

LCAT ELISA
Product No. 317941

Storage: 2-10°C

For Research Use Only!

INTENDED USE

The LCAT ELISA kit is intended for the quantitative determination of lecithin-cholesterol acyltransferase (LCAT) in human serum and plasma by utilizing a two-step sandwich method of enzyme-linked immuno-sorbent assay (ELISA).

EXPLANATION OF THE TEST

High density lipoprotein (HDL) plays an important role in reverse cholesterol transport, a process by which excess cholesterol from peripheral tissues is returned to the liver for use or excretion.

There is a strong inverse correlation between plasma HDL cholesterol concentration and the incidence of atherosclerosis. Lecithin-cholesterol acyltransferase (LCAT) performs a central role in HDL metabolism by catalyzing the formation of cholesteryl esters on HDL through the transfer of fatty acids from the *sn*-2 positions of phosphatidylcholine (PC) to cholesterol.

Two classes of genetic deficiencies are known: familial LCAT deficiency (FLD) and fish-eye disease (FED). FLD is caused by either null or missense mutations; in Class 1 defects, null mutations cause total loss of catalytic activity and virtual absence of LCAT mass, whereas in Class 2, missense mutations are characterized by loss of activity and either normal, reduced, or absent LCAT mass. FED is caused by missense mutations only; these mutations affect either LDL or HDL activity in Class 3 defects, and LCAT mass is reduced. In Class 4 defects, the missense mutations are associated with partial loss of activity against HDL only, and reduced LCAT mass. Direct measurement of the enzyme mass and activity may contribute to the differentiation of LCAT defects.

PRINCIPLE OF THE METHOD

Test wells are coated with anti-LCAT monoclonal antibody (mAb (36486)), which binds with LCAT in the sample. After the first incubation and washes to remove all of the unbound material, horseradish peroxidase (HRP)-labeled anti-LCAT mAb (36487) is added. The enzyme labeled mAb (36487) binds with LCAT immobilized on the well by the coated mAb (36486). After the second incubation and subsequent washes, the antibody / LCAT / enzyme complex is incubated with a substrate solution and terminated with a stop reagent. The intensity of color that develops in the enzyme reaction is measured by using a microplate reader. The absorbance is proportional to the concentration of LCAT in the sample.

REAGENTS

| | Reagent | Composition | Amount |
|------|-------------------------------------|--|---------|
| MTP | LCAT-mAb coated wells | anti-LCAT mAb (L86) coated 96-well plate | 1 plate |
| DILA | Dilution buffer (a) | Citrate buffer (pH 5.0) | 100 mL |
| DILB | Dilution buffer (b) | Phosphate buffer (pH 7.2) | 6 mL |
| CON | Enzyme-labeled mAb concentrate (7x) | HRP-labeled anti-LCAT mAb (L87) | 1 mL |
| WASH | Wash buffer concentrate (10x) | Phosphate buffer (pH 7.2) | 100 mL |
| SUB | Substrate (lyophilized) | o-Phenylenediamine | 2 vials |
| SUBB | Substrate buffer | H ₂ O ₂ in citrate buffer (pH 5.0) | 15 mL |
| STOP | Stop reagent | 7.7% H ₂ SO ₄ | 10 mL |
| STD | Standard (lyophilized) | Human plasma | 1 vial |

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measurement at 492 nm
- 8-channel pipette capable of delivering 50-200 µL
- 1-channel pipette capable of delivering 20-1000 µL
- 4. Deionized or distilled water.
- Plastic test tubes
- Volumetric flask of cylinder (1000 mL)
- Absorbent paper towels
- Micro-plate shaker with horizontal, circular movement, if available
- Plate washer, automated or manual, if available

REAGENT PREPARATION AND STORAGE

Unprepared reagents are stable for 2 years when stored at 2-10°C.

