CREATIVE BIOMART®
Assay Kit

LCAT Activity Fluorometric Assay Kit

Product Information

Cat.No. Kit-0507

Product Overview

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.

Storage

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.

Size

100 assays

Handling

Briefly centrifuge vials before opening.

Kit Components

Substrate Reagent: 100 µl, -20°C
READ Reagent: 30 ml, 4°C
Assay Buffer: 20 ml, 4°C

Materials Required but Not Supplied

- 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 for assay validation

Assay Protocol

All samples and standards should be run in duplicate.

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Single Tube Method
1. Mix 1 mL of LCAT Substrate Reagent with 200 mL of Assay Buffer and LCAT source (3–5 mL of plasma or serum).
2. Incubate for 4–8 hours at 37 °C.
3. Add 100 mL of the incubated mixture to 300 mL of Read Reagent and then vortex. Measure the fluorescent label (lex = 340/lem = 390 and 470 nm).
Note: Do not incubate the assay with Read Reagent - it will inactivate LCAT.

Microplate Method
1. Combine 4 mL of sample (plasma) and 0.5 ml of Substrate Reagent into well of polypropylene reaction plate. Bring to final volume of 100 ml 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 mL of Read reagent to wells of polypropylene plate, mixing well by pipetting.
4. Transfer 200 mL of the reaction mixture from the polypropylene plate to a black fluorescence microplate.
5. Measure the increase in fluorescence of samples using a fluorometer (lex = 340/lem = 390 and 470 nm).
6. Determine the ratio (lem 470/lem 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.

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