BioVendor Group

ENG

Instructions for Use:

# HUMAN LECITHIN-CHOLESTEROL ACYLTRANSFERASE (LCAT) ELISA

Catalogue number:

**RD191122200R**

**For research use only!**



BioVendor R&D®

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CONTENT

## HISTORY OF CHANGES

| Previous version | Current Version |
|---|---|
| ENG.005.A | ENG.006.A |
| "History of changes" added | |
| Chapter 7: Quantity - Biotin Labelled Antibody Conc. (50x) | |
| 0.28 ml | 0.26 ml |
| Chapter 9: A sentence "Centrifuge liquid containing microtube vials before opening" added. | |

## 1. INTENDED USE

The RD191122200R Human Lecithin:cholesterol acyltransferase (LCAT) ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human lecithin-cholesterol acyltransferase.

**Features**

- **It is intended for research use only**
- The total assay time is less than 3.5 hours
- The kit measures LCAT in serum, plasma (EDTA, citrate) and cerebrospinal fluid (CSF)
- Assay format is 96 wells
- Standard is recombinant protein based
- Quality Controls are human serum based
- Components of the kit are provided ready to use, concentrated or lyophilized



## 2. STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.

## 3. INTRODUCTION

Human Lecithin: cholesterol acyltransferase (LCAT) is a glycoprotein with a molecular mass of approximately 58 kDa. It is the key enzyme responsible for esterification of free cholesterol to cholesteryl esters in circulating plasma lipoproteins, primarily in high density lipoprotein (HDL). The tertiary structure of LCAT is maintained by two disulfide bridges, similar to lipases and other proteins of the α/β hydrolase fold family. Mature LCAT protein is synthesized from a 440 residue precursor by following cleavage of a 24 residue signal peptide. The mature protein contains 416 amino acids and is heavily N-glycosylated.
LCAT is abundant in blood plasma and it is present in various organs, including liver, brain and testes. In plasma LCAT is associated with ApoD which frequently co-purify. A recent study suggests that LCAT can act as an antioxidant and prevent the accumulation of oxidized lipid in plasma lipoproteins. 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. The role of LCAT in atherosclerosis is unclear. Dullaart *et al*. showed that plasma LCAT activity is elevated in metabolic syndrome and may be a marker of subclinical atherosclerosis. Sethi *et al*. demonstrated that low lecithin-cholesterol acyltransferase (LCAT) activities and high pre-ß1-HDL concentrations are strong positive risk markers for ischemic heart disease and are independent of HDLcholesterol. Miida *et al*. demonstrated that plasma pre- β1-HDL concentration increase in subjects with low LCAT activity. They also reported that patients with coronary artery disease (CAD) had higher pre-ß1-HDL concentrations than did normolipidemic subjects. Holleboom *et al*. showed that low plasma LCAT levels (reflecting low LCAT activity) are not associated with an increased risk of future (CAD) in the general population. However, other studies showed a positive association of LCAT levels with carotid atherosclerosis in patients with the metabolic syndrome as well as in control subjects whereas, LCAT activity was reduced in patients with CAD and in patients with acute myocardial infarction. In summary, LCAT activity might be reduced in the acute phase of a myocardial infarction.
Mutations of LCAT on chromozome 16 resulting in homozygous or compound heterozygous form can cause two major phenotypes: FLD (familial LCAT deficiency) and FED ( Fish Eye Disease). Patients with FLD have a complete loss of both α-LCAT activity and β-LCAT activity and an increased proportion of unesterified cholesterol in plasma. In FED is partial loss of α-LCAT activity with normal elevated free cholesterol in plasma. Both FLD and FED are characterized by the development of corneal opacities.

**Areas of investigation:**
Lipoprotein metabolism
Cardiovascular disease
Metabolic syndrome
Atherosclerosis

## 4. TEST PRINCIPLE

In the Biovendor Human LCAT ELISA, standards, quality controls and samples are incubated in microtitration wells pre-coated with polyclonal anti-human LCAT antibody. After a 60 minute incubation followed by washing, biotin labelled polyclonal anti-human LCAT antibody is added and incubated with the captured LCAT for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of LCAT. A standard curve is constructed by plotting absorbance values against LCAT concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

## 5. PRECAUTIONS

- **For professional use only**
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. These materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents
- This kit contains components of animal origin. These materials should be handled as potentially infectious
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth

