Human Anti-Ox LDL Antibody ELISA Kit (Sandwich ELISA)

User Manual

Catalog No. LS-F67367

It is important that you read this entire manual carefully before starting your experiment.

This kit is for Research Use Only and not for Diagnostic Use.
This kit is not approved for use in humans or for clinical diagnosis.

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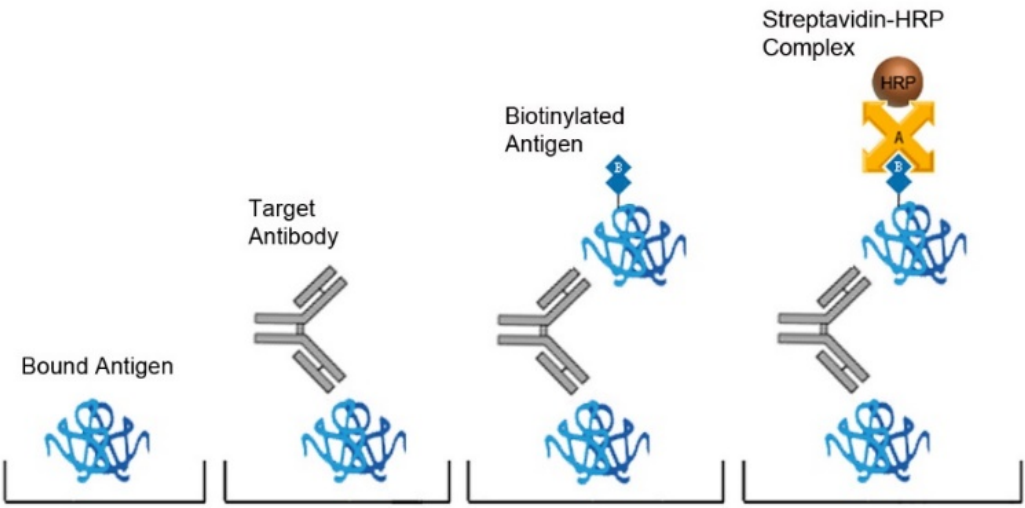
ASSAY SPECIFICATIONS

Target:	Anti-Ox LDL Antibody
Synonyms:	Anti-Ox LDL Antibody, Anti-Oxidized Low Density Lipoprotein Antibody
Specificity:	Antibody. No significant cross-reactivity or interference between Anti-Ox LDL Antibody and analogs was observed. This claim is limited by existing techniques therefore cross-reactivity may exist with untested analogs.
Sample Types:	This kit is recommended for use with serum, plasma and tissue homogenates. Use with other sample types is not supported.
Detection:	Colorimetric – 450nm (TMB)
Detection Range:	0.938-60 mIU/ml
Performance:	Intra-Assay CV (<8%); Inter-Assay CV (<10%)
Limitations:	This kit is for **Research Use Only** and is not intended for diagnostic use. This kit is not approved for use in Human or for clinical diagnosis.

ASSAY PRINCIPLE

This antibody detection assay is based on the sandwich ELISA principle. Each well of the supplied microtiter plate has been pre-coated with capture antigen. Controls or samples containing the target antibody are added to the wells and the antibody binds to the capture antigen. Unbound control or sample is washed away. A biotin-conjugated antigen is then added which binds to the captured antibody. Unbound antigen is washed away. An Avidin-Horseradish Peroxidase (HRP) conjugate is then added which binds to the biotin. Unbound Avidin-HRP conjugate is washed away. A TMB substrate is then added which reacts with the HRP enzyme resulting in color development. A sulfuric acid stop solution is added to terminate color development reaction and then the optical density (OD) of the well is measured at a wavelength of 450 nm ± 2 nm. An OD standard curve is generated using known antigen concentrations; the OD of an unknown sample can then be compared to the standard curve in order to determine its antigen concentration.

ASSAY PRINCIPLE IMAGE



KIT COMPONENTS

Component	Quantity
Coated 96-well Strip Plate	12 x 8 Strips
Standard (Lyophilized)	2 vials
Sample Diluent	1 vial x 20ml
Biotin-labeled Antigen (100x)	1 vial x 120ul
Antigen Dilution Buffer	1 vial x 10ml
HRP-Streptavidin Conjugate (100x)	1 vial x 120 µl
HRP-Streptavidin Conjugate Diluent	1 vial x 10 ml
Wash Buffer (25x)	1 vial x 30 ml
TMB Substrate	1 vial x 10 ml
Stop Solution	1 vial x 10 ml
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KIT STORAGE

The unopened kit can be stored at 2-8°C through the expiration date. Once opened, the kit can be stored at 2-8°C for 6 months. HRP-Conjugate Antibody (Concentrated), Substrate, and Biotin-labeled Antigen (Concentrated) should be protected from light. Unused strips should be kept in a sealed bag with the desiccant provided to minimize exposure to damp air.

OTHER REQUIRED SUPPLIES

- Microplate reader with 450nm wavelength filter or dual-wavelength (450/630nm)
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
- High-precision pipette and sterile pipette tips
- Eppendorf tubes
- 37°C incubator
- Deionized or distilled water

SAMPLE COLLECTION

This assay is recommended for use with serum or plasma. No other sample types are supported. The sample collection protocols below have been provided for your reference.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2–8°C within 30 minutes of collection. Collect the supernatant for assaying.

Serum - Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g at 4°C. Collect the supernatant for assaying. The suspended fibrous protein may cause a false positive result if not fully precipitated.

SAMPLE COLLECTION NOTES

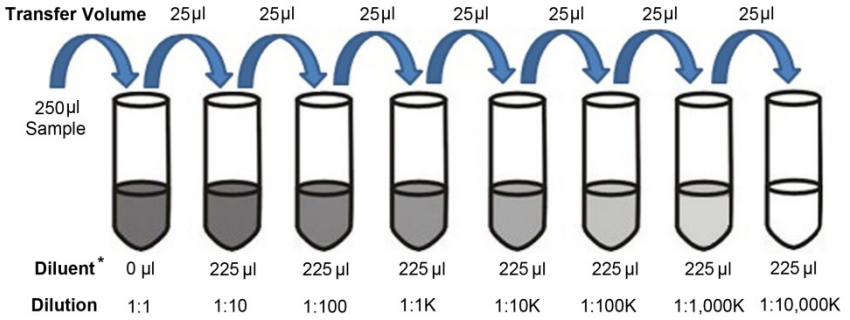
1. LSBio recommends that samples are used immediately upon preparation. Alternatively, samples stored at 2-8°C should be used within 7 days. For long-term storage sample aliquots should be prepared and stored at -20°C if used within 1 month, or -80°C if used within 3 months. Long term storage can result in protein degradation and denaturation, which may result in inaccurate results.
2. Avoid repeated freeze/thaw cycles for all samples.
3. Prior to the assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates.
4. Tubes for blood collection should be disposable and non-endotoxin. Samples with high hemolysis or high lipid content are not suitable for ELISA assay.
5. In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.
6. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.
7. Due to factors including cell viability, cell number, or sampling time, samples from cell culture supernatant may not be detected by the kit.
8. Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.
9. Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
10. LSBio is responsible for the quality and performance of the kit components but is NOT responsible for the performance of customer supplied samples used with the kit.

SAMPLE PREPARATION

The resulting Optical Density (OD) values of your sample must fall within the OD values of the standard curve in order for the calculated antigen concentration to be accurate. In many cases samples will need to be diluted in order to lower the antigen concentration to sufficient levels. Information about antigen concentrations within various sample types may be available from the published literature; however, it is often necessary to run a dilution series of each sample type. The following will prepare sufficient volumes to run the Sample dilution series in triplicate. In the case of small volume samples, a preliminary step dilution, such as 1:5 or 1:10, can be made using PBS (0.02mol/L pH7.0-7.2) as the diluent.

