

HUMAN SOLUBLE LECTIN - LIKE OXIDIZED LOW- DENSITY LIPOPROTEIN RECEPTOR-1 (LOX-1) ULTRASENSITIVE ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF
HUMAN SOLUBLE LOX-1 CONCENTRATIONS IN
SERUM AND EDTA PLASMA



ALWAYS REFER TO LOT SPECIFIC PROTOCOL
PROVIDED WITH EACH KIT FOR
INSTRUCTIONS. PROTOCOL MUST BE
READ AND CHECK ALL ITEMS OF EACH KIT
BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN
DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	HUMAN SOLUBLE LOX-1 ULTRASENSITIVE ELISA KIT
Catalog No.	SK00006-08
Lot No.	
Formulation	96 T
Standard range	7.8 - 500 pg/mL
Sensitivity	1.5 pg/mL
Sample Volume	100 µL
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Serum, EDTA Plasma,
Specificity	Human sLOX-1
Calibration	Human sLOX-1 Rec. (HEK293)
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 9%
Storage	2 – 8° C for 2 months. More information check page 2
This kit contains sufficient materials to run approximately 35-40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Soluble LOX-1 Ultrasensitive ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human soluble LOX-1 from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human soluble LOX-1 recombinant derived from HEK293 and monoclonal antibodies raised against this protein (derived from HEK293). Results from this immunoassay have shown to accurately quantify recombinant human sLOX-1 derived from human cells and human natural soluble LOX-1 in the samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human soluble LOX-1. The capture antibody can bind to the human soluble LOX-1 in the standard and samples. After washing the plate of any unbound substances, a biotinylated monoclonal antibody against human soluble LOX-1 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human soluble LOX-1 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

_This ELISA kit should not be used beyond the expiration date on the kit label.

_Do not mix reagents with those from other lots or sources.

_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_Each laboratory must determine the optimal dilution factors for the samples being assayed.

_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
LOX-1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with monoclonal antibody against human LOX-1.	006-08-01	1 plate
LOX-1 Standard – 2000 pg of recombinant human soluble LOX-1 in a buffered protein base with preservative; lyophilized.	006-08-02	1 vial
Detection Antibody Concentrate – 1.05 mL of 10-fold concentrate of biotinylated monoclonal antibody against human LOX-1 with preservative; lyophilized.	006-08-03	1 vial
Positive Control – one vial of recombinant human soluble LOX-1; lyophilized.	006-08-04	1 vial
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	SAHRP	1 vial
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	DB03	1 bottle
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	DB108A	1 bottle
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	DB08B	1 bottle
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant with preservative.	WB01	1 bottle
TMB Substrate Solution -11 mL of TMB substrate solution.	TMB01	1 bottle
Stop Solution – 11 mL of 0.25M HCl.	S-STOP	1 bottle
Plate Sealer	EAPS	1

Plastic Pouch	P01	1
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STORAGE

Unopened Kit: Store at 2 – 8° C for up to 2 months. For longer storage up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and Antibody & HRP Diluent Solution should be stored at -20° C. Streptavidin-HRP Conjugate and TMB Substrate Solution should be stored only at 2 – 8°C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (350 – 400 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) (Aviscera Bioscience’s Catalog No. 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Plasma and serum samples DO NOT need to be diluted in this assay. However, if samples are higher than the 500 pg/mL maximum standard point, then a 2-fold dilution or greater might be needed.

Optimal dilutions should be determined by each laboratory for each application. Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

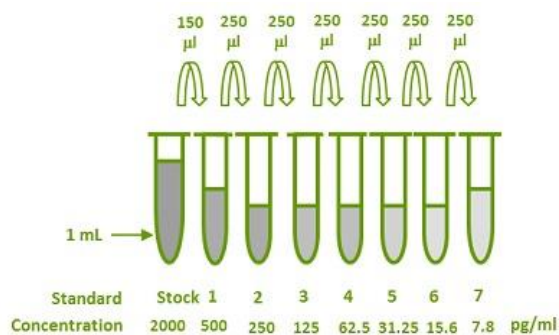
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Dilution Buffer (DB03) - Dilution Buffer (DB03) is highly viscous, warm in 30 - 37° C water bath until liquid flows more freely.

LOX-1 Standard - Reconstitute the LOX-1 standard with 1mL of **Dilution Buffer (DB03)**. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of **Dilution Buffer (DB03)** into tubes #2 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **500 pg/mL standard** serves as the high standard. The **Dilution Buffer (DB03)** serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 mL	2000 pg/mL
# 1	150µL of stock	450µL	500 pg/mL
# 2	250µL of 1	250µL	250 pg/mL
# 3	250µL of 2	250µL	125 pg/mL
# 4	250µL of 3	250µL	62.5 pg/mL
# 5	250µL of 4	250µL	31.25 pg/mL
# 6	250µL of 5	250µL	15.6 pg/mL
# 7	250µL of 6	250µL	7.8 pg/mL

Store the stock solution at -70 °C for a few days.



