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Product Information

LCAT Activity Assay Kit
Supplied by Roar Biomedical, Inc

Catalog Number **MAK107**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

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 of a cholesteryl ester. This enzymatic activity occurs on the surface of high density lipoproteins (HDL). The cholesteryl esters formed by LCAT may be packed into the core of HDL.

The LCAT Activity Assay Kit is a fluorometric assay useful for measuring the 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.

The emission spectrum of the Substrate Reagent has two distinct peaks, 390 nm and 470 nm. The relative intensity of the peaks depends upon the concentration of hydrolyzed and intact substrate present in the assay. If the substrate is intact, the flourophors are in close proximity and some energy of the excited states is dissipated 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 with a decrease of the 470 nm emission peak. It is important to measure both 390 and 470 nm emission because the fluorescence of the substrate is affected by several assay variables.

Components

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

Substrate Reagent Catalog Number MAK107A	0.1 mL
Read Reagent Catalog Number MAK107B	30 mL
LCAT Assay Buffer Catalog Number MAK107C	20 mL

Reagents and Equipment Required but Not Provided.

- 96 well polypropylene plates for assay set up
- 96 well U-bottom black plates for fluorescence assays
- 37 °C water bath incubator
- Fluorescence multiwell plate reader
- Iodoacetate (Catalog Number 57858) for assay validation

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.

Storage/Stability

The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended. Components are stable for 1 year, if stored properly.

Procedure
All samples and standards should be run in duplicate.

Single Tube Method

1. Mix 1 µL of LCAT Substrate Reagent with 200 µL of Assay Buffer and LCAT source (3–5 µL of plasma or serum).

2. Incubate for 4–8 hours at 37 °C.

3. Add 100 µL of the incubated mixture to 300 µL of Read Reagent and then vortex. Measure the fluorescent label (λ_{ex} = 340/λ_{em} = 390 and 470 nm).

Note: Do not incubate the assay with Read Reagent - it will inactivate LCAT.

Microplate Method

1. Combine 4 µL of sample (plasma) and 0.5 µl of Substrate Reagent into well of polypropylene reaction plate. Bring to final volume of 100 µl with LCAT Assay buffer.

2. Incubate for 2.5 hours at 37 °C.
   Note: The microplate incubator must be able to rapidly raise the assay temperature to 37 °C. Large, humidified air incubators may cause problems by slowly increasing the temperature from 25 °C to only 34 °C after three hours. Floating the plate in a water bath is recommended, rather than using an air incubator.

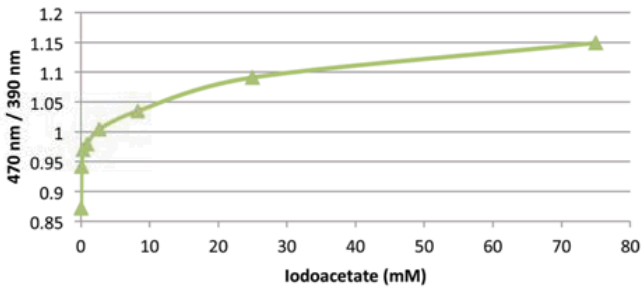
3. Add 200 µL of Read reagent to wells of polypropylene plate, mixing well by pipetting.

4. Transfer 200 µL of the reaction mixture from the polypropylene plate to a black fluorescence microplate.

5. Measure the increase in fluorescence of samples using a fluorometer (λ_{ex} = 340/λ_{em} = 390 and 470 nm).

6. Determine the ratio ($\lambda_{em\ 470}/\lambda_{em\ 390}$) to compare plasma LCAT activity among samples.
   Note: The fluorescence of the substrate is affected by several assay variables, such as viscosity and oxygen quenching as well as matrix effects. Better results are achieved with a ratio of the two emission intensities. Color quenching from compounds introduced into the assay or using a hemolyzed sample is also eliminated when a ratio is used.

Figure 1.
Iodoacetate titration into LCAT Activity Assay



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

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