*** The final dilution should always be made using the same buffer that is used to dilute the Standards, and/or generate the standard curve.**

Running duplicate or triplicate wells for each sample is recommended



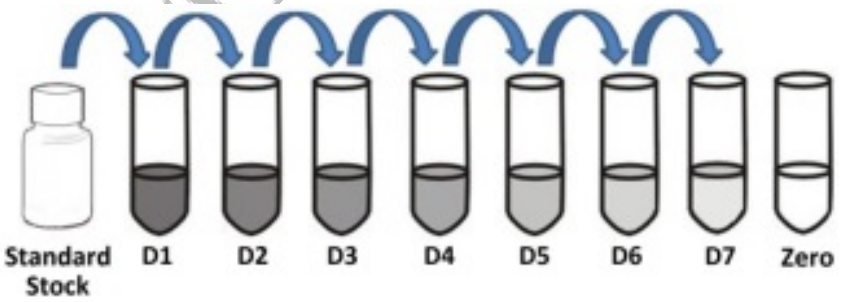
Standard Preparation

The following are instructions for the preparation of a Standard dilution series which will be used to generate the standard curve. The standard curve is then used to determine the concentration of target antigen in unknown samples (see the **Calculation of Results** section). The following will prepare sufficient volumes to run the Standard dilution series in duplicate. Reconstituted Standard and prepared standard dilutions should be used immediately and not stored for future use.

Standard Stock Solution (60 mIU/ml): Reconstitute 1 vial of Standard supplied with the kit with 1ml of Sample Diluent. Agitate gently at room temperature for 15 minutes prior to use.

D1 (60 ng/ml):	Pipette 300µl of Stock Standard into 0µl of Sample Diluent
D2 (30 ng/ml):	Pipette 300µl of D1 into 300µl of Sample Diluent
D3 (15 ng/ml):	Pipette 300µl of D2 into 300µl of Sample Diluent
D4 (7.5 ng/ml):	Pipette 300µl of D3 into 300µl of Sample Diluent
D5 (3.75 ng/ml):	Pipette 300µl of D4 into 300µl of Sample Diluent
D6 (1.875 ng/ml):	Pipette 300µl of D5 into 300µl of Sample Diluent
D7 (0.938 ng/ml):	Pipette 300µl of D6 into 300µl of Sample Diluent

Zero Standard (0 ng/ml): Use Sample Diluent alone



REAGENT PREPARATION

Bring all reagents to room temperature (18-25°C) before use.

1x Wash Buffer: If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved. Prepare 750 ml of Working Wash Buffer by diluting the supplied 30 ml of 25x Wash Buffer Concentrate with 720 ml of deionized or distilled water.

1x HRP-Streptavidin Conjugate Working Solution: Calculate the required amount needed before beginning the experiment (100µl/well) and include a 100µL excess. Dilute the HRP Conjugate Antibody to the working concentration using the HRP Conjugate Diluent (1:100).

1x Biotin-conjugated Antigen Working Solution: Calculate the required amount needed before beginning the experiment (100µl/well) and include a 100µL excess. Dilute the Biotin-conjugated Antigen to the working concentration using the Biotin-Conjugated Antigen Dilution Buffer (1:100).

REAGENT PREPARATION NOTES

1. It is highly recommended that standard curves and samples are run in duplicate within each experiment.

2. Once resuspended, standards should be used immediately, and used only once. Long-term storage of reconstituted standards is NOT recommended.

3. All solutions prepared from concentrates are intended for one-time use. Do not reuse solutions.

4. Do not prepare Standard dilutions directly in wells.

5. Prepared reagents may adhere to the tube wall or cap during transport; centrifuge tubes briefly before opening.

6. All solutions should be gently mixed prior to use.

7. Reconstitute stock reagents in strict accordance with the instructions provided.

8. To minimize imprecision caused by pipetting, ensure that pipettes are calibrated. Pipetting volumes of less than 10µl is not recommended.

9. TMB Substrate solution is easily contaminated; sterility precautions should be taken. TMB Substrate solution should also be protected from light.

10. Do not substitute reagents from one kit lot to another. Use only those reagents supplied within this kit.

11. Due to the antigen specificity of the antibodies used in this assay, native or recombinant proteins from other manufacturers may not be detected by this kit.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature without additional heating (except for TMB Substrate) and mixed thoroughly by gently swirling before pipetting (avoid foaming). Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C. Prepare all reagents and samples as directed in the previous sections. Duplicate or triplicate wells are recommended.