## 6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

## 7. REAGENT SUPPLIED

| Kit Components | State | Quantity |
|---|---|---|
| Antibody Coated Microtiter Strips | ready to use | 96 wells |
| Biotin Labelled Antibody Conc. (50x) | concentrated | 0.26 ml |
| Streptavidin-HRP Conjugate | ready to use | 13 ml |
| Master Standard | lyophilized | 2 vials |
| Quality Control HIGH | lyophilized | 2 vials |
| Quality Control LOW | lyophilized | 2 vials |
| Dilution Buffer | ready to use | 50 ml |
| Biotin-Ab Diluent | ready to use | 13 ml |
| Wash Solution Conc. (10x) | concentrated | 100 ml |
| Substrate Solution | ready to use | 13 ml |
| Stop Solution | ready to use | 13 ml |

## 8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 5-1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
- Software package facilitating data generation and analysis (optional)

## 9. PREPARATION OF REAGENTS

**All reagents need to be brought to room temperature prior to use.**

**Centrifuge liquid containing microtube vials before opening.**

**Always prepare only the appropriate quantity of reagents for your test.**

**Do not use components after the expiration date marked on their label.**

### Assay reagents supplied ready to use:

**Antibody Coated Microtiter Strips**

Stability and storage:
Return the unused strips to the provided aluminium zip-sealed bag with desicant and seal carefully. Remaining Microtiter Strips are stable 3 months when stored at 2-8°C and protected from the moisture.

**Streptavidin-HRP Conjugate**

**Dilution Buffer**

**Biotin-Ab Diluent**

**Substrate Solution**

**Stop Solution**

Stability and storage:

Opened reagents are stable 3 months when stored at 2-8°C.

## Assay reagents supplied concentrated or lyophilized:

### Master Standard

**Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!**
Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the human LCAT in the stock solution is **160 ng/ml.**

Prepare set of standards using Dilution Buffer as follows:

| Volume of Standard | Dilution Buffer | Concentration |
|---|---|---|
| Stock | - | 160 ng/ml |
| 250 µl of stock | 250 µl | 80 ng/ml |
| 250 µl of 80 ng/ml | 250 µl | 40 ng/ml |
| 250 µl of 40 ng/ml | 250 µl | 20 ng/ml |
| 250 µl of 20 ng/ml | 250 µl | 10 ng/ml |
| 250 µl of 10 ng/ml | 250 µl | 5 ng/ml |
| 250 µl of 5 ng/ml | 250 µl | 2.5 ng/ml |

**Prepared Standards are ready to use, do not dilute them.**

Stability and storage:
**Do not store the diluted Standard solutions.**

### Quality Controls HIGH, LOW

**Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!**
Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
**Reconstituted Quality Controls are ready to use, do not dilute them.**

Stability and storage:
**Do not store the reconstituted Quality Controls.**

Note:
Concentration of analyte in Quality Controls need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid.Quality Controls serve just for control that the kit works in accordance with IFU and CoA and that ELISA test was carried out properly.

**Biotin Labelled Antibody Conc. (50x)**

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (50x) with 49 parts Biotin-Ab Diluent. Example: 20 µl of Biotin Labelled Antibody Concentrate (50x) + 980 µl of Biotin-Ab Diluent for 1 strip (8 wells).

Stability and storage:
Opened Biotin Labelled Antibody Conc. (50x) is stable 3 months when stored at 2-8°C.
**Do not store the diluted Biotin Labelled Antibody solution.**

**Wash Solution Conc. (10x)**

Dilute Wash Solution Conc. (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.

Stability and storage:
The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

## 10. PREPARATION OF SAMPLES

The kit measures human LCAT in serum, plasma (EDTA, citrate) and cerebrospinal fluid.

Samples should be assayed immediately after collection or should be stored at -20°C. Thoroughly mix thawed samples just prior to the assay and avoid repeated freeze-thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

**Serum and plasma samples:**

Dilute samples just prior to the assay 1 200x with Dilution Buffer in two steps as follows:

**Dilution A (30x):**
Add 5 µl of sample into 145 µl of Dilution Buffer. Mix well (not to foam). Vortex is recommended.

**Dilution B (40x):**
Add 10 µl of Dilution A into 390 µl of Dilution Buffer to prepare final dilution 1 200x. Mix well (not to foam). Vortex is recommended.

