Version 28-02/25

User's Manual

Human Soluble LDLR ELISA Kit

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics
Address: 45-1 Ramsey Road, Shirley, NY 11967, USA
Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) Fax: 1-631-938-8221
Email: info@creative-diagnostics.com Web: www.creative-diagnostics.com

PRODUCT INFORMATION

Intended Use

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.

Principles of Testing

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.

Reagents And Materials Provided

1. sLDLR Microplate: 1 plate, 96 well microplate precoated with an anti-human soluble LDLR monoclonal antibody
2. sLDLR Standard: 1 vial, 3000pg/vial of recombinant human soluble LDLR in a buffered protein base with preservative; lyophilized.
3. Detection Antibody-HRP Conjugate: 1 vial, 55 uL/vial of 200-fold concentrated solution of antibody conjugated to HRP against soluble LDLR.
4. Positive Control: 1 vial, one vial of recombinant human soluble LDLR; lyophilized (optional).
5. Dilution Buffer: 2 bottle, 60 mL of buffered protein based solution with preservative.
6. Wash Buffer: 1 bottle, 50 mL of 10-fold concentrated buffered surfactant, with preservative.
7. TMB Substrate Solution: 1 bottle, 11 mL of TMB substrate solution.
8. Stop Solution: 1 bottle, 11 mL of 0.5M HCl.
9. Plate Sealer: 1 piece
10. Plastic Pouch: 1 piece

Materials Required But Not Supplied

 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.

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 200-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.

Opened/Reconstituted Reagents: Reconstituted Standard (stock) solution SHOULD BE STORED at -20°C or -70°C for up to one month.

Microplate Wells: Return unused microplate strips to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

Specimen Collection And Preparation

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 uL of sample + 90 uL 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

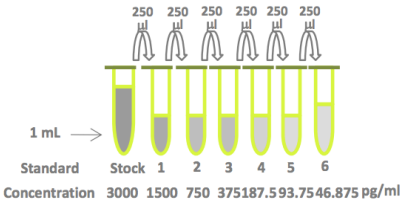
Bring all reagents to room temperature before use.

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 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 3000 pg/mL. 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	1.0 ml	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 0.5 mL of Dilution Buffer. Note: Positive Control could be reused within a few days if stored at -20°C or -70°C.

Detection Antibody-HRP Conjugate - Pipette 10.945 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 55 uL of 200-fold concentrated stock solution to prepare working solution. Note: 1x working solution of Detection Antibody-HRP conjugate should be used within a few days (protect from light). DO NOT FREEZE.

Assay Procedure

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. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 ul per well of Dilution Buffer to Blank wells.
4. Add 100 ul 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).
5. Aspirate wells and wash 4 times with 300 ul of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
6. Add 100 ul 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.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 uL of Substrate Solution to each well. Incubate for 8-15 minutes on microplate shaker at room temperature. Protect from light.
9. Add 100 uL 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.
12. Determine the optical density of each well within 15 minutes using a microplate reader set to 450 nm.

Calculation

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (xaxis) 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 Standard Curve

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 (0.098)
46.875	0.035
93.75	0.079
187.5	0.172
375	0.351
750	0.689
1500	1.402
3000	2.562

Precision

Intra-assay Precision: 4-6%

Inter-assay Precision: 8-12%

Detection Range

46.875-3000 pg/mL

Sensitivity

30 pg/mL

Specificity

PROTEINS CROSS-REACTIVITY (%)

Human soluble LDLR 100

Human PCSK9 0

Human ATGL 0

Human Endothelial Lipase

Precautions

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.

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. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.

_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.