1. Wash plate 2 times before adding Standard, Blank or Sample to wells. Wash by adding approximately 350µl of 1x Wash Buffer using a squirt bottle, multi-channel pipette, manifold dispenser or automated washer. Allow each wash to sit for 1-2 minutes before completely aspirating. After the last wash, aspirate to remove any remaining Wash Buffer then invert the plate and tap against clean absorbent paper.

2. Add 100µl of Standard, Blank, or Sample per well, cover with a plate sealer, and incubate for 90 minutes at 37°C.

3. Aspirate to remove liquid then invert the plate and tap against clean absorbent paper. Wash each well 2 times as outlined in step 1.

4. Add 100µl of 1x Biotinylated Detection Antibody working solution to each well, cover with a plate sealer, and gently agitate to ensure thorough mixing. Incubate for 60 minutes at 37°C.

5. Aspirate the liquid from each well and wash 3 times as outlined in step 1.

6. Add 100µl of 1x HRP-Streptavidin Conjugate working solution to each well, cover with a new plate sealer, and incubate for 30 minutes at 37°C.

7. Aspirate the liquid from each well and wash 5 times as outlined in step 1.

8. Add 90µl of TMB Substrate solution to each well, cover with a new plate sealer, and incubate for 10-20 minutes at 37°C. Protect from light and monitor periodically until optimal color development has been achieved. Do not exceed 30 minutes.

9. Add 50µl of Stop Solution to each well. The blue color will change to yellow immediately. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. The Stop Solution shouldAdd

50 µl of **Stop Solution** to each well. The blue color will change to yellow immediately. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. The Stop Solution should be added to wells in the same order and timing as was the Substrate solutions.

10. Determine the optical density (OD value) of each well within 10 minutes using a microplate reader set to 450 nm.

ASSAY PROCEDURE NOTES

1. **ELISA Plate**: Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be placed in a sealed bag containing desiccant, and stored at 4°C.
2. **Solutions**: To avoid cross-contamination, change pipette tips between additions of each control, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. **Applying Solutions:** All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.
4. **Assay Timing**: The interval between adding sample to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experiment's accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.
5. **Incubation**: To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods of time between incubation steps. Do not let wells dry out at any time during the assay. Strictly observe the recommended incubation times and temperatures.
6. **Washing**: Proper washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper during the washing process. Do not put absorbent paper directly into the reaction wells.
7. **Controlling Substrate Reaction Time**: After the addition of the Substrates, periodically monitor the color development. Stop color development before the color become too deep by adding Stop Solution. Excessively strong color will result in inaccurate absorbance reading.

8. **Reading**: The microplate reader should be preheated and programmed prior to use. Prior to taking OD readings, remove any residual liquid or fingerprints from the underside of the plate and confirm that there are no bubbles in the wells.
9. **Reaction Time Control**: Control reaction time should be strictly followed as outline.
10. **Stop Solution**: The Stop Solution is contains an acid, therefore proper precautions should be taken during its use, such as protection of the eyes, hands, face and clothing.
11. **Mixing**: During incubation times, the use of a micro-oscillator at low frequency is recommended. Sufficient and gentle mixing is particularly important in producing reliable result.
12. Kits from different batches may be a little different in detection range, sensitivity and color developing time. Please perform the experiment exactly according to the supplied instructions.
13. Due to inter- and intra-assay variability, it is recommended that appropriate carry over controls be included between assays.
14. Prior to running valuable samples LSBio recommends that the user runs a preliminary experiment using the supplied controls in order to validate the assay.
15. To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.
16. The kit should not be used beyond the expiration date on the kit label.

ASSAY PROCEDURE SUMMARY

PREPARE ALL REAGENTS, SAMPLES AND STANDARDS.

WASH PLATE 2 TIMES BEFORE ADDING STANDARD, BLANK OR SAMPLE TO WELLS

ADD 100 µl OF SAMPLE, STANDARD, OR BLANK TO EACH WELL AND INCUBATE FOR 90 MINUTES AT 37°C.