**CSF sample:**

Dilute samples 20x with Dilution Buffer just prior to the assay, e.g. 10 µl of sample + 190 µl of Dilution Buffer for singlets, or preferably 15 µl of sample + 285 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Samples should be stored at -20°C, or preferably at -70°C for long-term storage. Avoid repeated freeze/thaw cycles.
**Do not store the diluted samples**.

See Chapter 13 for stability of serum samples when stored at 2-8°C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of human LCAT.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

## 11. ASSAY PROCEDURE

1. Pipet **100 µl** of Standards, Quality Controls, Dilution Buffer (=Blank) and diluted samples, preferably in duplicates, into the appropriate wells. See *Figure 1* for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add **100 µl** of Biotin Labelled Antibody solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 µl** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 min**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for **10 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 - 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. **The absorbance should be read within 5 minutes following step 12.**

Note: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine LCAT concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were “in range” at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

| | strip 1+2 | strip 3+4 | strip 5+6 | strip 7+8 | strip 9+10 | strip 11+12 |
|---|---|---|---|---|---|---|
| **A** | **Standard 160** | **QC HIGH** | Sample 7 | Sample 15 | Sample 23 | Sample 31 |
| **B** | **Standard 80** | **QC LOW** | Sample 8 | Sample 16 | Sample 24 | Sample 32 |
| **C** | **Standard 40** | Sample 1 | Sample 9 | Sample 17 | Sample 25 | Sample 33 |
| **D** | **Standard 20** | Sample 2 | Sample 10 | Sample 18 | Sample 26 | Sample 34 |
| **E** | **Standard 10** | Sample 3 | Sample 11 | Sample 19 | Sample 27 | Sample 35 |
| **F** | **Standard 5** | Sample 4 | Sample 12 | Sample 20 | Sample 28 | Sample 36 |
| **G** | **Standard 2.5** | Sample 5 | Sample 13 | Sample 21 | Sample 29 | Sample 37 |
| **H** | **Blank** | Sample 6 | Sample 14 | Sample 22 | Sample 30 | Sample 38 |

*Figure 1: Example of a work sheet*

## 12. CALCULATIONS

Most microtiter plate readers perform automatic calculations of analyte concentration. The Standards curve is constructed by plotting the absorbance (Y) of Standards against the known concentration (X) of Standards, using the four-parameter algorithm. Results are reported as concentration of LCAT ng/ml in samples.

Alternatively, the logit log function can be used to linearize the standard curve, i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.

**The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay, e.g. 5 ng/ml (from standard curve) x 1 200 (dilution factor) = 6 µg/ml.**

