GENLISA® Human Oxidized Low Density Lipoprotein, OxLDL ELISA

REF : KBH4091

Ver 1.0

RUO

Enzyme Immunoassay for the Quantitative Determination of Human Oxidized Low Density Lipoprotein, OxLDL in serum, plasma and other biological samples

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

For Research Use Only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Krishgen Biosystems Private Limited is strictly prohibited.

REF KBH4091 Σ 96 tests

Krishgen Biosystems Private Limited
For US/Europe Customers: toll free +1(888)-970-0827 | tel +1(562)-568-5005
For Asia/India Customers: +91(22)-49198700
Email: sales1@krishgen.com | http://www.krishgen.biz / www.krishgenbio.com

Introduction:

The GENLISA® ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a sandwich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody. Double antibodies are used in this kit.

Intended Use:

The GENLISA® Human Oxidized Low Density Lipoprotein, OxLDL ELISA kit is used as an analytical tool for quantitative determination of Human Oxidized Low Density Lipoprotein, OxLDL in serum, plasma and other biological samples.

Principle:

The method employs sandwich ELISA technique. Anti-Human Oxidized Low Density Lipoprotein, OxLDL monoclonal antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and Human Oxidized Low Density Lipoprotein, OxLDL present in the sample are bound by the antibodies. Anti-Human OxLDL antibody:HRP Conjugate is added. After washing microwells in order to remove any non-specific binding, the Chromogenic substrate solution is added to microwells and color develops proportionally to the amount of Human Oxidized Low Density Lipoprotein, OxLDL in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Anti-Human OxLDL Antibody Coated Microtiter Plate (96 wells) – 1 no.
2. Human OxLDL Standard (2000, 1000, 500, 250, 125, 62.5 pg/ml) - 6 vials x 0.3 ml
3. Anti-Human OxLDL antibody:HRP Conjugate – 10 ml
4. Sample Dilution - 6 ml
5. (20X) Wash Buffer – 25 ml
6. Chromogenic substrate A – 6 ml
7. Chromogenic substrate B – 6 ml
8. Stop Solution – 6 ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Deionized (DI) water
3. Wash bottle or automated microplate washer
4. Clean tubes and Eppendorf tubes
5. Precision single and multi-channel pipette and disposable tips.
6. 37°C incubator
7. Timer.

Handling/Storage:

1. All reagents should be stored as indicated on the component label.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to 2ºC - 8ºC. Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Sample Preparation and Storage:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
2. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. **Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.
4. **Urine-** Collect urine in a sterile container, centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
5. **Cell Culture Supernatant-** Collect sample in a sterile container. Centrifuge for 20-mins at 2000-3000 rpm. Remove the supernatant carefully. When examining the components within the cell, dilute cell suspension with PBS (pH 7.2-7.4), if cell concentration is greater than 1 million/ml. Damage the cells by repeated freeze-thaw cycles to release intracellular components. Centrifuge for 20-min at 2000-3000 rpm. If precipitation appears, centrifuge again.
6. **Cell Lysate – for adherent cells-** Remove the culture medium and wash with PBS, normal saline or serum-free medium. Add an appropriate amount of lysis buffer and gently aspirate to ensure through cell contact. Typically cells will be lysed within 10 seconds. For suspension cells: Centrifuge to collect cells, then wash with PBS, normal saline, or serum-free medium. Add lysis buffer and aspirate to disperse cells. Gently tap with fingers to complete lysis. After complete lysis, centrifuge at 10,000 – 14,000xg for 3-5 minutes. Collect the supernatant immediately for analysis or aliquoting, then store at -20°C.

Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Before use, all components should be rewarmed at least 60 min to ensure full rewarmed to room temperature.
2. Bring all reagents to Room temperature before use.
3. To make **Wash Buffer (1X)**; dilute **25 ml of 20X Wash Buffer in 475 ml of DI water**.
4. **Working Chromogenic Substrate:** Mix Substrate Solutions A and B thoroughly in a 1:1 ratio prior to use, and use the prepared mixture within 15 minutes.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Human Oxidized Low Density Lipoprotein, OxLDL. High Dose Hook Effect is due to excess of antigen for very high concentrations of Human Oxidized Low Density Lipoprotein, OxLDL present in the sample.
3. Human Oxidized Low Density Lipoprotein, OxLDL concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
4. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Human Oxidized Low Density Lipoprotein, OxLDL.
5. It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. All reagents and components should be restored to room temperature first. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.

2. Prepare the working solution of each component of the kit according to the method described in the previous reagent preparation.

3. Take out the required strips from the aluminium foil bag, and seal the remaining strips in a self-sealing bag and put them back in the refrigerator.

4. Set standard, sample wells on the pre-coated plate respectively, and then, record the positions.

5. Add **50 ul** of different concentration of standard product to each standard well.

6. Add **50 ul of sample** to be tested to the sample well and do not add blank well.

7. Pipette **100 ul Anti-Human OxLDL antibody:HRP Conjugate** to all wells.

8. Cover the plate with a sealer and incubate for **60 minutes** at **37°C.**

9. Aspirate and wash plate 5 times with diluted **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

10. Pipette **100 ul Working Chromogenic Substrate** to all wells.

11. Cover the plate with a sealer and incubate at **37°C** for **15 minutes in dark**.

12. Pipette **50 ul** of **Stop Solution** to all wells. The wells should turn from blue to yellow in color.

13. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Graph Paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Human Oxidized Low Density Lipoprotein, OxLDL concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Human Oxidized Low Density Lipoprotein, OxLDL Concentration.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or 4-PL is best recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Performance Characteristics of the Kit:
This kit has been validated. Please view the details herein below.

Standard Calibration Range:
62.5 pg/ml – 2000 pg/ml.

Sensitivity:
Limit Of Quantification:
It is defined as the lowest detectable concentration that can be determined with an acceptable repeatability and the LOQ was found to be 10 pg/ml.

Specificity:
This assay has high sensitivity and excellent specificity for detection of Human Oxidized Low Density Lipoprotein, OxLDL. No significant cross-reactivity or interference between Human Oxidized Low Density Lipoprotein, OxLDL and analogues was observed.

Recovery Rate:
The recovery rate is between 85% and 115%.

Precision:
Intra-Assay: CV<10%
Inter-Assay: CV<15%

Linearity:
The correlation coefficient r value of the calibration dose response curve is greater than or equal to 0.9900.

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Source materials maybe derived from Human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed.
- Do not smoke, eat or drink while handling kit material.
- Always use protective gloves.
- Never pipette material by mouth.
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all Ratand individual regulations to the use of this kit.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Interpolated Concentration
1A 2A	Blank Blank			
1B 2B	Standard No.1 Standard No.1			
1C 2C	Standard No.2 Standard No.2			
1D 2D	Standard No.3 Standard No.3			
1E 2E	Standard No.4 Standard No.4			
1F 2F	Standard No.5 Standard No.5			
1G 2G	Standard No.6 Standard No.6			
1H 2H	Sample Sample			
3A 4A	Sample Sample			
3B 4B	Sample Sample			

LIMITED WARRANTY

Krishgen Biosystems Private Limited does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems Private Limited, or against damages resulting from such non-Krishgen Biosystems Private Limited made products or components. Krishgen Biosystems Private Limited passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems Private Limited.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems Private Limited shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems Private Limited shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems Private Limited be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems Private Limited with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Krishgen Biosystems Private Limited. 2026

THANK YOU FOR USING A KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS PRIVATE LIMITED | OUR REAGENTS | YOUR RESEARCH |

SYMBOLS KEY

MTP	Coated Microtiter Plate (96 wells)
STD	Standard
HRP CONJ	Conjugate Horseradish Peroxidase
SAMP DIL	Sample Dilution
20X WASH BUF	(20X) Wash Buffer
SUB A	Chromogenic Substrate A
SUB B	Chromogenic Substrate B
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature