oxLDL-β_2GPI (human) ELISA Kit

Item No. 10007893

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity
1	oxLDL-β_2GPI Calibrator Serum*	1 vial/200 µl
2	oxLDL-β_2GPI High Control Serum*	1 vial/200 µl
3	oxLDL-β_2GPI Low Control Serum*	1 vial/200 µl
4	oxLDL-β_2GPI Sample Diluent	2 bottles/50 ml
5	oxLDL-β_2GPI Biotin-Antibody Conjugate	1 bottle/15 ml
6	oxLDL-β_2GPI HRP-Streptavidin Conjugate (HRP-SA)	1 bottle/15 ml
7	oxLDL-β_2GPI HRP Substrate	1 bottle/20 ml
8	oxLDL-β_2GPI Stop Solution	1 bottle/20 ml
9	oxLDL-β_2GPI Wash Buffer Concentrate	2 bottles/50 ml
10	Anti-oxLDL-β_2GPI (murine) coated, 96-well ELISA Plate	1 plate
11	96-Well Cover Sheet	1 cover

*CAUTION: May contain sodium azide as a preservative.
If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.

WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

Human source material used to prepare the Calibrators and Controls included in this kit has been tested and shown to be negative for antibodies to HBsAg, HCV, and HIV 1 & 2 by FDA required tests. However, all human blood derivatives and samples, should be handled as potentially infectious material.

Do not pipette by mouth.

Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.

Certain components of this product may contain Sodium Azide as a preservative. Sodium Azide has been reported to form lead and copper azides when left in contact with these metals. These metal azides are explosive. Any solutions containing Sodium Azide must be thoroughly flushed with copious amounts of water to prevent the build-up of exploisive metal azides in the plumbing sytem.

Wear disposable gloves while handling kit reagents and wash hands thoroughly afterwards.

The HRP substrate solution can cause irritation to the eyes and skin. Absorption through the skin is possible. Use gloves when handling substrate and wash thoroughly after handling. Keep reagent away from ignition sources. Avoid contact with oxidizing agents.

Certain components are labeled with the following: Harmful if swallowed. If swallowed, seek medical advice immediately and show container or label. Irritating to eyes and skin. Avoid contact with skin and eyes. In case of contact with eyes, flush affected areas with copious amounts of water and seek medical advice. Wear suitable protective clothing.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed at 2-8°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm.
2. Adjustable pipettes and a repeat pipettor.
3. A source of 'UltraPure' water. Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. *NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000).*

INTRODUCTION

Background

Atherosclerosis is characterized by a gradual thickening of arterial walls due to the excessive accumulation of lipids, a process that leads to reduced elasticity, decreased blood supply, thrombus (clot) formation and tissue or organ damage. Experimental evidence indicates that pro-inflammatory factors and dyslipidemia are the main contributors to the development of atherosclerosis.[1-5] Low-density lipoprotein (LDL) is the principal form of cholesterol that accumulates in atherosclerotic lesions or plaques, but LDL must be first modified into an oxidized structure (oxLDL). The most significant pro-atherogenic mechanism for modifying LDL into oxLDL is oxidative stress.[6,7] In addition, chronic oxidative stress produces endothelium and platelet dysfunction, which in combination with the accumulation of oxLDL in the arterial wall, leads to the development of an arterial prothrombotic state referred to as athero-thrombosis.[8-13]

Unlike native LDL, oxLDL binds to β_2GPI to form oxLDL-β_2GPI complexes. The interaction between oxLDL and β_2GPI is initially mediated by electrostatic forces, producing unstable or dissociable complexes. This initial interaction is followed by the formation of stable (non-dissociable) complexes mediated by covalent bonds. Stable complexes are regarded as pathogenic and more clinically relevant.[14-17] Both oxLDL and β_2GPI have been demonstrated in atherosclerotic lesions by immunostaining, along with immunoreactive lymphocytes and immunoglobulins.[6,18] The *in vitro* macrophage uptake of oxLDL-β_2GPI complexes is mediated by scavenger receptors, and this uptake is significantly enhanced in the presence of anti-β_2GPI antibodies, a process likely mediated by Fcγ receptors.[17] This mechanism is physiologically relevant in explaining the development of foam cells within the atherosclerotic lesions or plaques. Thus, oxLDL-β_2GPI complexes have been implicated as proatherogenic antigens and may represent a serologic risk factor and/or a significant contributor to the development of athero-thrombosis.[19-21]

The premature (or accelerated) development of clinical atherosclerosis in patients with systemic autoimmune diseases cannot be fully explained by the traditional risk factors or disease treatment, *i.e.*, steroids.[22-25] Elevated serum levels of oxLDL-β_2GPI complexes have been demonstrated in patients with SLE, SSc and APS, disorders characterized by a pro-thrombotic predisposition and significant vascular complications.[26-29] Atherosclerotic cardiovascular disease is also common in patients with type 2 DM.[12,13] About half of type 2 DM patients showed elevated serum levels of oxLDL-β_2GPI complexes, compared to age and sex matched healthy controls. Thus, the presence of circulating oxLDL-β_2GPI complexes in patients with autoimmune diseases and DM suggest a possible pathogenic role in the development of athero-thrombotic complications.

Principle of the Assay

The test is performed as an indirect ELISA. Diluted serum or plasma samples, calibrators, and controls are incubated in microwells coated with purified anti-human monoclonal antibody directed only to complexed β_2GPI. Incubation allows the oxLDL-β_2GPI antigen complex present in the samples to react with the immobilized antibody. After the removal of unbound serum or plasma proteins by washing, anti-human apoB100 (LDL) monoclonal antibodies, conjugated to biotin, are added to form complexes with the bound antigen. Following another washing step, horseradish peroxidase (HRP)-conjugated Streptavidin (SA) is added to form complexes with the bound biotin-conjugated antibody. Following another washing step, the bound HRP-SA conjugate is assayed by the addition of tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) chromogenic substrate. Color develops in the wells at an intensity proportional to the serum or plasma concentration of the oxLDL-β_2GPI antigen complex. Results are calculated against a calibration curve prepared from the calibrator provided in the kit.

Definition of Key Terms

Blank: background absorbance caused by TMB. The blank absorbance should be subtracted from the absorbance readings of all the other wells.

Standard Curve: a plot of the absorbance *versus* concentration for a series of wells containing various known amounts of analyte.

PRE-ASSAY PREPARATION

NOTE: Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. UltraPure water may be purchased from Cayman (Item No. 400000).

Wash Solution Preparation

Measure 50 ml of Wash Buffer Concentrate (20X) and dilute to 1 liter with UltraPure water. The pH of the final solution should be 7.35 ± 0.1. Store unused wash solution at 2-8°C. Discard if the solution shows signs of microbial growth or contamination.

Specimen Collection and Preparation

Serum, EDTA plasma, or citrated plasma (3.2%) are the preferred sample matrixes. Blood should be collected by venipuncture, allowed to clot, and the serum separated from the cells by centrifugation. If not tested immediately, specimens should be stored at 2-8°C. If specimens are to be stored for more than one week, then freeze at -20°C or below. Avoid repeated freezing and thawing. Do not use hemolyzed, icteric, or lipemic serum as these conditions may cause aberrant results. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

If EDTA or citrated plasma is to be used, blood should be collected by venipuncture and the plasma separated from the cells immediately by centrifugation at 1,500 x g for 10 minutes. The supernatant must be carefully removed after centrifugation to avoid contamination with platelets. Repeating the centrifugation and separation steps may be advisable to minimize platelet contamination. If not tested immediately, plasma samples should be stored as described for serum.

ASSAY PROTOCOL

NOTE: All dilutions of the Calibrators, Controls, and samples should be made just prior to use in the assay.