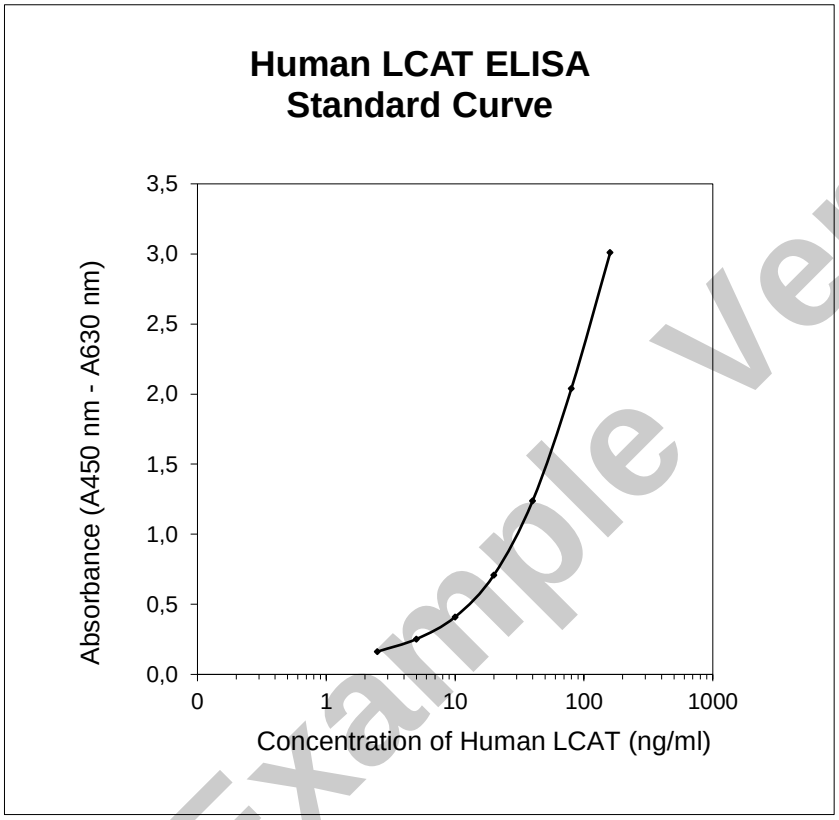


*Figure 2: Typical Standard Curve for Human LCAT ELISA.*

# 13. PERFORMANCE CHARACTERISTICS

**Typical analytical data of BioVendor Human LCAT ELISA are presented in this chapter.**

## Sensitivity

Limit of detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3xSD_{blank}$) is calculated from the real human LCAT values in wells and is 0.27 ng/ml.
* Dilution Buffer is pipetted into Blank wells.

## Limit of Assay

Results exceeding human LCAT level of 160 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the LCAT concentration.

## Specificity

The antibodies used in this ELISA are specific for human LCAT.
Determination of LCAT does not interfere with hemoglobin (1mg/ml), bilirubin (170 µmo/l) and triglycerides (5.0 mmol/l).

Serum of several mammalian species were measured in the assay. See results below.
For details please contact us at info@biovendor.com

| Mammalian serum sample | Observed crossreactivity |
|---|---|
| Bovine | no |
| Cat | no |
| Dog | no |
| Goat | no |
| Hamster | no |
| Horse | no |
| Monkey | yes |
| Mouse | yes |
| Pig | no |
| Rabbit | no |
| Rat | no |
| Sheep | no |

**Presented results are multiplied by respective dilution factor**

## Precision

Intra-assay (Within-Run) (n=8)

| Sample | Mean (µg/ml) | SD (µg/ml) | CV (%) |
|---|---|---|---|
| 1 | 28.74 | 0.60 | 2.1 |
| 2 | 31.82 | 0.97 | 3.0 |

Inter-assay (Run-to-Run) (n=6)

| Sample | Mean (µg/ml) | SD (µg/ml) | CV (%) |
|---|---|---|---|
| 1 | 21.98 | 2.40 | 10.9 |
| 2 | 30.41 | 0.28 | 0.9 |

## Spiking Recovery

Serum samples were spiked with different amounts of human LCAT and assayed.

| Sample | Observed (µg/ml) | Expected (µg/ml) | Recovery O/E (%) |
|---|---|---|---|
| Serum 1 | 22.62 | - | - |
| | 34.26 | 34.62 | 98.9 |
| | 45.10 | 46.62 | 96.7 |
| | 70.53 | 70.62 | 99.9 |
| Serum 2 | 27.27 | - | - |
| | 38.50 | 39.27 | 98.0 |
| | 48.04 | 51.27 | 93.7 |
| | 72.93 | 75.27 | 96.9 |
| CSF 1 | 0.50 | - | - |
| | 1.26 | 1.30 | 96.7 |
| | 0.82 | 0.90 | 90.2 |
| | 0.59 | 0.70 | 84.1 |
| CSF 2 | 0.48 | - | - |
| | 1.16 | 1.28 | 90.0 |
| | 0.77 | 0.88 | 87.5 |
| | 0.56 | 0.68 | 83.4 |

## Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

| Sample | Dilution | Observed (µg/ml) | Expected (µg/ml) | Recovery O/E (%) |
|---|---|---|---|---|
| Serum 1 | - | 43.11 | - | - |
| | 2x | 22.35 | 21.55 | 103.7 |
| | 4x | 11.43 | 10.78 | 106.0 |
| | 8x | 5.74 | 5.39 | 106.6 |
| Serum 2 | - | 34.56 | - | - |
| | 2x | 17.42 | 17.28 | 100.8 |
| | 4x | 8.70 | 8.64 | 100.7 |
| | 8x | 4.27 | 4.32 | 98.9 |
| CSF 1 | - | 1.44 | - | - |
| | 2x | 0.72 | 0.71 | 102.8 |
| | 4x | 0.35 | 0.35 | 97.4 |
| | 8x | 0.19 | 0.18 | 102.8 |
| CSF 2 | - | 0.87 | - | - |
| | 2x | 0.44 | 0.44 | 101.1 |
| | 4x | 0.21 | 0.22 | 94.8 |
| | 8x | 0.11 | 0.11 | 97.2 |

