RAT/MOUSE ENDOTHELIAL LIPASE (EL) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF RAT/MOUSE ENDOTHELIAL LIPASE CONCENTRATIONS IN SERUM AND EDTA PLASMA

PURCHASE INFORMATION:

ELISA Name	Rat/Mouse Endothelial Lipase (EL) ELISA
Catalog No.	SK00276-02
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 and EDTA plasma
Specificity	Rat/Mouse Endothelial Lipase
Intra-assay Precision	6-8%
Inter-assay Precision	8-12%
Storage	4 °C

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INTRODUCTION

Rat/Mouse Endothelial Lipase ELISA employs the quantitatively competitive enzyme immunoassay technique in which rat/mouse Endothelial Lipase present in samples compete with a fixed amount of biotinylated 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 rat/mouse Endothelial Lipase bound in the initial step. The sample values are read off the standard curve.

LIMITATIONS OF THE PROCEDURE

_ 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.

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 an antibody against EL	**276-02-01**	**1 plate**
EL Standard – 500 ng/vial of recombinant Endothelial Lipase in a buffered protein base with preservatives; lyophilized.	**276-02-02**	**2 vials**
Biotin Solution - 550 μL/vial, 10-fold concentrated of Endothelial Lipase biotinylated with preservatives; lyophilized.	**276-02-03**	**1 vial**
Positive Control – one vial of recombinant Endothelial Lipase, lyophilized (optional)	**276-02-04**	**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 CAN NOT BE STORED at 2-8°C. Streptavidin-HRP Conjugate 200-fold Concentrate 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 the 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 (code: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per mL of sample.

SAMPLE PREPARATION

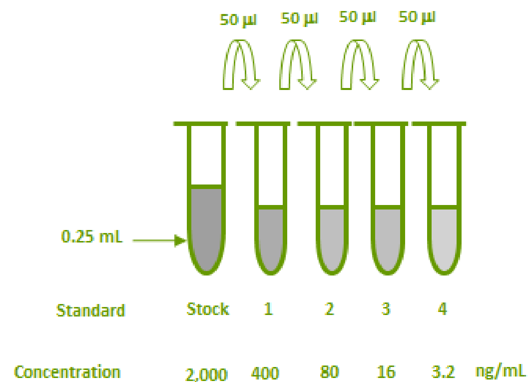
Serum and plasma samples may not need to be diluted.
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

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 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 550 µl of Dilution Buffer to make 10-fold concentrated solution. Transfer it to 4.95 mL of Dilution Buffer to prepare **1X Biotin Solution.**

Streptavidin-HRP Conjugate - Pipette 11.94 mL of **HRP Diluent Solution** 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 uL of Dilution Buffer to make working solution.

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 duplicate.

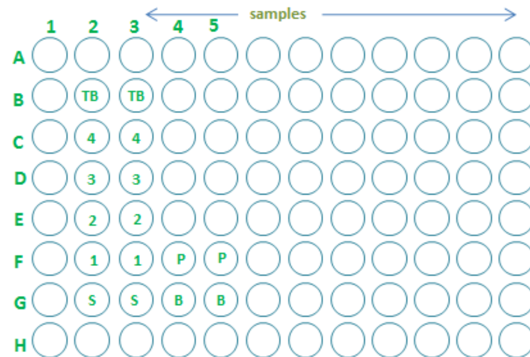
1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Leave well G4, G5 as Blank. **DO NOT ADD ANY BIOTIN SOLUTION OR DILUTION BUFFER INTO BLANK 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). **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 samples wells. Do NOT add into blank 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 4-8 minutes at room temperature. **Protect from light**.

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 Endothelial Lipase levels. Such samples require further external predilution according to expected Endothelial Lipase values with Dilution Buffer in order to precisely quantify the actual 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 (0.065)
Total Binding	0.959
0.64 (optional)	0.940
3.2	0.874
16	0.834
80	0.518
400	0.253
2000	0.083

- Lot No.:
- Positive Control: 90 - 170 ng/mL

SPECIFICITY

Rat/Mouse Endothelial Lipase ELISA kit recognizes recombinant and endogenous rat/mouse 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.

Proteins	Cross-reactivity
Rat Endothelial Lipase	100%
Mouse Endothelial Lipase	100%
Human Endothelial Lipase	100%
Human Adiponectin	0
Rat SPARC	0

The capture antibody has been selected for its ability to recognize human Endothelial Lipase in direct ELISAs and western blots. It has cross-reactivity with rat/mouse Endothelial Lipase in these formats. The rat/mouse samples can be detected by this antibody in ELISA and Western Blots. The detection antibody was raised by a peptide fragment of human Endothelial Lipase which is completely identical to rat/mouse Endothelial Lipase. The data also indicates that rat/mouse samples were bound to antibody that was used in this kit formulation condition. Its dynamic dilution curves were parallel to the standard curves obtained using the ELISA standard. That means rat/mouse samples cross-react with this ELISA kit.

LINEARITY

To assess the linearity of the assay, pooled research rat **plasma** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
1X	521.118	521.118	100
2X	255.948	511.896	98.23

To assess the linearity of the assay, pooled research rat **serum** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
1X	581.142	581.142	100
2X	303.287	606.574	104

To assess the linearity of the assay, pooled research mouse **plasma** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
1X	183.289	183.289	100
5X	30.936	154.68	84

To assess the linearity of the assay, pooled research mouse **serum** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
1X	292.877	292.877	100
5X	69.279	346.395	118

SUMMARY OF ASSAY PROCEDURE

Prepare reagents, samples and standards
⇩
Add 50 µl 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.
⇩
Add 50 µl 1X Biotin Solution to each well. Incubate 2 hours on the plate shaker at RT.
⇩
Aspirate and wash 4 times.
⇩
Add 100 µl Streptavidin-HRP conjugate working solution to all wells. 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 4-8 min on the bench top. **Protect from light.**
⇩
Add 100 µl Stop Solution to each well. Read 450nm within 15 min