Roar

Product Information

Roar LCAT Activity Assay Kit, 100 assays

Lecithin:Cholesterol Acyltransferase Activity Assay Kit
Catalog No. RB-LCAT

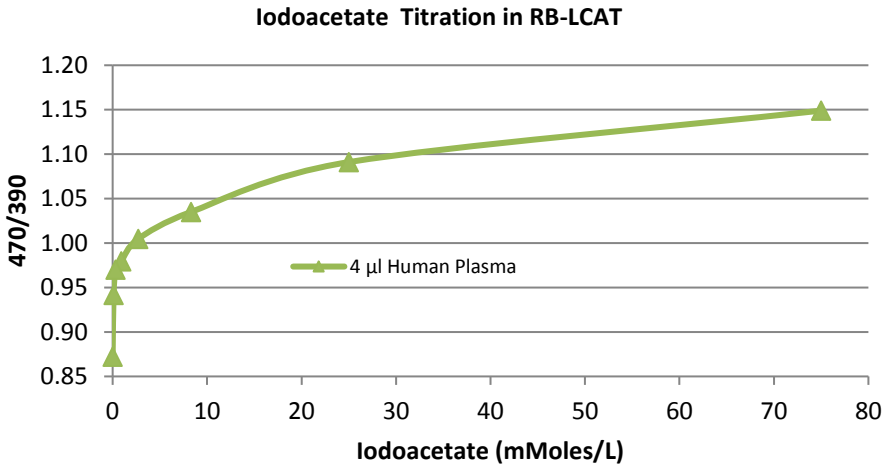
Assay Method:	Fluorometric
Number of Assays:	100 assays in 100 µl total incubation volume
Kit Contents:	Substrate Reagent: 100 µl READ Reagent: 30 ml Assay Buffer: 20 ml
Storage and Handling:	Substrate Reagent: -20°C READ Reagent: 4°C Assay Buffer: 4°C If stored properly, components are stable for up to 1 year.
Instrumentation:	Fluorescence spectrophotometer: Excitation: 340 nm / Emission: 390 and 470 nm

Introduction

The plasma protein lecithin:cholesterol acyltransferase (LCAT) catalyzes the transfer of an acyl group from the sn2 position of phosphatidylcholine to the 3-hydroxyl group of cholesterol resulting in the formation cholesteryl ester. This enzymatic activity occurs on the surface of high density lipoprotein (HDL). The cholesteryl esters formed by LCAT may be packed into the core of HDL.

The **Roar RB-LCAT Activity Assay Kit** is a fluorometric assay useful for measuring phospholipase activity of LCAT. The assay may be validated by inhibition of LCAT with iodoacetate or another spectrally benign (not Ellman's reagent) LCAT inhibitor.

Applications for this method include high-throughput screening, mechanism of action studies and structure-activity relationship (SAR) work. This assay, along with Roar's RB-LCFC assay, provides a complete set of tools for LCAT mechanism studies by detection of phospholipase and acyltransferase activity.
Intra- and interassay coefficients of variation of the assay: <5% (Nakhjavani, 2011; Harangi, 2009; Kassai, 2007)



Overview of the Assay

The emission spectrum of the Substrate Reagent has two distinct peaks, 390 nm and 470 nm. The relative intensity of the peaks depend upon the concentration of hydrolized and intact substrate present in the assay. If the substrate is intact, the flourophors are in close proximity and some energy of their excited state is disipated by radiationless transitions.

The emission intensity is predominately at the less energetic 470 nm peak. After hydrolysis of the substrate by LCAT, the fluorophors are not able to energetically interact and a shift in intensity is seen in the emission spectrum as an increase in 390 nm emission at the expense of the 470 nm emission peak.

It is important to measure both 390 and 470 nm emission because this label is affected by many assay variables.

Method 1. Using a Cuvette

1. Mix 1 µl LCAT Substrate Reagent with Assay Buffer and LCAT source to 200 µl.

2. Incubate for 4 to 8 hours at 37 °C.

3. Add 100 µl of the incubated mixture to 300 µl of READ Reagent and then vortex. Read the fluorescent label at 340 nm excitation and emission at 390 nm and at 470 nm. NOTE: Do not incubate the assay with READ Reagent - it will inactivate LCAT.

Method 2. Microplate Method - We recommend round bottom, black microplates (Corning, Costar #3792)

1. Set up the assay in a polypropylene microplate with 0.5 µl Substrate Reagent plus LCAT source in a total volume of 100 µl with Assay Buffer. For example, use 4 µl of normal human plasma.

2. Incubate for 2.5 hours at 37 °C.

3. Add 200 µl of READ Reagent to the polypropylene plate and mix by aspiration/dispense with pipet.

4. Transfer 200 µl of the mixture from the polypropylene plate to a black fluorescence compatible microplate and read the plate at 340 nm excitation, 390 nm and 470 nm emission.

5. Determine the change in emission ratio of 470 nm/390 nm over time to compare LCAT activity among samples using an excitation wavelength of 340 nm.

Related Product

Roar LCFC-LCAT Activity Assay Kit (cat. #RB-LCFC), 100 assays: useful for measuring the acyltransferase activity of LCAT resulting in the formation of cholesteryl ester.

For Research Use Only. Not for Diagnostic or Therapeutic Purposes.

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