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two blanks (Blk) and a six point standard curve (including the blank) run in duplicate. Each sample should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 1, below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see **Analysis**, page 14, for more details). We suggest you record the contents of each well on the template sheet provided (see page 19).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	Blk	1	1	9	9	17	17	25	25	33	33
B	S1	S1	2	2	10	10	18	18	26	26	34	34
C	S2	S2	3	3	11	11	19	19	27	27	35	35
D	S3	S3	4	4	12	12	20	20	28	28	36	36
E	S4	S4	5	5	13	13	21	21	29	29	37	37
F	S5	S5	6	6	14	14	22	22	30	30	38	38
G	C1	C1	7	7	15	15	23	23	31	31	39	39
H	C2	C2	8	8	16	16	24	24	32	32	40	40

Blk = Blank
S1-S5 = Standards 1-5
C1-C2 = Controls
1-40 = Samples

Figure 1. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Procedural Notes

1. Bring serum or plasma samples and kit reagents to room temperature (18-26°C) and mix well before using; avoid foaming. Return all unused samples and reagents to refrigerated storage as soon as possible.
2. The plate reader should be programmed to air blank.
3. Good washing technique is critical for optimal performance of the assay. Adequate washing is best accomplished by directing a forceful stream of wash solution from a plastic squeeze bottle with a wide tip into the bottom of the microwells. An automated microtiter plate washing system can also be used.
4. IMPORTANT: Failure to adequately remove residual Wash Solution can cause inconsistent color development of the Substrate Solution.
5. Use a multichannel pipettor capable of delivering to 8 or 12 wells simultaneously when possible. This speeds the process and provides more uniform incubation and reaction times for all wells.
6. Careful controlled timing of all steps is critical. All Calibrator, Controls, and samples must be added within a five minute period. Batch size of samples should not be larger than the amount that can be added within this time period.
7. For all incubations, the start of the incubation period begins with the completion of reagent or sample addition.
8. Addition of all samples and reagents should be performed at the same rate and in the same sequence.
9. Incubation temperatures above or below normal room temperature (18-26°C) may contribute to inaccurate results.

10. Avoid contamination of reagents when opening and removing aliquots from the primary vials.
11. Do not use kit components beyond expiration date.
12. Do not use kit components from different kit lot numbers.

Assay Procedure

1. Remove any microwell strips that will not be used from the frame. Store them with the desiccant pouch in the resealable bag provided.
2. Prepare a six-point calibration curve: Label six tubes for Calibrators 1-6.

   In tube #1, prepare a 1:100 dilution of Calibrator in Sample Diluent by adding 10 µl Calibrator to 1,000 µl Sample Dilutent.

   Add 500 µl of Sample Diluent (yellow) to tubes # 2-6.

   Remove 250 µl from tube # 1, transfer to tube # 2 and mix well.

   Repeat this 3-fold serial dilution series through tube 5.

   Tube # 6 containing only Sample Diluent (yellow) is to be used as a reagent blank. A reagent blank control should be run on each plate. These wells will be treated the same as sample wells in subsequent assay steps.

   The value of Calibrator 1 is indicated on the vial label. The value of Calibrator dilutions 2-5 are calculated by dividing Calibrator 1 value by each Calibrator dilution factor (DF).

Calibrator #	DF	Volume to Add (µl)	Volume to Sample Diluent (µl)	Calibrator value
1	-	10 µl Calibrator	1,000 µl	5.00
2	3	250 µl Calibrator 1	500 µl	1.67
3	9	250 µl Calibrator 2	500 µl	0.56
4	27	250 µl Calibrator 3	500 µl	0.19
5	81	250 µl Calibrator 4	500 µl	0.06
6 (Reagent Blank)	-	-	500 µl	0.00

