HUMAN ENDOTHELIAL LIPASE (EL) ELISA KIT

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

PURCHASE INFORMATION:

ELISA Name	Human Endothelial Lipase (EL) ELISA
Catalog No.	SK00276-01
Lot No.	
Formulation	96 T
Standard range	3.2-2000 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
Intra-assay Precision	6-8%
Inter-assay Precision	8-12%
Storage	4 °C

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INTRODUCTION

Human Endothelial Lipase ELISA employs the quantitatively competitive enzyme immunoassay technique in which 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. Following 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. The sample values are read off the standard curve.

LIMITATIONS OF THE PROCEDURE

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_ 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.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
EL Microplate – 96 well microplate precoated with antibody against EL	**276-01-01**	**1 plate**
EL Standard – 500 ng/vial of recombinant Endothelial Lipase in a buffered protein base with preservatives; lyophilized.	**276-01-02**	**2 vials**
Biotin Solution-1.1 mL/vial, 10-fold concentrated of Endothelial Lipase biotinylated with preservatives; lyophilized.	**276-01-03**	**1 vial**
Positive Control – two vials of recombinant Endothelial Lipase , lyophilized (optional)	**276-01-04**	**2 vials**
Streptavidin-HRP Conjugate - 60 µl/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Dilution Buffer- 60 mL of buffered protein based solution with preservatives	**DB18**	**1 bottle**
HRP Diluent Solution-12 mL of buffered protein based solution with preservatives	**DB01**	**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 12 months. For longer storage, unopened Standard, Positive Control and Biotin Solution Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Biotin Solution COULD BE STORED at -20 °C or -70°C for up to one month. Reconstituted Biotin Solution (1.1mL) CAN NOT BE STORED at 2-8°C. Streptavidin-HRP Conjugate 200-fold concentrated and other components may be stored at 2 - 8°C for up to 12 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8° C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 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.

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.
Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

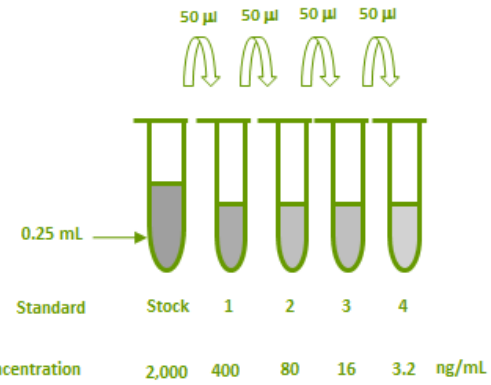
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

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 Wash Buffer.
Standard - Refer to vial label for reconstitution volume. Reconstitute the Standard with 0.25 ml 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 #4. 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	0.25 ml	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



Biotin Solution - Reconstitute the Biotin Solution with 1.1mL of Dilution Buffer to make 10-fold concentrated solution. Transfer 550 µL to 4.95 mL of Dilution Buffer to prepare **1X Biotin Solution.**

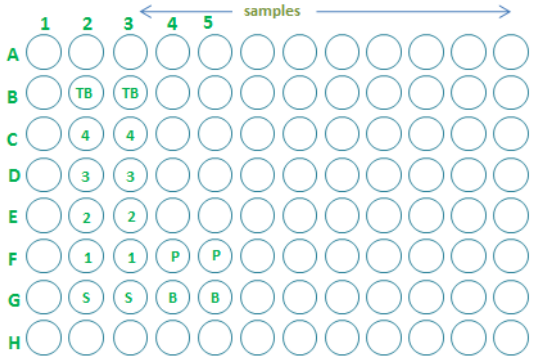
Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

Positive Control- Reconstitute the positive control with 250 µL of Dilution Buffer to make working solution. **Note:** Positive Control working solution should be used immediately.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Leave well G4, G5 as Blank. **DO NOT ADD ANY BIOTIN SOLUTION INTO BLANK WELLS.**
4. Set B2, B3 as total binding (TB). Add 50 µl per well of Dilution Buffer.
5. Add 50 µl per well of standard solution from #4 to S (reverse order of serial dilution) to the appropriate wells (C2, C3 to G2, G3). Add 50 µl per well of positive control into wells F4, F5. 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). Note: Standard, Blank and PC should be assayed in duplicates. **DO NOT ASPIRATE AND WASH. PROCEED IMMEDIATELY TO NEXT STEP.**
7. Add 50 µl per well of 1X Biotin Solution into total binding, standard, PC and samples wells. Seal plate and incubate at room temperature for 2 hours on micro-plate 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. 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 each well. Incubate for 30-90 seconds 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 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 micro-plate 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.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. Coli*-expressed recombinant human Endothelial Lipase.

SENSITIVITY

Twenty-five assays using Dilution Buffer as matrix were evaluated and the minimum detectable dose of EL was 0.7-2.5 ng/mL.

TYPICAL DATA

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

Standard (ng/mL)	Average OD450 (Corrected)
Blank	0 (0.043)
Total Binding	1.425
0.64 (optional)	1.391
3.2	1.383
16	1.319
80	0.802
400	0.396
2000	0.146

- Lot No.:
- Positive Control: 16 - 32 ng/mL

SPECIFICITY

Human Endothelial Lipase ELISA kit recognizes recombinant and endogenous human Endothelial Lipase. The factors listed below were prepared at 20 µg/ml in Dilution Buffer, and assayed for cross reactivity. Preparations of the following factors were assayed for interference. No significant cross-reactivity or interference was observed.

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 standards

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Add 50µl of standard, samples, positive control to each well. 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 each well. 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. 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 each well. Incubate 30-90 seconds on the plate shaker. **Protect from light.**

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Add 100 µl Stop Solution to each well. Read 450nm within 15 min