Positive Control - Reconstitute the Positive Control with refer to lot of **Dilution Buffer (DB03)**. Discard the positive control after use. It is for one time use only.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody Diluent Solution (DB108A)** to produce a 10-fold concentrated stock solution. For 96 wells test, freshly pipette 9.45 mL of **Antibody Diluent Solution (DB108A)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. For partial strip test, freshly prepare 900 µL per strip of working solution. Store the stock solution at -20 ~ -70 °C for a few days.

Streptavidin-HRP Conjugate - For 96 wells test, freshly pipette 10.89 mL of **HRP Diluent Solution (DB08B)** into a 15 mL centrifuge tube and transfer 110 µL of 100-fold concentrated stock solution to prepare working solution (**protect from light**). *The working solution of Streptavidin-HRP Conjugate should be freshly prepared and used within 20-30 minutes.* For partial strip test, freshly prepare 900 µL per strip of working solution. Store the stock solution (100-fold concentrated) at 2 ~ 8 °C for 8 months.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL per well of **Dilution Buffer** to blank wells (B).
3. Add 100 µL of **Standard dilutions** in reverse order of serial dilution (7-1), **samples**, or **positive control (P)** per well. Check page 5 for more information. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1x Wash Buffer** (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of **Substrate Solution** to each well. Incubate for 20-25 minutes on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well using a microplate reader set to 450 nm within 3 minutes.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log or 4-Parameter curve fit.

Readings of sample concentration exceeding that of the standard 500 pg/mL may result in inaccurate, low human sLOX-1 levels. Such samples require further external pre-dilution according to expected

human sLOX-1 values with Dilution Buffer in order to precisely quantify the actual human sLOX-1 level.

SPECIFICITY

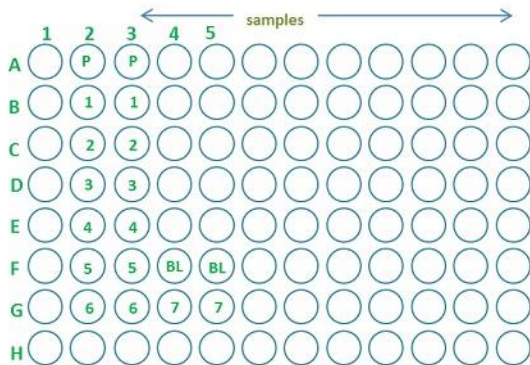
PROTEINS	CROSS-REACTIVITY
Human soluble LOX-1 (NS0 derived)	100%
Human soluble LOX-1 (HEK293 derived)	100%
Mouse soluble LOX-1	0
Human CD36	0
Human sRAGE	0
Human CD94	0
Human LDL	0
Human VLDL	0

The human LOX-1 extracellular domain recombinant derived from E. Coli or sf21 cells may not be detected by this ELISA Kit.

TYPICAL DATA

This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)*
Blank	0 (0.069)
7.8	0.042
15.6	0.082
31.25	0.170
62.5	0.355
125	0.736
250	1.459
500	2.449



Human Soluble LOX-1 Ultrasensitive ELISA Kit

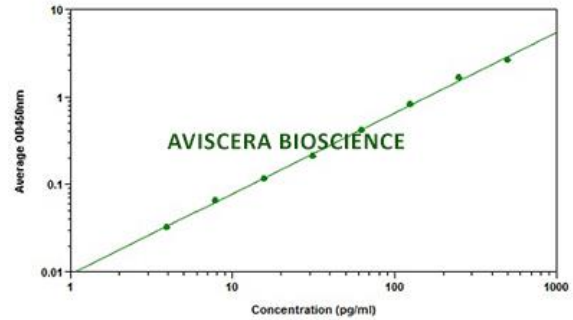
Catalog No.: SK00006-08 Size: 96 T

Sensitivity: 1.5 pg/mL

Standard Range: 7.8 ~ 500 pg/mL

Calibration: rh LOX-1 (HEK293) Rec.

Sample Type: Serum, Plasma, Cell Cultures



SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µL of standard dilutions, samples, or positive control to each well. Incubate 2 hours on plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Detection Antibody working solution to each well. Incubate 2 hours on plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Streptavidin-HRP Conjugate working solution to each well. Incubate 60 min on plate shaker at RT. Protect from light.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate Solution to each well. Incubate for 20-25 minutes on plate shaker at RT. Protect from light.
↓
Add 100 µL Stop Solution to each well. Read at 450nm within 3 minutes.