Table 1. Example calibrator calculations

3. Duplicate determinations are recommended. Prepare a 1:100 dilution of the controls and samples in Sample Diluent (yellow), *e.g.*, 10 µl sample added to 1,000 µl Sample Diluent equals a 1:100 sample dilution.
4. Mix thoroughly, and add 100 µl of the dilutions (6 Calibrators which include the reagent blank, controls and samples) to the appropriate microwells.
5. Incubate 60 minutes at room temperature (18-26°C). After the incubation is complete, carefully invert the microwells and empty the sample fluid. Do not allow samples to contaminate other microwells.
6. Wash four times with wash solution. Each well should be completely filled with Wash Solution per wash. Invert microwells between each wash to empty fluid. Use a snapping motion of the wrist to shake the liquid from the wells. The frame must be squeezed at the center on the top and bottom to retain microwell modules during washing. Blot on absorbent paper to remove residual wash fluid. Do not allow wells to dry out between steps.
7. Add 100 µl Biotin-Antibody Conjugate Solution (green) to each well.
8. Incubate for 30 minutes at room temperature (18-26°C). After the incubation is complete, carefully invert the microwells and empty the conjugate solution.
9. Wash four times as in step 6.
10. Add 100 µl HRP-Streptavidin Conjugate solution (blue) to each well.
11. Incubate for 30 minutes at room temperature (18-26°C) as in step 8.
12. Wash four times as in step 6.
13. Add 100 µl Substrate (TMB, H_2O_2) to each well and incubate for 30 minutes at room temperature (18-26°C). Add the substrate to the wells at a steady rate. Blue color will develop in wells with positive samples.
14. Add 100 µl Stop Solution (0.25 M sulfuric acid) to each well to stop the enzyme reaction. Be sure to add Stop Solution to the wells in the same order and at the same rate as the Substrate was added. Blue substrate will turn yellow and colorless substrate will remain colorless. Air blank or zero the plate reader. Read the O.D. of each well at 450 nm (and 650 nm reference if dual beam). The O.D. values should be measured within five minutes after the addition of Stop Solution.

ANALYSIS

Many plate readers come with data reduction software that plots data automatically. Alternatively a spreadsheet program can be used. We recommend using a 4-parameter curve fit calculation.

Calculations

1. Calculate the mean O.D. values for the duplicates of the Calibrator Solution dilutions, Reagent Blank, Controls and samples.
2. Plot the mean O.D. obtained for each Calibrator (x-axis) against the corresponding Calibrator Level value (y-axis) using a 4-parameter curve fit calculation.
3. Ensure that all quality control parameters have been met (see Quality Control) before reporting test results.
4. A new calibration curve should be prepared with every test run.

Quality Control

1. The mean O.D. of the reagent blank (zero point) should be less than 0.100. Readings greater than 0.100 may indicate possible reagent contamination or inadequate plate washing.
2. The oxLDL-β_2GPI antigen complex values obtained for the control sera should be within the ranges indicated on the container labels. Occasional small deviations outside these ranges are acceptable.
3. O.D. values for the duplicates of the controls or samples should be within 20% CV of the mean O.D. value for samples with absorbance readings greater than 0.200.
4. Each laboratory should periodically determine their own normal range for the appropriate population being tested.
5. Samples with oxLDL-β_2GPI antigen complex values greater than 5 Units/ml may be reported as "greater than 5 Units/ml" or diluted and re-assayed. Correct the values obtained multiplying by the dilution factor.

Limitations of the Test

oxLDL-β_2GPI complexes may result from oxidative stress caused by various underlying inflammatory conditions. Chronic vascular inflammation characteristic of systemic autoimmune diseases and chronic hyperglycemia of DM are known causes of oxidative stress, however, other transient conditions such as certain systemic bacterial infections may cause oxidative stress and elevated oxLDL-β_2GPI complexes. oxLDL-β_2GPI complexes have been detected in some patients with syphilis and infective endocarditis.

The capture monoclonal antibody used in the assay only binds β_2GPI complexed with oxLDL. The assay does not detect native or unmodified LDL, as this form of LDL does not bind to β_2GPI.

The assay uses Mg^{++} in the Sample Diluent to dissociate electrostatically bound complexes, thus favoring the detection of stable (covalently bound) and possibly pathogenic oxLDL-β_2GPI complexes.

Testing samples containing excess hemoglobin, lipids, and/or bilirubin is not recommended as these substances may interfere with the results of the assay.

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Related Products

CD36 Monoclonal Antibody - Item No. 188150
CD36 Polyclonal Antibody - Item No. 100011
Cholesterol Assay Kit - Item No. 10007640
Cholesterol Cell-Based Detection Assay Kit - Item No. 10009779
LDL Receptor Polyclonal Antibody - Item No. 10007665
LDL Uptake Cell-Based Assay Kit - Item No. 10011125
Lipid Droplets Fluorescence Assay Kit - Item No. 500001
Triglyceride Assay Kit - Item No. 10010303

Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

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A												
B												
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NOTES

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