Roar

Product Information

Roar LPL Activity Assay Kit, 100 assays

Lipoprotein Lipase Activity Assay Kit
Catalog no. RB-LPL

Introduction

Lipoprotein lipase (LPL) hydrolyzes triglycerides associated with VLDL. The **Roar LPL Activity Assay Kit** includes a non-fluorescent substrate emulsion that becomes intensely fluorescent upon interaction with LPL and pre-hydrolyzed substrate for use as a standard to convert the fluorescence intensity reading to moles of reactant formed. The assay is not specific for LPL and will also detect hepatic lipase activity. Intra- and inter-assays coefficients of variation (CV): < 5% (Notarnicola, 2012, *Lipids in Health and Disease*).

Kit Components

LPL Substrate Emulsion: 125 µl
Standard (Pre-hydrolyzed Substrate): 100 µl
LPL Assay Buffer: 20 ml

Storage and Handling

Store kit components at 4°C.
If stored properly, components are stable for up to 1 year. DO NOT FREEZE.

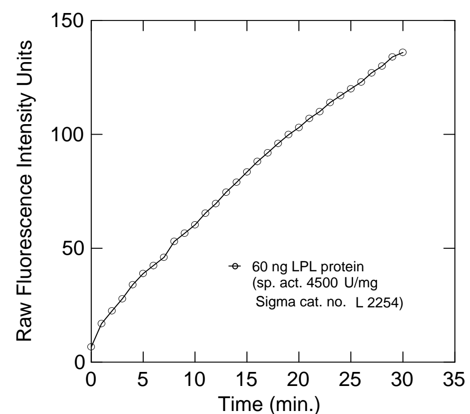
Materials Required, But Not Supplied

- Lipoprotein lipase source
- Distilled water to dilute assay buffer
- Fluorimeter with 370 nm excitation / 450 nm emission capability
- Black microplates (top-reading plate readers only) – two examples are the U-bottom, black microplates from Thermo Scientific #7205 and #7005

Assay Method

1. Vortex substrate emulsion before use.
2. Pre-mix substrate emulsion and assay buffer for all assays using 1 µl substrate per assay total volume of 200 µl. See: Assay Notes 1.
3. Distribute the mix among all the wells.
4. Add lipoprotein lipase source and buffer blanks incubate from 25°C - 37°C for 15 to 60 minutes. See: Assay Notes 2.
5. Read assay at 370 nm excitation / 450 nm emission

LPL Activity at 25º C



Assay Notes:

1. The substrate is in a liquid crystalline state and difficult to pipette accurately in small volumes. We have provided enough substrate for 125 assays to reduce the level of frustration. For an easy way to pipette the substrate use a wide barrel pipette tip, or simply cut the end off of a standard pipette tip.
2. 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 assay buffer and/or incubation at 25°C in order for the assay to provide a linear response. The assay is very sensitive and provides a wide linear window that allows you to make adjustments on the fly. We recommend first time users start with a lower temperature, ~15 minute incubation time points and multiple reads to work out a set of conditions.
3. Many of the references citing the use of our kit provide detailed descriptions of assay conditions used with a variety of samples including cell lysates, homogenization protocols for different tissue samples, as well as, pre- and post-heparin plasma or serum. The following papers caught our attention and provide particularly helpful details in the Methods sections:

   Yamazaki H, Arai M, Matsumura S, Inoue K, Fushiki T. Intracranial administration of transforming growth factor-beta3 increases fat oxidation in rats. Am J Physiol Endocrinol Metab. 2002;283(3):E536-44.

   Mizunoya W, Haramizu S, Shibakusa T, Okabe Y, Fushiki T. Dietary conjugated linoleic acid increases endurance capacity and fat oxidation in mice during exercise. Lipids. 2005;40(3):265-271.

   Cerne D, Melkic E, Trost Z, Sok M, Marc J. Lipoprotein lipase activity and gene expression in lung cancer and in adjacent noncancer lung tissue. Exp Lung Res. 2007;33(5):217-225.

   Gordts PLSM, Reekmans S, Lauwers A, Van Dongen A, Verbeek L, Roebroek AJM. Inactivation of the LRP1 intracellular NPxYxxL motif in LDLR-deficient mice enhances postprandial dyslipidemia and atherosclerosis. Arterioscler Thromb Vasc Biol. 2009;29(suppl):1258-1264.

