 8.5, with 4 mM sodium deoxycholate (Catalog Number 30970) and 0.1 mM linoleic acid (Catalog Number L1376) with different concentrations of Orlistat in Step 2.
2. Orlistat dissolved in DMSO was added to final concentrations of 16.8, 3.36, 0.67, 0.13, 0.027, and 0 μM.
3. After incubation of LPL with Orlistat for 10 minutes at 20 °C, 10 μL aliquots were removed and the activity was determined according to the assay procedure.

Figure 1.
LPL Activity Assay Inhibition with Orlistat



Reference

1. Lookene, A., et al., Interactions of lipoprotein lipase with the active-site inhibitor tetrahydrolipstatin (Orlistat). *Eur. J. Biochem*., **222**, 395-403 (1994).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	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	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the 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

Notice

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