HUMAN SOLUBLE LOW-DENSITY LIPOPROTEIN RECEPTOR (LDLR) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE LDLR CONCENTRATIONS IN SERUM AND PLASMA

THIS PROTOCOL IS PROVIDED FOR DEMONSTRATION ONLY. ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN SOLUBLE LDLR ELISA KIT**
Catalog No.	SK00418-01
Lot No.:	20112855
Formulation	96 T
Standard range	46.875 - 3000 pg/mL
Sensitivity	20 pg/mL
Sample Volume	100 µL of diluted samples
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum, EDTA Plasma
Specificity	Human sLDLR
Calibration	Human sLDLR recombinant (HEK293 cells)
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 - 8° C
This kit contains sufficient materials to run approximately 40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Soluble LDLR ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human LDLR from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human LDLR and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural LDLR samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human soluble LDLR. The capture antibody can bind to the human soluble LDLR in the standard and samples. After washing the plate of any unbound substances, a monoclonal antibody-HRP conjugate against human soluble LDLR is added to the wells. 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 LDLR 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 with a pretest.
_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
sLDLR Microplate – 96 well microplate precoated with an anti-human soluble LDLR monoclonal antibody.	**418-01-01**	**1 plate**
sLDLR Standard – refer to lot specific of recombinant human soluble LDLR in a buffered protein base with preservative; lyophilized.	**418-01-02**	**2 vials**
Detection Antibody-HRP Conjugate – refer to lot specific of concentrated solution of antibody conjugated to HRP against soluble LDLR.	**418-01-03**	**1 vial**
Positive Control – one vial of recombinant human soluble LDLR; lyophilized (optional).	**418-01-04**	**1 vial**
Dilution Buffer - 60 mL of buffered protein based solution with preservative.	**DB10**	**1 bottle**
Antibody Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB78**	**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 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody-HRP Conjugate 100-fold concentrated solution should be stored at -20° C or -70° C. Substrate Solution can be stored at 2 – 8° C for up to 8 months (**DO NOT FREEZE** and **PROTECT FROM LIGHT**). All other components can be stored at 2 – 8° C for up to 8 months. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 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

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) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and EDTA plasma samples may require a 200 ~ 400-fold dilution.

A suggested 10 -fold dilution is 10 µL of sample + 90 µL of Dilution Buffer. Then, to make a 200-fold dilution is 12 µL of 10-fold diluted samples + 228 µl of Dilution Buffer. To make a 400-fold dilution is 6 µL of 10-fold diluted sample + 234 µL of Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application with a pretest. 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.

sLDLR Standard - Reconstitute the sLDLR standard with refer to lot specific of Dilution Buffer. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer. The **3000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	Refer to lot specific	3000 pg/ml
# 1	250µl of stock	250µl	1500 pg/ml
# 2	250µl of 1	250µl	750 pg/ml
# 3	250µl of 2	250µl	375 pg/ml
# 4	250µl of 3	250µl	187.5 pg/ml
# 5	250µl of 4	250µl	93.75 pg/ml
# 6	250µl of 5	250µl	46.875 pg/ml

Positive Control - Reconstitute the Positive Control with refer to lot specific of Dilution Buffer.

Detection Antibody-HRP Conjugate - Pipette refer to lot specific of Antibody Diluent solution **(DB78)** into a 15 mL centrifuge tube and transfer refer to lot specific of concentrated stock solution to prepare working solution **(protect from light)**. **DO NOT FREEZE.**

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.

3. Add 100 µl per well of standard solutions from #6 to #S (reverse order of serial dilution), positive control or samples. Cover with plate sealer and incubate at room temperature for 2 hours on microplate shaker (250 rpm).
4. Aspirate wells and wash 4 times with 300 µl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
5. Add 100 µl per well of 1x Detection Antibody-HRP conjugate working solution. Cover with plate sealer and incubate at room temperature for 1 hour on microplate shaker (250 rpm). **Protect from light.**
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Substrate Solution to each well. Incubate for refer to lot specific on microplate shaker at room temperature. **Protect from light**.
8. 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.
9. Determine the optical density of each well using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

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

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.067)
46.875	0.037
93.75	0.070
187.5	0.131
375	0.248
750	0.469
1500	0.858
3000	1.503

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human soluble LDLR (HEK293 cells)	100
Human PCSK9	0
Human ATGL	0
Human Endothelial Lipase	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples or positive control to the well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl per well 1x Detection Antibody-HRP conjugate working solution to each well. Incubate 1 hour on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate refer to lot specific on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450nm.