Elabscience

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5th Edition, revised in June, 2014

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Human PPAR-δ (Peroxisome Proliferator Activated Receptor Delta) ELISA Kit

Catalog No: E-EL-H2397

96T

This manual must be read attentively and completely before using this product.

May you have any problems, please contact our Technical Service Center for help.

Phone: 86-27-87385095

Email: techsupport@elabscience.com

techelabscience@gmail.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Intended use

This ELISA kit applies to the in vitro quantitative determination of Human PPAR-δ concentrations in serum, plasma and other biological fluids.

Sensitivity

The minimum detectable dose of Human PPAR-δ is 0.094ng/mL (The sensitivity of this assay, or lowest detectable limit (LDL) was defined as the lowest protein concentration that could be differentiated from zero).

Detection Range

0.156-10ng/mL

Specificity

This kit recognizes natural and recombinant Human PPAR-δ. No significant cross-reactivity or interference between Human PPAR-δ and analogues was observed.

Note：

Limited by existing techniques, cross reaction may still exist, as it is impossible for us to complete the cross-reactivity detection between Human PPAR-δ and all the analogues.

Repeatability

Coefficient of variation were<10%.

Statement: Thank you for choosing our products. This product is produced by using raw material from world-renowned manufacturer and professional manufacturing technology of ELISA kits. Please read the instructions carefully before use and check all the reagent compositions! If in doubt, please contact Elabscience Biotechnology Co., Ltd.

Storage: All the reagents in the kit should be stored according to the labels on vials. Unused wells should be returned to the foil pouch with the desiccant pack and resealed along entire edge of zip-seal. Substrate Reagent shouldn't be kept at -20℃ (Check!). Exposure of reagents to strong light should be avoided in the process of incubation and storage. All the taps of reagents should be tightened to prevent evaporation and microbial contamination. If not to store reagents according to above suggestions, erroneous results may occur.

Kit Components：

Item	Specifications	Storage
Micro ELISA Plate	8 wells ×12 strips	4℃
Reference Standard	2 vials	4℃
Reference Standard & Sample Diluent	1vial 20mL	4℃
Concentrated Biotinylated Detection Ab	1vial 120μL	4℃
Biotinylated Detection Ab Diluent	1vial 10mL	4℃
Concentrated HRP Conjugate	1vial 120μL	4℃(shading light)
HRP Conjugate Diluent	1vial 10mL	4℃
Concentrated Wash Buffer (25×)	1vial 30mL	4℃
Substrate Reagent	1vial 10mL	4℃(shading light)
Stop Solution	1vial 10mL	4℃
Plate Sealer	5pieces	
Manual	1 copy	
Certificate of Analysis	1 copy	

Test principle

This ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to PPAR-δ. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for PPAR-δ and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain PPAR-δ, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of PPAR-δ. You can calculate the concentration of PPAR-δ in the samples by comparing the OD of the samples to the standard curve.

Sample collection and storage

Samples should be clear and transparent and be centrifuged to remove suspended solids.

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4℃ before centrifugation for 15 minutes at 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8℃ within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Hemolysis samples are not suitable for ELISA assay!

Cell culture supernate: Centrifuge supernate for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8℃. Collect the clear supernate and carry out the assay immediately.

Tissue homogenates: You'd better get detailed references from other literatures before assay aiming at different tissue types. For general information, hemolysis blood may affect the result, so you should mince the tissues to small pieces and rinse them in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (the volume depends on the weight of the tissue) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5minutes at 5000×g to get the supernate.

Other biological fluids: Centrifuge samples for 20 minutes at 1000×g at 2 - 8℃. Collect the supernatant and carry out the assay immediately.

Note:

1. Samples should be used within 7 days when stored at 2-8℃, otherwise samples must be divided and stored at -20℃ (≤1month) or -80℃ (≤6months) to avoid the loss of bioactivity and contamination. Avoid repeated freeze-thaw cycles.
2. Please take the samples to room temperature (18-25℃) without extra heating before performing the assay.
3. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

Sample preparation

1. Elabscience is only responsible for the kit itself, but not for the samples consumed during the experiment. The user should calculate the possible amount of the samples needed in the whole test. Reserving sufficient samples in advance is recommended.
2. If the samples are not mentioned in this manual, a pre-experiment to determine the validity of the kit is necessary.
3. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected Elisa results due to the impacts of certain chemicals.
4. Due to the possibility of mismatching between antigen from other origins and antibodies used in

our kits, some native or recombinant proteins from other manufacturers may not be detected by our kits.

