HUMAN ENDOTHELIAL LIPASE (EL) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN ENDOTHELIAL LIPASE CONCENTRATIONS IN SERUM, PLASMA AND CELL CULTURE SUPERNATES

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.

PURCHASE INFORMATION:

ELISA Name	HUMAN ENDOTHELIAL LIPASE (EL) ELISA
Catalog No.	SK00276-01
Lot No.	
Formulation	96 T
Standard range	0.64-2000 ng/mL
Sensitivity	0.7-2.5 ng/mL
Sample Volume	50 µL
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum, EDTA plasma, Cell Culture Supernates
Specificity	Human Endothelial Lipase
Calibration	Human Endothelial Lipase recombinant
Intra-assay Precision	6 - 8%
Inter-assay Precision	8 - 12%
Storage	2 – 8° C

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INTRODUCTION

This Human Endothelial Lipase ELISA kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Endothelial Lipase from cell culture supernates, serum and plasma in a competitive EIA format.

This immunoassay contains recombinant and biotinylated recombinant human Endothelial Lipase, and an antibody raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural Endothelial Lipase.

ASSAY OVERVIEW

This assay employs the quantitative competitive EIA format. Human Endothelial Lipase present in samples compete with a fixed amount of biotinylated human Endothelial Lipase for sites on an antibody specific against Endothelial Lipase. After a wash to remove any unbound standard, samples and biotin conjugate, a Streptavidin conjugated to horseradish-peroxidase (HRP) is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when Stop Solution is added. The intensity of the color measured is in inverse proportion to the amount of human Endothelial Lipase bound in the initial step. 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.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute 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.
_ If samples generate values higher than the highest standard, dilute the samples with dilution buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
EL Microplate – 96 well microplate precoated with antibody against EL.	**276-01-01**	**1 plate**
EL Standard – 1000 ng/vial of recombinant Endothelial Lipase in a buffered protein base with preservative; lyophilized.	**276-01-02**	**1 vial**
Biotin Solution – 550 µL/vial, 10-fold concentrate of biotinylated Endothelial Lipase with preservative; lyophilized.	**276-01-03**	**1 vial**
Positive Control – one vial of recombinant Endothelial Lipase; lyophilized (optional).	**276-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer - 60 mL of buffered protein based solution with preservative.	**DB18**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB06**	**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 6 months. For longer storage, unopened Standard, Positive Control and Biotin Solution Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard (stock) and Biotin concentrated solution

COULD BE STORED at -20° C or -70° C for up to one month. Reconstituted Biotin concentrated solution CANNOT BE STORED at 2 – 8° C. Streptavidin-HRP Conjugate 100-fold concentrated solution **(protect from light)** and other components may be stored at 2 – 8° C for up to 6 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

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

SAMPLE PREPARATION

Serum and Plasma samples do not require dilutions. **Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.**

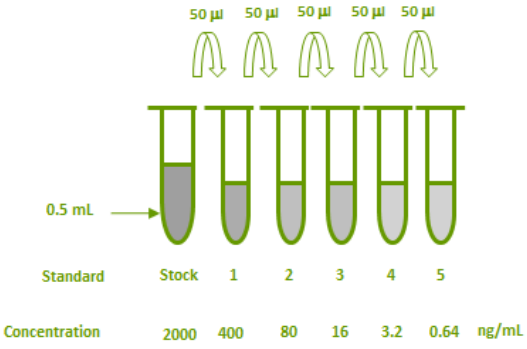
REAGENT PREPARATION

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.

EL Standard - Reconstitute the EL standard with 500 µL of Dilution Buffer. This reconstitution produces a stock solution of 2000 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 200 µL of the appropriate Dilution Buffer into tubes #1 to #5. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **2000 ng/mL** standard serves as the high standard.

Tube	Standard	Dilution Buffer	Concentration
stock	powder	500µl	2000 ng/ml
# 1	50µl of stock	200µl	400 ng/ml
# 2	50µl of 1	200µl	80 ng/ml
# 3	50µl of 2	200µl	16 ng/ml
# 4	50µl of 3	200µl	3.2 ng/ml
# 5	50µl of 4	200µl	0.64 ng/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.

