Roar

Product Information

Roar LCFC-LCAT Activity Assay Kit, 100 assays

Catalog No. RB-LCFC
PATENTS PENDING

Assay Method:	Fluorometric
Number of Assays:	100 assays in 140 µl total assay volume
Kit Contents:	LCFC Reagent: 10 ml Assay buffer: 5 ml 100 mM tris pH 7.4
Storage and Handling:	Store kit components at 4°C. If stored properly, components are stable for up to 1 year. DO NOT FREEZE.
Instrumentation:	Fluorescence spectrophotometer: cuvette or microplate reading formats Excitation: 320 nm / Emission: 405 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 forming cholesteryl ester. This activity occurs on the surface of high density lipoprotein (HDL) and the cholesteryl esters formed by LCAT are incorporated into the core of HDL.

The Roar LCFC-LCAT Activity Assay Kit is a homogeneous, fluorometric assay useful for measuring the acyltransferase activity of LCAT resulting in the formation of cholesteryl ester. The method detects free cholesterol without the use of cholesterol oxidase, peroxidase or the generation of hydrogen peroxide and, therefore, is not affected by iodoacetate or other LCAT inhibitors.[1]

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

Advantages

- Non-enzymatic determination of cholesteryl ester formation
- Not affected by LCAT inhibitors or other chemical compounds
- Dilution or non-dilution type assay
- Assay components stable for up to one year

Materials Required, But Not Supplied

- Fluorimeter with appropriate wavelength capabilities (Ex: 320 nm; Em: 405 nm)
- 37°C water bath / incubator
- Source of LCAT (plasma, serum)
- Sodium iodoacetate (optional)

[1] **Harris WS, Rayford A, LCAT Inhibitors Interfere with Enzymatic Determination of Cholesterol and Triglycerides. Lipids. 1990;25:341-43**

Assay Method 1[†] (dilution-type assay)

This protocol introduces a dilution factor that may affect components in the sample influencing LCAT activity.

Protocol:

Prepare the LCAT inhibitor solution with 31 mg/ml sodium iodoacetate using some of the supplied 100 mM tris buffer.

In a fluorescence-compatible microplate:

1. Add 10 µl of the iodoacetate solution to the wells of column 1.
2. Add 10 µl tris buffer to the wells of column 2.
3. Add 20 µl plasma to the wells of columns 1 and 2.
4. Gently vortex the plate.
5. Seal the plate and incubate for 60 minutes at 37°C.
6. After the 60 minute incubation, chill the plate on crushed ice for ~15 minutes.
7. Remove the cover and add 10 µl of tris buffer to column 1 (the wells containing iodoacetate and plasma).
8. Add 10 µl of iodoacetate to the wells in column 2 (containing tris buffer and plasma).
9. Add 100 µl Roar LCFC reagent to all the wells and leave the plate at room temperature for 30 minutes.
10. Read the plate at 320 nm excitation 405 nm emission.

Example: Dilution assay results

	Column A (Control)	**Column B**	**% Control**
	Iodoacetate	**Tris Buffer**	**(B / A) x 100**
	Raw FIU	**Raw FIU**	
Human Sample 1	26644	20542	77
Human Sample 2	25030	15192	61
Human Sample 3	25909	20277	78
Human Sample 4	26594	24133	91

Column A with the LCAT inhibitor has a higher fluorescence signal than Column B. The fluorescence intensity is directly related to the free cholesterol concentration present in the sample. After incubation, an active plasma sample without LCAT inhibitor has less free cholesterol due to the cholesterol-to-cholesteryl ester conversion by LCAT.

[†] The use of iodoacetate was adapted from: Albers, J.J., Chen, C.H. and Lacko, A.G. (1986) Isolation, Characterization, and Assay of Lecithin-Cholesterol Acyltransferase , *Meth. Enzymol*. 129, 763-783.

Assay Method 2 (non-dilution assay)

This protocol maintains physiological concentrations of all components in the sample.

Protocol:

In two fluorescence-compatible microplates (Plate A and Plate B):

1. Add 20 µl plasma to the wells of column 1 of Plate A and Plate B.
2. Seal the plates and incubate Plate A at 4℃ and Plate B at 37℃ for 60 minutes.
3. Remove Plate B from the incubator and place it with Plate A at 4℃ for ~15 minutes.
4. Remove the sealers from both plates and add 100 µl of Roar LCFC reagent to all of the wells.
5. Let the plates sit on the bench at room temperature for 30 minutes.
6. Read the plates at 320 nm excitation 405 nm emission.

Example: Non-dilution assay results

	Average FIU – Plate A (4℃)	Average FIU – Plate B (37℃)	% Control (B/A) x 100
Plasma	13886	11109	80

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RB-LCFC Cited References
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- Liu C, Gaudet D, Miller YI. Deficient cholesterol esterification in plasma of apoc2 knockout zebrafish and familial chylomicronemia patients. *PLoS ONE*. **2017**;12(1):e0169939.