ASPIRATE, WASH 2 TIMES,THEN AND ADD 100 µl OF 1X BIOTIN-DETECTION ANTIBODY AND INCUBATE FOR 60 MINUTES AT 37°C.

ASPIRATE AND WASH 3 TIMES

ADD 100 µl OF 1X HRP-STREPTAVIDIN CONJUGATE AND INCUBATE FOR 30 MINUTES AT 37°C.

ASPIRATE AND WASH 5 TIMES

ADD 90 µl of TMB SUBSTRATE SOLUTION AND INCUBATE FOR 10-20 MINUTES AT 37°C

ADD 50 µl OF STOP SOLUTION.

READ IMMEDIATELY AT 450NM.

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Low signal	Too brief incubation times.	Ensure sufficient incubation time.
	Incorrect assay temperature.	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes.	Check pipettes and ensure correct preparation.
	Improper dilution.	
Deep color but low value	Plate reader settings not optimal.	Verify the wavelength and filter setting in the plate reader.
		Turn on and warm-up the plate reader prior to use.

Troubleshooting Guide (continued)

Problem	Possible Cause	Solution
Large CV	Inaccurate pipetting.	Check pipettes.
High background	Plate is insufficiently washed.	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer.	Make fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit.	All the reagents should be stored according to the instructions.
	Stop solution not added.	Stop solution should be added to each well before measurement.

ASSAY USAGE AND SUPPORT

This kit is for **Research Use Only** and is not intended for diagnostic use. This kit is not approved for use in humans or for clinical diagnosis. This kit should not be used beyond the expiration date printed on the lot specific kit label.

Warning: This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion. Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

The LifeSpan Guarantee: LifeSpan guarantees the integrity of all components contained with an immunoassay kit, and that the standards provided will produce a standard curve sufficient for the quantification of target antigen concentrations that fall within the specified range of the kit. Due to the variable nature of sample types and preparations, LifeSpan cannot guarantee that the target antigen will be detectable in customer-supplied samples. For this reason, LifeSpan strongly recommends that customers conduct validation experiments, using positive control samples generated in a similar manner to the experimental samples, before using valuable research specimens. Due to the perishable nature of ELISA kits, orders of greater than 5 units of a single catalog number cannot be returned upon shipment, and are not eligible for refund.

Technical Support: LifeSpan's knowledgeable staff scientists are available to answer any questions about this kit. Email your detailed questions to Technical.Support@LSBio.com.

RETURNS, REFUNDS, CANCELLATIONS

Any problems with LifeSpan products must be reported to LifeSpan within 10 days of product receipt. The customer must obtain written authorization from LifeSpan before returning items. To request that goods be returned, please contact LifeSpan Technical Support. If an error by LifeSpan Biosciences results in shipment of an incorrect order, LifeSpan will, at its option, either ship a replacement order at no charge, or credit the customer's account for the original product shipped in error. Returns and cancellations may be subject to a 30% restocking fee. Conditions & Warranty: All LifeSpan products are intended for Research Use Only and are not for use in human therapeutic or diagnostic applications. The information supplied with each product is believed to be accurate, but no warranty or guarantee is offered for the products, because the ultimate conditions of use are beyond LifeSpan's control. The information supplied with each product is not to be construed as a recommendation to use this product in violation of any patent, and LifeSpan will not be held responsible for any infringement or other violation that may occur with the use of its products. Under no event will LifeSpan be responsible for any loss of profit or indirect consequential damage, including, but not limited to, personal injuries resulting from use of these products. LifeSpan's liability to any user of Products for damages that do not result from any fault of the user, will be limited to replacement of the Product(s) only, and in no event shall LifeSpan's liability exceed the actual price received by LifeSpan for the Product(s) at issue. LifeSpan shall not be liable for any indirect, special, incidental or consequential damages. LIFESPAN FURTHER DISCLAIMS ANY AND ALL EXPRESS AND IMPLIED OR STATUTORY WARRANTIES WITH RESPECT TO THE PRODUCTS, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. LifeSpan disclaims any and all responsibility for any injury or damage which may be caused by the fault of the user.

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