## Effect of sample matrix

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals.
Results are shown below:

| Volunteer No. | Serum (µg/ml) | Plasma (µg/ml) | | |
|---|---|---|---|---|
| | | EDTA | Citrate | Heparin |
| 1 | 28.91 | 24.74 | 21.36 | 27.01 |
| 2 | 25.51 | 26.97 | 24.43 | 31.27 |
| 3 | 34.08 | 34.83 | 28.26 | 29.03 |
| 4 | 27.92 | 24.80 | 22.38 | 25.64 |
| 5 | 34.00 | 34.36 | 31.34 | 33.76 |
| 6 | 30.38 | 28.36 | 26.99 | 27.15 |
| 7 | 31.13 | 27.03 | 26.67 | 27.20 |
| 8 | 32.83 | 34.79 | 32.59 | 38.87 |
| 9 | 25.90 | 25.05 | 22.28 | 32.19 |
| 10 | 22.20 | 18.74 | 15.59 | 18.55 |
| **Mean (µg/ml)** | **29.28** | **27.97** | **25.19** | **29.07** |
| **Mean Plasma/Serum (%)** | | **95.5** | **86.0** | **99.3** |
| **Coefficient of Determination $R^2$** | **-** | **0.83** | **0.8** | **0.34** |



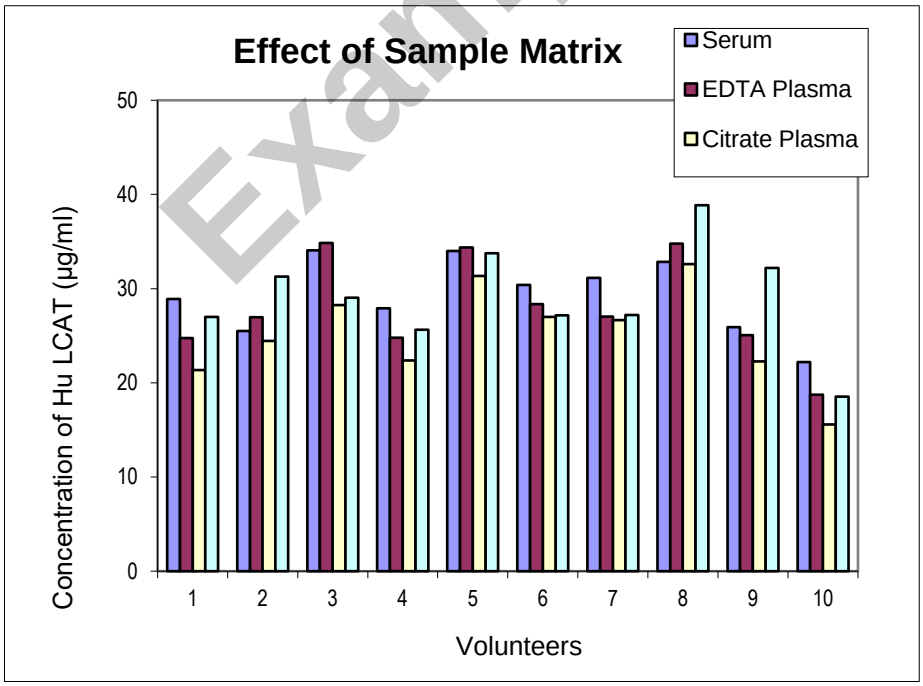


*Figure 3: LCAT levels measured using Human LCAT ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.*

### Stability of samples stored at 2-8°C

Samples are not stable at 2-8°C even for one day. Therefore they should be stored at - 20˚C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and thimerosal, resulting in the final concentration of 0.03% and 0.01%, respectively.

| Sample | Incubation Temp, Period | Serum (µg/ml) | Plasma (µg/ml) | | |
|---|---|---|---|---|---|
| | | | EDTA | Citrate | Heparin |
| 1 | -20°C | 28.18 | 27.27 | 25.36 | 29.35 |
| | 2-8°C, 1 day | 29.40 | 29.60 | 22.61 | 30.22 |
| | 2-8°C, 7 days | 39.14 | 32.22 | 23.87 | 26.25 |
| 2 | -20°C | 35.42 | 36.39 | 21.78 | 34.29 |
| | 2-8°C, 1 day | 26.03 | 32.37 | 26.68 | 27.24 |
| | 2-8°C, 7 days | 61.68 | 29.02 | 26.72 | 36.75 |
| 3 | -20°C | 30.69 | 30.71 | 28.34 | 35.03 |
| | 2-8°C, 1 day | 33.06 | 38.53 | 31.37 | 33.36 |
| | 2-8°C, 7 days | 37.96 | 36.19 | 30.13 | 33.97 |