1. Working wash buffer: Dilute the wash buffer concentrate (5) with 900 mL of distilled water. The resulting working wash buffer is stable for 1 month at 2-10°C.
2. Working anti-LCAT mAb HRP conjugate: Dilute the HRP-labeled mAb concentrate with 6 mL of the dilution buffer (b). The resulting working anti-LCAT mAb HRP conjugate is stable for two weeks at 2-10°C.
3. Working substrate solution: Just prior to use, reconstitute the substrate (lyophilized) (6) by adding 6 mL of the substrate buffer (7) to the substrate vial. Since the substrate is light sensitive, it should not be exposed to excessive light. The resulting working substrate solution should be used within 1 hour after reconstitution.
4. Standard: Reconstitute the standard (lyophilized) (9) by adding 1.0 mL of the dilution buffer (a) to the standard vial. The content of LCAT is indicated on the label. The stock solution is stable for 2 weeks at 2-10°C. Prepare the serial standard dilutions immediately prior to use to obtain a calibration curve in the following manner.

| LCAT Content | a | a/2 | a/4 | a/8 | a/16 | a/32 | 0 µg/mL |
|---------------------|-----|-----|-----|-----|------|------|---------|
| LCAT Stock sol. | 150 | 150 | 150 | 150 | 150 | 150 | 0 µL |
| | | ↗ | ↗ | ↗ | ↗ | | |
| Dilution buffer (a) | 0 | 150 | 150 | 150 | 150 | 150 | 150 µL |

5. Other: Seal extra strips with plate tape sealer and store at 2-10°C for future use. When stored at 2-10°C, the dilution buffer (a), the substrate buffer, and the stop reagent are stable until the expiration date on the label.

PREPARATION OF SAMPLES

Samples must be diluted to 1:100 with the dilution buffer (a) (sample 10 µL + the dilution buffer (a) 1000 µL) before they are added to the plate. When the obtained absorbance exceeds the range of the calibration curve, dilute the sample with higher volume of the dilution buffer (a) and repeat the measurement. The dilution ratio should not be less than 1:20.

ASSAY PROCEDURE

| Reagent | Vol. | Procedure |
|---|--------|--|
| Samples or Standard | 50 µl | Add sample to the center of each test well. All standards should be tested twice. Incubate the covered plate for 2 hours at room temp. |
| Thoroughly remove solution from wells | | |
| Working wash buffer | 350 µl | Wash wells 3 times. Thoroughly remove droplets. |
| Working anti-LCAT MoAb HRP conjugate | 50 µl | Add to each test well. Incubate the covered plate for 1 hour at room temp. |
| Thoroughly remove solution from wells | | |
| Working wash buffer | 350 µl | Wash well 3 times. Thoroughly remove droplets. |
| Working substrate solution | 50 µl | Add to each test well. Incubate the covered plate for 15 minutes at room temp. |
| Stop reagent | 50 µl | Add to each test well. |
| Read the absorbance of each well at 492 nm. | | |

CALCULATION OF RESULTS

Calculate the Δ absorbance by subtracting the absorbance of the 0 $\mu\text{g/mL}$ calibrator from those of other calibrators and unknown samples. Plot the Δ absorbance of the calibrators against the calibrator concentration on log-log or semi-log graph paper. Draw a smooth curve through these points to construct the calibration curve. Read the concentrations for the diluted unknown samples from the calibration curve.

PROCEDURAL NOTES

1. For research use only.
2. A standard curve must be run with each assay.
3. Read absorbences just after completion of the assay.
4. The human plasma used in the calibrator has been tested and found to be negative for hepatitis B surface antigen (HbsAg), antibodies to hepatitis C virus (HCV), and antibodies to human immunodeficiency virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if they are capable of transmitting infectious disease.
5. Stop reagent (7.7% H_2SO_4) is poisonous and can cause severe burns. Do not ingest. Avoid contact with skin, eyes, and clothing. If contact occurs, immediately wash the area thoroughly with water.
6. All residual wash buffers must be drained from the wells by aspiration or by decantation followed by tapping the plate forcefully on absorbent paper.

REFERENCE

1. A new enzyme-linked immunosorbent assay with two monoclonal antibodies to specific epitopes measures lecithin-cholesterol acyltransferase in human serum. Kobori K et al., *Atherosclerosis, supplements*, 2001; 2: 74.
2. A new enzyme-linked immunosorbent assay with two monoclonal antibodies to specific epitopes measures human lecithin-cholesterol acyltransferase. Kobori K et al., *J Lipid Res*, 2002; 43: 325-334.