5. Influenced by factors including cell viability, cell number or sampling time, molecular from cells culture supernatant may not be detected by the kit.
6. Grossly hemolyzed samples are not suitable for use in the assay.
7. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Other supplies required

Microplate reader with 450nm wavelength filter
High-precision transferpettor, EP tubes and disposable pipette tips
37℃ Incubator
Deionized or distilled water
Absorbent paper
Loading slot for Wash Buffer

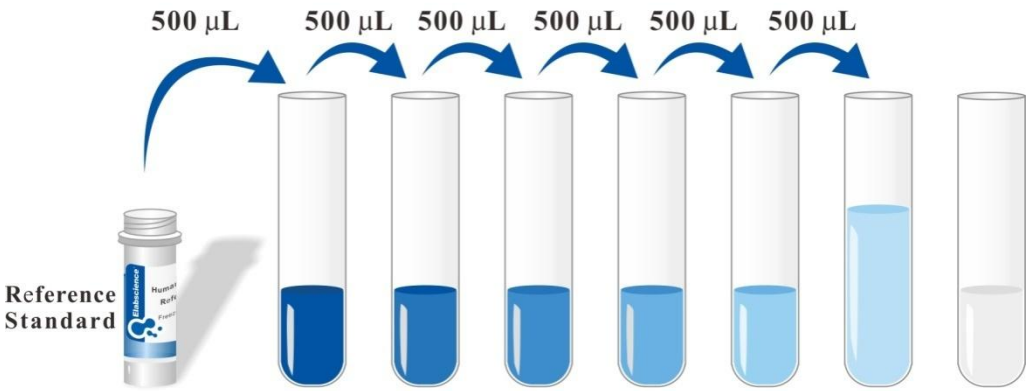
Reagent preparation

Bring all reagents to room temperature (18-25℃) before use.

Wash Buffer - Dilute 30 mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4℃. If crystals have formed in the concentrate, you can warm it with 40℃ water bath (Heating temperature should not exceed 50℃) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

Standard – Prepare standard within 15 minutes before use. Centrifuge at 10,000×g for 1 minute, and reconstitute the Standard with **1.0mL** of Reference Standard &Sample Diluent. Tighten the lid, let it stand for 10 minutes and turn it upside down for several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a stock solution of 10ng/mL. Then make serial dilutions as needed (making serial dilution in the wells directly is not permitted). The recommended concentrations are as follows:**10、5、2.5、1.25、0.625、0.313、0.156、0 ng/mL**. If you want to make standard solution at the concentration of 5ng/mL, you should take0.5mL standard at 10ng/mL, add it to an EP tube with 0.5mL Reference Standard &Sample Diluent, and mix it. Procedures to prepare the remained concentrations are all the same. The undiluted standard serves as the highest standard (10ng/mL). The Reference Standard &Sample Diluent serves as the zero (0 ng/mL).

(Standards can also be diluted according to the actual amount， such as 200μL/tube)



1	2	3	4	5	6	7	8	tube
10	5	2.5	1.25	0.625	0.313	0.156	0	ng/mL

Biotinylated Detection Ab – Calculate the required amount before experiment (100µL/well). In actual preparation, you should prepare 100~200µL more. Centrifuge the stock tube before use, dilute the concentrated Biotinylated Detection Ab to the working concentration using Biotinylated Detection Ab Diluent (1:100).

Concentrated HRP Conjugate – Calculate the required amount before experiment (100µL/well). In actual preparation, you should prepare 100~200µL more. Dilute the Concentrated HRP Conjugate to the working concentration using Concentrated HRP Conjugate Diluent (1:100).

Substrate Reagent: As it is sensitive to light and contaminants, so you shouldn't open the vial until you need it! The needed dosage of the reagent can be aspirated with sterilized tips and the unused residual reagent shouldn't be dumped back into the vial again.

Note: Please don't prepare the reagent directly in the Diluent vials provided in the kit. Contaminated water or container for reagent preparation will influence the result.

Washing Procedure:

1. **Automated Washer:** Add 350µL wash buffer into each well, the interval between injection and suction should be set about 60s.
2. **Manual wash:** Add 350µLWash Buffer into each well, soak it for 1~2minutes. After the last wash, decant any remaining Wash Buffer by inverting the plate and blotting it dry by rapping it firmly against clean and toweling absorbent paper on a hard surface.

Assay procedure

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.** It's recommended that all samples and standards be assayed in duplicate.

1. **Add Sample:** Add 100μL of Standard, Blank, or Sample per well. The blank well is added with Reference Standard & Sample diluent. Solutions are added to the bottom of micro ELISA plate well, avoid inside wall touching and foaming as possible. Mix it gently. Cover the plate with sealer we provided. Incubate for 90 minutes at 37℃.
2. **Biotinylated Detection Ab:** Remove the liquid of each well, don't wash. Immediately add 100μL of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer. Gently tap the plate to ensure thorough mixing. Incubate for 1 hour at 37℃.
3. **Wash:** Aspirate each well and wash, repeating the process three times. Wash by filling each well with Wash Buffer (approximately 350μL) (a squirt bottle, multi-channel pipette, manifold dispenser or automated washer are needed). Complete removal of liquid at each step is essential. After the last wash, remove remained Wash Buffer by aspirating or decanting. Invert the plate and pat it against thick clean absorbent paper.
4. **HRP Conjugate:** Add 100μL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 minutes at 37℃.
5. **Wash:** Repeat the wash process for five times as conducted in step 3.
6. **Substrate:** Add 90μL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for about 15 minutes at 37℃. Protect the plate from light. The reaction time can be shortened or extended according to the actual color change, but not more than 30minutes. When apparent gradient appeared in standard wells, user should terminate the reaction.
7. **Stop**: Add 50μLof Stop Solution to each well. Then, the color turns to yellow immediately. The order to add stop solution should be the same as the substrate solution.
8. **OD Measurement**: Determine the optical density (OD value) of each well at once, using a micro-plate reader set to 450 nm. User should open the micro-plate reader in advance, preheat the instrument, and set the testing parameters.
9. After experiment, put all the unused reagents back into the refrigerator according to the specified storage temperature respectively until their expiry.

