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Product Information

LCFC-LCAT Acyltransferase Activity Assay
Supplied by Roar Biomedical, Inc

Catalog Number **MAK306**
Storage Temperature 2–8 °C; DO NOT FREEZE

TECHNICAL BULLETIN

Product Description

The plasma protein lecithin:cholesterol acyltransferase (LCAT) catalyzes the transfer of the 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 are incorporated into the core of HDL.

The LCFC-LCAT Acyltransferase Activity Assay is a fluorometric assay useful for measuring the acyltransferase activity of LCAT in serum or plasma. The method detects changes in LCAT free cholesterol (LCFC) levels in the sample without the use of cholesterol oxidase, peroxidase, or the generation of hydrogen peroxide. Detection is not affected by iodoacetate or other LCAT inhibitors. LCAT activity results in a fluorometric (λ_{ex} = 320/λ_{em} = 405 nm) product proportional to the amount of free cholesterol present.

The procedure can be run at two different sample concentrations. In Assay Method 1 (Dilution Assay), iodoacetate is used to inhibit the LCAT activity in the control wells. The sample is not diluted in Assay Method 2 (Non-dilution Assay), maintaining the physiological concentrations of all components in the sample. Temperature is used to inhibit the LCAT activity in the control wells in Assay Method 2.

Applications for this method include high-throughput screening, mechanism of action studies, and structure-activity relationship (SAR) work without the risk of compound interference.

Components

The kit is sufficient for 100 assays in 120–140 µL total assay volume.

LCFC Reagent Catalog Number MAK306A	10 mL
LCFC Assay Buffer Catalog Number MAK306B	5 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plates. It is recommended to use black U-bottom plates.
- Adhesive plate seals
- 37 °C water bath incubator
- Fluorescence multiwell plate reader
- Sodium iodoacetate (Catalog Number 57858 or equivalent) for use with Assay Method 1 (Dilution Assay)

Precautions and Disclaimer

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.

Storage/Stability

The kit is shipped on wet ice. Storage at 2–8 °C, protected from light, is recommended. Do not freeze. Components are stable for 2 years if stored properly.

Procedure
All samples and controls should be run in duplicate.

Assay Method 1 (Dilution Assay)

1. Prepare the LCAT Inhibitor Solution by diluting 31 mg of sodium iodoacetate with 1,000 µL of Assay Buffer.

2. Set up the Enzymatic Reactions in duplicate wells of a 96 well plate as shown in Table 1.

Table 1.
Enzymatic Reactions

Reagent	Controls	Samples
LCAT Inhibitor Solution	10 µL	–
Assay Buffer	–	10 µL
Sample	20 µL	20 µL

3. Seal plate with an adhesive plate seal and gently vortex the plate.

4. Incubate for 60 minutes at 37 °C.

5. Chill the plate on crushed ice for 15 minutes.

6. Remove the plate seal and add reagents to the sample and control wells as shown in Table 2.

Table 2.
Development Reactions

Reagent	Controls	Samples
LCAT Inhibitor Solution	–	10 µL
Assay Buffer	10 µL	–
LCFC Reagent	100 µL	100 µL

7. Incubate for 60 minutes at room temperature.

8. Measure the fluorescence of all wells (λ_{ex} = 320/ λ_{em} = 405 nm).

After incubation, the control wells with LCAT Inhibitor have more free cholesterol than the sample wells without LCAT Inhibitor due to the cholesterol-to-cholesteryl ester conversion by LCAT. The fluorescence intensity is directly related to the free cholesterol concentration.

Assay Method 2 (Non-dilution Assay)

1. Add 20 µL of sample to wells of *two separate 96 well plates*. (Plate A (Control) and Plate B (Sample)). Seal the plates with adhesive plate seals.

2. Incubate Plate A for 60 minutes at 4 °C. Incubate Plate B for 60 minutes at 37 °C.

3. Transfer Plate B to the 4 °C incubator. Incubate Plate A and Plate B at 4 °C for 15 additional minutes.

4. Remove the plate seals and add 100 µL of LCFC Reagent to all sample and control wells on Plate A and Plate B.

5. Incubate Plate A and Plate B at room temperature for 30 minutes.

6. Measure the fluorescence of all wells (λ_{ex} = 320/ λ_{em} = 405 nm).

After incubation, the control wells (Plate A) incubated at 4 °C have more free cholesterol than the sample wells (Plate B) incubated at 37 °C due to the cholesterol-to-cholesteryl ester conversion by LCAT. The fluorescence intensity is directly related to the free cholesterol concentration.

Results
Calculations
To report LCAT acyltransferase activity of a sample, calculate the ratio of its fluorescence intensity units (FIU_{sample}) with respect to FIU measured for the control ($FIU_{control}$):

$$\% \text{ of Control} = 100\% \times (FIU_{sample}/FIU_{control})$$

Measurements in the example refer to the average FIU calculated for duplicates.

Example:

$FIU_{control}$: 26644

FIU_{sample}: 20542

% of Control = 100% × (20542/26644) = 77.1%

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

This product is supplied by Roar Biomedical, Inc., US Patent No. 10,495,652.

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