Datasheet for ABIN579133

LIPG ELISA Kit

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Overview

Quantity:	96 tests
Target:	LIPG
Reactivity:	Rat
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	This immunoassay kit allows for the specific measurement of rat EL concentrations in cell culture supernates of smooth muscle cells, serum, and plasma.
Sample Type:	Cell Culture Supernatant, Serum, Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay recognizes recombinant and natural rat EL.
Cross-Reactivity (Details):	No significant cross-reactivity or interference was observed.
Characteristics:	Rattus norvegicus,Rat,Endothelial lipase,Endothelial-derived lipase,EDL,Lipg,3.1.1.3
Components:	Reagent (Quantity): Assay plate (1), 2 Standard (2), Sample Diluent (1 × 20ml), Assay Diluent A (1x10ml), Assay Diluent B (1x10ml), Detection Reagent A (1 × 120µl), Detection Reagent B (1 × 120µl), Wash Buffer (25 x concentrate) (1 × 30ml), Substrate (1x10ml), Stop Solution (1 x 10ml)

Target Details

Target:	LIPG

Target Details

Alternative Name:	Lipg (LIPG Products)
Background:	Endothelial lipase (EL) was initially cloned by both of these laboratories using entirely different strategies. Endothelial lipase was identified as a transcript that was upregulated in cultured rat umbilical vein endothelial cells undergoing tube formation, whereas the Rader group cloned endothelial lipase as a transcript that was upregulated in the rat macrophage-like cell line THP-1 exposed to oxidized LDL. Database searches revealed that endothelial lipase shows strong sequence similarity to lipoprotein lipase (44 percent identity) and hepatic lipase (41 percent identity), two well-characterized lipases that function at vascular endothelial surfaces. Critical motifs associated with lipase activity (GXSXG and the catalytic triad S169, D193, H274), and with heparin binding were strongly conserved. Interestingly, in contrast to both lipoprotein lipase and hepatic lipase, endothelial lipase has little triglyceride hydrolase activity in vitro but instead cleaves fatty acids from the sn-1 position of phosphatidylcho-line. In in vitro assays the enzyme is most active on lipids presented in HDL, although it will release fatty acids from all classes of lipoproteins. Consistent with this finding, adenovirus-mediated overexpression of endothelial lipase in LDL receptor-deficient mice reduced plasma concentrations of VLDL and LDL cholesterol by about 50 percent, whereas HDL-C decreased to almost zero in these animals. These data suggested that endothelial lipase may play a role in HDL catabolism.
Gene ID:	2987

Application Details

Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for EL has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any EL present is bound by the immobilized antibody. An enzyme-linked monoclonal antibody specific for EL is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of EL bound in the initial step. The color development is stopped and the intensity of the color is measured.
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 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer. Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent.

	This reconstitution produces a stock solution of 400 pg/ml. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions (Making serial dilution in the wells directly is not permitted). Please firstly dilute the stock solution to 200 pg/ml and the diluted standard serves as the high standard (200 pg/ml). The Sample Diluent serves as the zero standard (0 pg/ml). pg/mL 400 200 100 50 25 12.5 6.25 3.12 0 Detection Reagent A and B - Dilute to the working concentration using Assay Diluent A and B (1:100), respectively.
Sample Collection:	Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 × g. Remove serum and assay immediately or aliquot and store samples at -20 C or -80 C . Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 × g at 2 - 8 C within 30 minutes of collection. Store samples at -20 C or -80 C . Avoid repeated freeze-thaw cycles. Cell culture supernates and other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20 C or -80 C . Avoid repeated freeze-thaw cycles. Note: Serum, plasma, and cell culture supernatant samples to be used within 7 days may be stored at 2-8 C, otherwise samples must stored at -20 C (≤ 1 months) or -80 C (≤ 2 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay slowly bring samples to room temperature.
Assay Procedure:	Allow all reagents to reach room temperature (Please do not dissolve the reagents at 37 C directly.). All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored at 4 C until the kits expiry date. Prepare all reagents, working standards and samples as directed in the previous sections. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments. 1. Add 100 µ l of Standard, Blank, or Sample per well. Cover with the Plate sealer. Incubate for 2 hours at 37 C . 2. Remove the liquid of each well, don ' t wash. 3. Add 100 µ l of Detection Reagent A working solution to each well. Cover with the Plate sealer. Incubate for 1 hour at 37 C . Detection Reagent A working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform. 4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (approximately 400 µ l) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is

	essential to good performance. After the last wash, 4 remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. 5. Add 100 µ l of Detection Reagent B working solution to each well. Cover with a new Plate sealer. Incubate for 1 hours at 37 C . 6. Repeat the aspiration/wash five times as in step 4. 7. Add 90 µ l of Substrate Solution to each well. Cover with a new Plate sealer. Incubate within 30 minutes at 37 C . Protect from light. 8. Add 50 µ l of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. 9. Determine the optical density of each well at once, using a microplate reader set to 450 nm. Important Note:1. Absorbance is a function of the incubation time. Therefore, prior to starting the assay it is recommended that all reagents should be freshly prepared prior to use and all required strip-wells are secured in the microtiter frame. This will ensure equal elapsed time for each pipetting step, without interruption. 2. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards can be used only once. This assay requires pipetting of small volumes. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10 µ l for once pipetting. 3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. 4. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes. 5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent. 6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. 7. Duplication of all standards and specimens, although not required, is recommended. 8. Substrate Solution is easily contaminated. Please protect it from light.
Calculation of Results:	Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative,

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	construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the sRANKL concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. It is recommended to use some related software to do this calculation, such as curve expert 13.0. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
Restrictions:	For Research Use only

Handling

Handling Advice:	1. The kit should not be used beyond the expiration date on the kit label. 2. Do not mix or substitute reagents with those from other lots or sources. 3. If samples generate values higher than the highest standard, further dilute the samples 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. 4. This assay is designed to eliminate interference by soluble receptors, ligands, 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. 3.
Storage:	4 °C/-20 °C
Storage Comment:	The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20 °C upon being received. The other reagents can be stored at 4 °C.