Catalog NO.: FY-EH3936
Human PPAR-δ(Peroxisome Proliferator Activated Receptor Delta) ELISA Kit

For the quantitative determination of Human PPAR-δ concentrations in serum, plasma, and other biological fluids.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. The micro plate provided in this kit has been pre-coated with an antibody specific to Human PPAR-δ. Standards or samples are added to the appropriate microtiter plate wells then with a biotin-conjugated antibody specific to Human PPAR-δ. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain Human PPAR-δ, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450nm±3nm. The concentration of PPAR-δ in the samples is then determined by comparing the OD of the samples to the standard curve.

PERFORMANCE

★ Sensitivity:0.1 ng/mL.

★ Detection Range:0.16-10ng/mL.

★ No significant cross-reactivity or interference between Human PPAR-δ and analogues was observed.

★ Repeatability: Coefficient of variation is 10%.

PRECAUTIONS BEFORE THE EXPERIMENT

1. The detection range of the kit is different from the concentration range of the substance to be measured in the sample. It is recommended to estimate the concentration by looking up the reference before the experiment, and determine the actual concentration by pre-experiment. If the concentration is too high or too low, dilute or concentrate the sample appropriately.
2. The newly opened ELISA plate may contain water fog-like substances, which is a normal phenomenon and will not have any impact on the experimental results.
3. Standard working solution, Biotinylated Detection Ab working solution and HRP Conjugate working solution should not be reused.
4. The micro plate reader requires a filter with a wavelength of 450±3nm and a detector capable of detecting 450±3nm, and the optical density value is between 0 and 3.5.
5. Avoid cross-contamination, change the pipette head between each standard concentration addition, between sample addition, and between reagent addition. In addition, separate storage bottles should be used for each reagent.
6. In order to obtain accurate experimental results, it is necessary to ensure that the sealing plate membrane seals the enzyme label plate during incubation.
7. The substrate solution should remain colorless and free from light until it is added to the plate. When added to the plate, the substrate solution should change from colorless to a gradient of blue.
8. The stop solution should be added to the Micro ELISA Plate in the same order as the substrate solution. After the stop solution is added, the color of the solution in the well will change from blue to yellow. Green wells indicate that the stop solution is not sufficiently mixed with the substrate solution. If the color is uneven, shake the plate gently to mix the solution evenly.

REAGENTS&MATERIALS PROVIDED

Item	Specifications	Storage
Micro ELISA Plate (Dismountable)	96T: 8 wells ×12 strips 48T: 8 wells ×6 strips 24T: 8 wells ×3 strips	-20℃, 6 months
Reference Standard	96T: 2 vials 48T: 1 vial 24T: 1 vial	
Concentrated Biotinylated Detection Ab (100×)	96T: 1 vial,120μL 48T: 1 vial, 60μL 24T: 1 vial, 60μL	
Concentrated **HRP Conjugate** (100×)	96T: 1 vial, 120μL 48T: 1 vial, 60μL 24T: 1 vial, 60μL	-20℃, 6 months
Reference Standard & Sample Diluent	1 vial, 20 mL	2-8℃, 6 months
Biotinylated Detection Ab Diluent	1 vial, 13 mL	
HRP Conjugate Diluent	1 vial, 13mL	
Concentrated Wash Buffer (25×)	1 vial, 30 mL	
Substrate Reagent	1 vial, 10 mL	2-8℃ (Protect from light)
Stop Solution	1 vial, 10 mL	2-8℃, 6 months
Plate Sealer	5 pieces	RT
Product Description	1 copy	
Certificate of Analysis	1 copy	

OTHER SUPPLIES REQUIRED

Microplate reader with 450nm wavelength filter
Incubator capable of maintaining 37℃
Single or multi-channel pipettes with high precision
disposable pipette tips
EP tubes
Container for Wash Solution
Squirt bottle, manifold dispenser
Deionized or distilled water
Absorbent paper for blotting the microplate

SAMPLE COLLECTION&STORAGE

Serum: Allow blood samples to clot for 2 hours at room temperature or overnight at 2-8℃ before centrifugation for 15 min at 2000×g at 2-8℃. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 2000×g at 2-8℃ within 30 min of collection. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use. Avoid repeated freeze-thaw cycles.

Cell culture supernatant : Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Other Types of samples : Please check our official website for the collection and processing method(https://www.feiyuebio.cn/index/e31/66.html).