Biotin Solution - Reconstitute the Biotin Solution concentrate with 550 µL of Dilution Buffer to make 10-fold concentrated solution. Transfer 550 µL to 4.95 mL of Dilution Buffer to prepare **1x Biotin Solution.**

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent Solution (DB06)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days **(protect from light)**.

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, standard dilutions, positive control and samples as directed previously.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. **DO NOT ADD ANY SOLUTIONS INTO BLANK WELLS, EXCEPT STREPTAVIDIN-HRP WORKING SOLUTION.**
4. Add 50 µl per well of Dilution Buffer into Total Binding wells.
5. Add 50 µl per well of Standard solutions from #5 to #S (reverse order of serial dilution) to the wells. Add 50 µl per well of positive control into wells. Add 50 µl per well of samples into appropriate wells.
6. Seal plate and incubate at room temperature for 2 hours on microplate shaker (250-300 rpm). **DO NOT ASPIRATE AND WASH. PROCEED IMMEDIATELY TO NEXT STEP.**
7. Add 50 µl per well of 1x Biotin Solution into total binding, standard, positive control and sample wells. **Do not add 1x Biotin Solution into Blank wells.** Seal plate and incubate at room temperature for 2 hours on microplate shaker.
8. Aspirate wells and wash 4 times with 300 µl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
9. Add 100 µl of Streptavidin-HRP Conjugate working solution to **every well including blanks**. Seal plate and incubate at room temperature for 1 hour on microplate shaker. **Protect from light.**
10. Repeat the aspiration/wash as in step 8.
12. Add 100 µl of Substrate Solution to every well, including blanks. Incubate for 3 - 7 minutes on microplate shaker at room temperature. **Protect from light. *Be prepared to add stop solution due to the fast color development.**
13. Add 100 µl of Stop Solution to every well, including blanks. 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 OF RESULTS

Average the duplicate readings for each standard, positive control and samples, and subtract the average blank optical density. It is recommended to use software capable of generating a four parameter logistic (4-PL) curve-fit. The standard curve shows relationship between standard concentrations and corresponding O.D absorbance.

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

Calculation of samples with a concentration exceeding that of standard 2000 ng/mL may result in inaccurate, low human Endothelial Lipase levels. Such samples require further external predilution according to expected human Endothelial Lipase values with Dilution Buffer in order to precisely quantify the actual human Endothelial Lipase level.

TYPICAL DATA

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

Standard (ng/mL)	Average OD450 (Corrected)
Blank	0 (0.05)
Total Binding	1.212
0.64	1.206
3.2	1.179
16	1.043
80	0.600
400	0.245
2000	0.075

- Lot No.:
- Positive Control:

SPECIFICITY

Proteins	Cross-reactivity
Human Endothelial Lipase	100%
Human ATGL	0
Human Adiponutrin	0
Human gAdiponectin	0
Human SPARC	0
Human sCD36	0
Human Visfatin	0
Human FABP-4	0
Human OSF-2	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARD DILUTIONS

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Add 50 µl of standard, samples, or positive control to the wells. Incubate 2 hours on the plate shaker at RT. **DO NOT WASH OR ASPIRATE. PROCEED IMMEDIATELY TO NEXT STEP.**

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Add 50 µl 1x Biotin Solution to the wells, excluding blanks. Incubate 2 hours on the plate shaker at RT.

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Aspirate and wash 4 times.

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Add 100 µl Streptavidin-HRP conjugate working solution to all wells, including blanks. Incubate 1 hour on the plate shaker at RT. **Protect from light.**

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Aspirate and wash 4 times.

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Add 100 µl Substrate Solution to every well, including blanks. Incubate 3-7 minutes on the plate shaker at RT. **Protect from light.**

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Add 100 µl Stop Solution to every well, including blanks. Read 450nm within 15 min.