### Effect of Freezing/Thawing

Concentration of Human LCAT in serum and plasma samples declined more than 20% after repeated (3x) freeze/thaw cycles. It is strongly recommended to avoid repeated freezing/thawing of the samples.

| Sample | Number of f/t cycles | Serum (µg/ml) | Plasma (µg/ml) | | |
|---|---|---|---|---|---|
| | | | EDTA | Citrate | Heparin |
| 1 | 1x | 37.62 | 28.69 | 21.37 | 31.73 |
| | 3x | 27.30 | 32.24 | 29.68 | 29.60 |
| | 5x | 33.37 | 32.41 | 28.57 | 32.89 |
| 2 | 1x | 39.57 | 43.68 | 35.09 | 41.45 |
| | 3x | 37.15 | 33.75 | 32.03 | 42.51 |
| | 5x | 34.72 | 34.59 | 24.24 | 38.41 |
| 3 | 1x | 27.13 | 26.43 | 29.26 | 27.86 |
| | 3x | 33.59 | 31.00 | 25.99 | 29.21 |
| | 5x | 33.18 | 29.98 | 29.57 | 29.14 |

## 14. DEFINITION OF THE STANDARD

The recombinant human LCAT is used as the Standard. The human LCAT, produced in cell lines HEK 293, is 48.5 kDa protein containing 416 amino acid residues of the human LCAT and 13 AA extra.

## 15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 101 unselected donors (56 men + 45 women) 22-65 years old were assayed with the Biovendor Human LCAT ELISA in our laboratory:

### Age and Sex dependent distribution of LCAT

| Sex | Age (years) | n | Mean | SD | Min. | Max. |
|---|---|---|---|---|---|---|
| | | | LCAT(µg/ml) | | | |
| Men | 23-29 | 10 | 25.12 | 2.98 | 22.25 | 33.13 |
| | 30-39 | 18 | 23.20 | 3.32 | 17.51 | 29.94 |
| | 40-49 | 21 | 25.49 | 4.61 | 18.36 | 36.82 |
| | 50-65 | 7 | 25.82 | 1.94 | 23.31 | 28.82 |
| Women | 22-29 | 9 | 23.17 | 2.63 | 17.33 | 26.14 |
| | 30-39 | 14 | 26.92 | 5.19 | 19.51 | 35.00 |
| | 40-49 | 17 | 24.46 | 5.70 | 15.27 | 32.66 |
| | 50-61 | 5 | 30.18 | 4.56 | 23.85 | 36.28 |

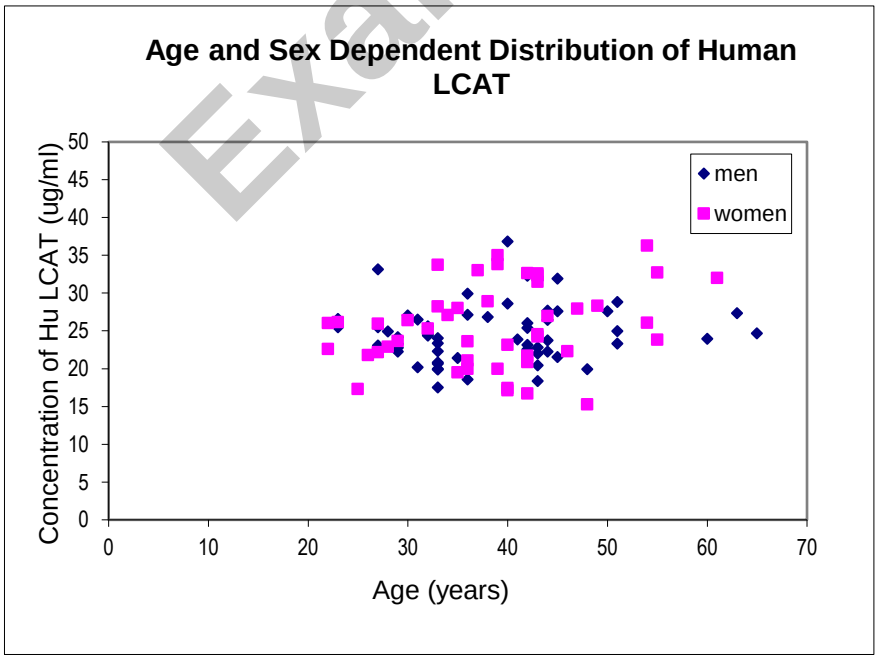


*Figure 4: Human LCAT concentration plotted against donor age and sex.*

### Reference range

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological reference ranges for LCAT levels with the assay.