Important Note:

1. **ELISA Plate**: The just opened ELISA Plate may appear water-like substance, which is normal and will not have any impact on the experimental results.
2. **Add Sample**: The interval of sample adding between the first well and the last well should not be too long, otherwise will cause different pre-incubation time, which will significantly affect the

experiment's accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. Parallel measurement is recommended.

3. **Incubation**: To prevent evaporation and 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. Do not let the strips dry at any time during the assay. Strict compliance with the given incubation time and temperature.
4. **Washing**: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper in the washing process. But don't put absorbent paper into reaction wells directly. Note that clear the residual liquid and fingerprint in the bottom before measurement, so as not to affect the micro-titer plate reader.
5. **Reagent Preparation**: As the volume of Concentrated Biotinylated Detection Ab and Concentrated HRP Conjugate is very small, liquid may adhere to the tube wall or tube cap when being transported. You better hand-throw it or centrifugal it for 1 minute at 1000rpm. Please pipette the solution for 4-5 times before pippeting. Please carefully reconstitute Standards, working solutions of Detection Ab and HRP Conjugate according to the instructions. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10μL for once pipetting. Do not reuse standard solution, working solution of Detection Ab and HRP Conjugate, which have been diluted. If you need to use standard repeatedly, you can divide the standard into a small pack according to the amount of each assay, keep them at -20～-80℃ and avoid repeated freezing and thawing.
6. **Reaction Time Control:** Please control reaction time strictly following this product description!
7. **Substrate:** Substrate Solution is easily contaminated. Please protect it from light.
8. **Stop Solution:** As it is an acid solution, please pay attention to the protection of your eyes, hands, face and clothes when using this solution.
9. **Mixing:** You'd better use micro-oscillator at the lowest frequency, as sufficient and gentle mixing is particularly important to reaction result. If there is no micro-oscillator available, you can knock the ELISA plate frame gently with your finger before reaction.
10. **Security:** Please wear lab coats and latex gloves for protection. Especially detecting samples of blood or other body fluid, please perform following the national security columns of biological laboratories.
11. Do not use components from different batches of kit (washing buffer and stop solution can be an exception).

12. To avoid cross-contamination, change pipette tips between adding of each standard level, between sample adding, and between reagent adding. Also, use separate reservoirs for each reagent. **Otherwise, the results will be inaccurate!**

Calculation of results

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Create a standard curve by plotting the mean OD value for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. It is recommended to use some professional software to do this calculation, such as curve expert 1.3 or 1.4. In the software interface, a best fitting equation of standard curve will be calculated using OD values and concentrations of standard sample. The software will calculate the concentration of samples after entering the OD value of samples. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it after appropriate dilution. The actual concentration is the calculated concentration multiplied dilution factor.

SUMMARY

1. Add 100μL standard or sample to each well. Incubate 90mintues at 37℃
2. Remove the liquid. Add 100μL Biotinylated Detection Ab. Incubate 1 hour at 37℃
3. Aspirate and wash 3 times
4. Add 100μL HRP Conjugate. Incubate 30 minutes at 37℃
5. Aspirate and wash 5 times
6. Add 90μL Substrate Reagent. Incubate 15 minutes at 37℃
7. Add 50μL Stop Solution. Read at 450nm immediately
8. Calculation of results

Troubleshooting

Problem	Causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by a gentle mix.
	Wells not completely aspirated	Completely aspirate wells between steps.
Low signal	Too brief incubation times	Ensure sufficient incubation time;
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes	Check pipettes and ensure correct preparation
	Improper dilution	
Deep color but low value	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
		Open the Plate Reader ahead to pre-heat
Large CV	Inaccurate pipetting	Check pipettes
High background	Concentration of detector too high	Use recommended dilution factor.
	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions
	Stop solution not added	Stop solution should be added to each well before measurement

Declaration:

1. Limited by current conditions and scientific technology, we can't completely conduct the comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. The final experimental results will be closely related to the validity of products, operation skills of the operators and the experimental environments. Please make sure that sufficient samples are available.
3. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions in the description!
4. Incorrect results may led by wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for Micro-plate reader. Please read the instruction carefully and adjust the instrument prior to the experiment.
5. Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled.
6. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some unexpected reasons such as transportation conditions, different lab equipments, and so on. Intra-assay variance among kits from different batches might arise from above reasons, too.
7. Valid period: 6 months.