   Yamazaki T, Kishimoto K, Ezaki O. The ddY mouse: A model of postprandial hypertriglyceridemia in response to dietary fat. J Lipid Res. 2012;53(10):2024-2037. doi: 10.1194/jlr.M023713; 10.1194/jlr.M023713.

   Dube E, Ethier-Chiasson M, Lafond J. Modulation of cholesterol transport by insulin-treated gestational diabetes mellitus in human full-term placenta. Biol Reprod. 2013;88(1):16, 1-10

Standardization

Use the Standard (Pre-hydrolyzed Substrate) included with the kit as a way to determine pmoles of hydrolyzed substrate produced in the assay from your samples.

You will be reading the standard from high to low concentration and then use the fluorescence intensity units (FIUs) measured to calculate the fluorescent label formed in your assays from hydrolysis of the substrate. Using linear regression, you can plug in the FIU reads from the assay (after subtracting the blank) to determine pmoles of substrate hydrolyzed.

1. In a separate plate (not your assay plate), make a serial dilution of the Standard to determine the appropriate range to use with your particular instrument. A 1:2500 dilution of the Standard in assay buffer and serially diluting 8 to 12 steps further should provide a reasonably thorough range for you to create a standard curve. After reading at 370 nm excitation / 450 nm emission you may find that the highest concentration of Standard is off the reader's scale, but the lower concentrations are within the range of the reader. For some very sensitive readers a starting dilution of 1:250000 may be necessary if the entire serial dilution is off scale.
2. The concentration of the Standard is 75.7 µmoles/ml. Plot a standard curve (FIU to nmoles of label) and determine the slope (m) and intercept (b).
3. Next, calculate the moles of substrate hydrolyzed from the FIU reads of your assay plate by subtracting the FIU of the blanks from the FIU of the samples. This will be the variable (y). Calculate moles of label in the assay by subtracting the intercept (b) from (y) and them dividing by the slope (m) of the standard curve.

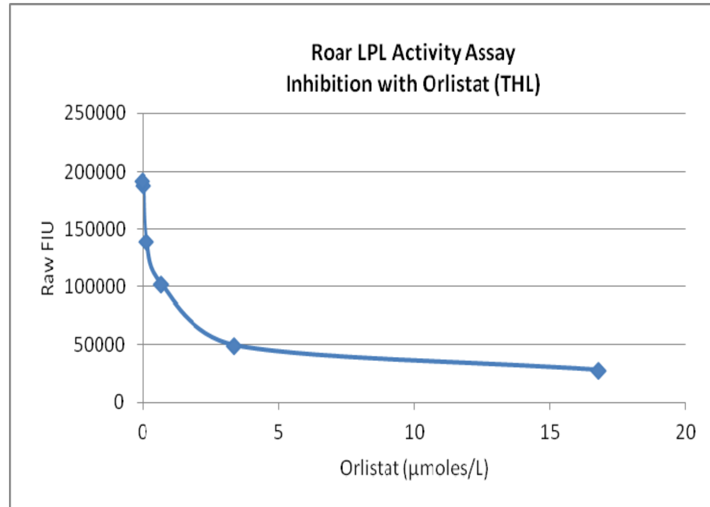
   <u>Do not incubate the Standard with the samples</u>. Unlike many conventional assay standards, the standard curve will not be treated like the samples – this standardization is a procedure to determine your instrument's response to the label.

Assay Validation

Important: LPL in ammonium sulfate (such as Sigma #L2254) will NOT be inhibited by orlistat (tetrahydrolipstatin (THL)). This is true whether activity measurements are made with the traditional nitrophenyl butyrate assay or Roar's LPL Activity Assay. The Sigma product must be re-isolated out of ammonium sulfate in order to be inhibited by orlistat.

Other sources of LPL without ammonium sulfate, including bacterial LPL (Sigma #62335) in PBS, work well to validate the assay with inhibitor.

Please contact us for more information if you are working with LPL inhibitors.



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Roar Biomedical, Inc., Audubon Biomedical Center, 3960 Broadway, New York, NY 10032 USA
Tel: +1 (212) 280-2983 ▪ Fax: +1 (212) 280-2968 ▪ info@roarbiomedical.com ▪ www.roarbiomedical.com

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