## 16. TROUBLESHOOTING AND FAQS

### Weak signal in all wells

Possible explanations:
- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

### High signal and background in all wells

Possible explanations:
- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

### High coefficient of variation (CV)

Possible explanation:
- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples

## 17. REFERENCES

**References to human LCAT:**


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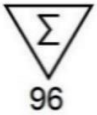
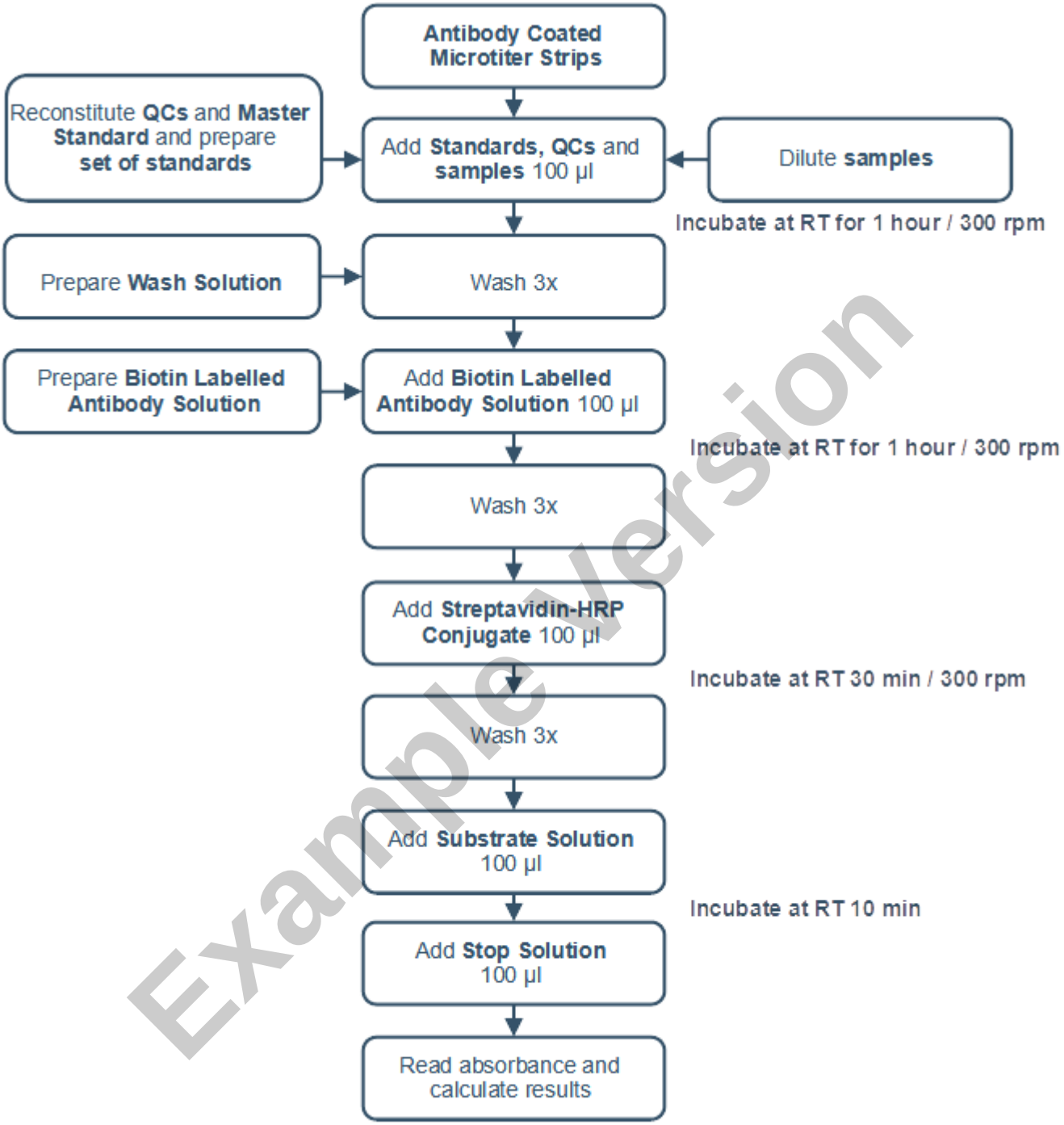
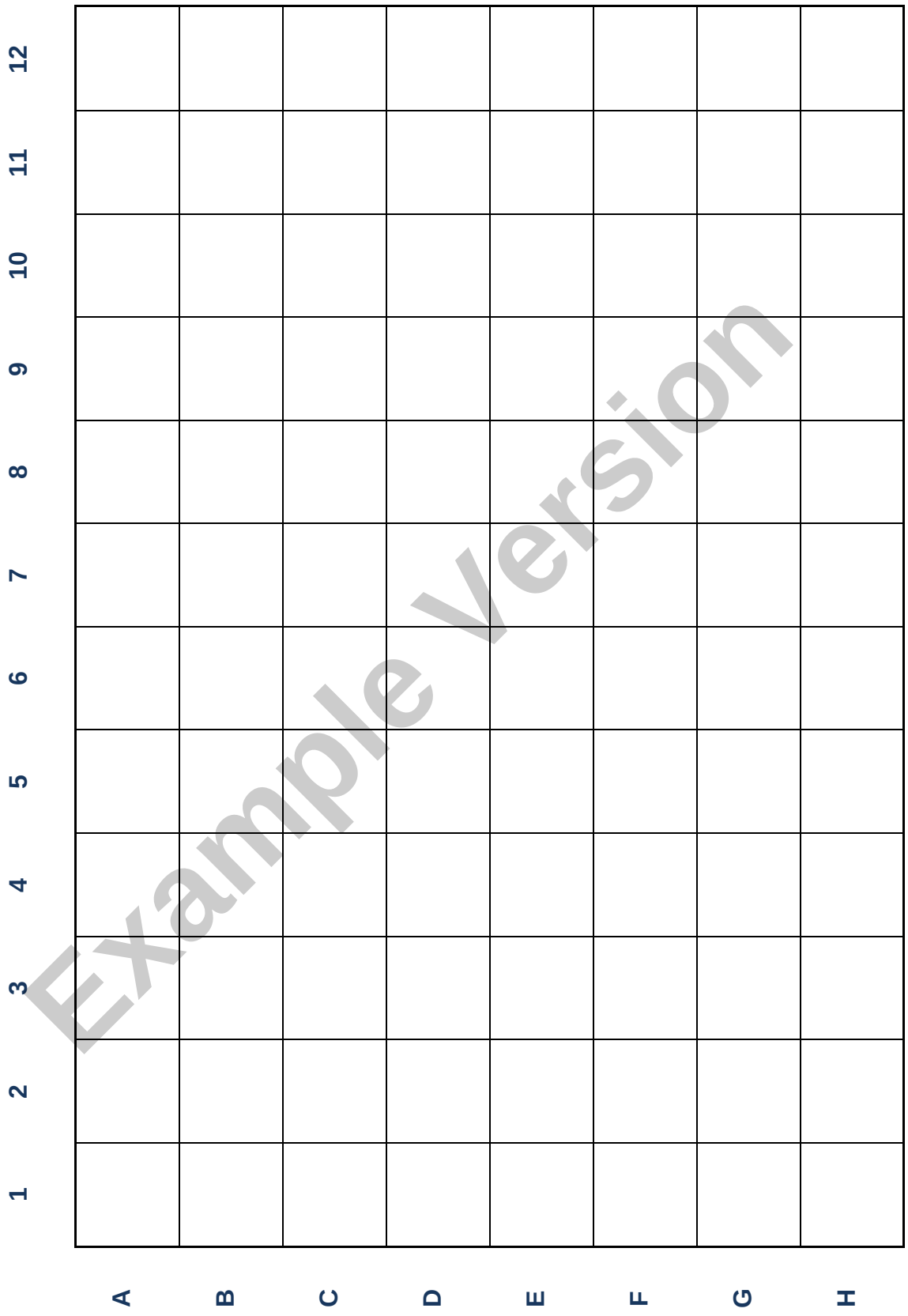
**For more references on this product see our web pages at www.biovendor.com.**

## 18. EXPLANATION OF SYMBOLS

| | |
|---|---|
|   | Catalogue number |
|   | Batch code |
|  | Caution |
|  | Use by date |
|   | Temperature limit |
|  | Manufacturer |
|   | Read electronic instructions for use - eIFU |
|   | The content is sufficient for 96 tests |
|  | Biological risks |

## 19. ASSAY PROCEDURE - SUMMARY





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