REAGENT PREPARATION

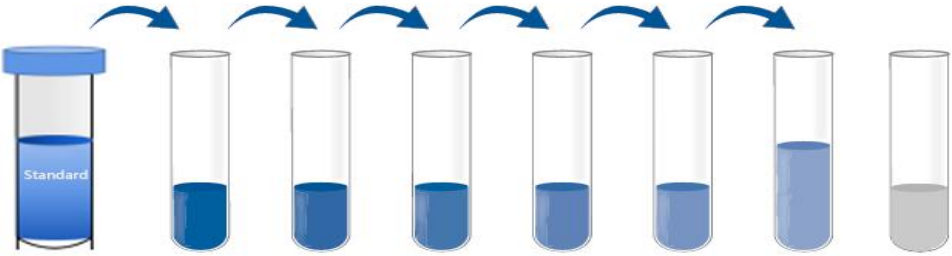
1. Allow all reagents to return to room temperature (18-25℃) before use.
2. **Wash Buffer:** Dilute 20 mL of Concentrated Wash Buffer with 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

3.**Standard working solution:**

①Centrifuge the Reference Standard at 10000×g for 1 min.

②Add 1mL of Reference Standard & Sample Diluent, let it stand for 10min and then mix gently, that is 10ng/mL of standard working solution.

③Prepare 7 EP tubes, add 500μL standard & sample diluent to each tube, take 500μL from 10ng/mL to make 5ng/mL, and so on, the last tube is blank (no more liquid needs to be drawn from the penultimate tube). Use it now!



10	5	2.5	1.25	0.63	0.32	0.16	0
ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL

4. **Biotinylated Detection Ab working solution**: Calculate the amount of Biotinylated Detection Ab required (100μL/ well). The actual preparation should be 100-200μL. Centrifuge the concentration tube before use and dilute 100× concentrated Detection Ab to 1× working solution (e.g. 10μL + 990μL Biotinylated Detection Ab Diluent).
5. **HRP Conjugate working solution**: Calculate the amount of HRP Conjugate required (100μL/ well). The actual preparation should be 100-200μL. Centrifuge the concentration tube before use and dilute 100× concentrated HRP Conjugate to 1× working solution (e.g. 10μL + 990μL HRP Conjugate diluent).

ASSAY PROTOCOL

1. Determine wells for diluted standard, blank and sample. Add **100μL** each dilution of **standard, blank** and **sample** into the appropriate wells . Cover the plate with the sealer provided in the kit. Incubate for **90 min** at **37°C**. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

2. Decant the solution from each well add **300μL** of **wash buffer** to each well. Soak for **0.5 min** and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step **3 times**. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.

3. Immediately add **100μL** of **Biotinylated Detection Ab working solution** to each well. Cover the plate with a new sealer. Incubate for **60 min** at **37°C**.

4. Decant the solution from each well, repeat the wash process for **3 times** as conducted in step 2.

5. Add **100μL** of **HRP Conjugate working solution** to each well. Cover the plate with a new sealer. Incubate for **30 min** at **37°C**.

6. Decant the solution from each well, repeat the wash process for **3 times** as conducted in step 2.

7. Add **100μL** of **Substrate Reagent** to each well. Cover the plate with a new sealer. Incubate for about **15 min** at **37°C**. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than **30 min**. Preheat the Microplate Reader for about **15 min** before OD measurement.

8. Add **50μL** of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.

9. Determine the optical density (OD value) of each well at once with a micro plate reader set to **450 nm**.

SUMMARY FOR PROCEDURE

Respectively add standard and sample 100μL/ well, and incubate at 37℃ for 90 minutes

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Wash the plate three times

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Add Biotinized Detection Ab 100μL/ well and incubated at 37℃ for 60 min

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Wash the plate three times

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Add HRP Conjugate 100μL/ well and incubate at 37℃ for 30 min

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Wash the plate three times

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Add substrate 100μL/ well and incubate at 37℃ for 15 minutes (avoid light and not exceed 30 minutes)

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Add stop solution 50μL/ well

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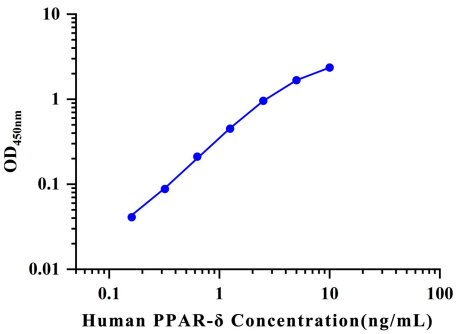
Read OD value

EXAMPLE DATA

For each test, a standard curve must be set for each plate. The standard curves below are for example purposes only.

ng/mL	Corrected OD	Typical Standard Curve
0	0	
0.16	0.041	
0.32	0.088	
0.63	0.211	
1.25	0.45	
2.5	0.957	
5	1.673	
10	2.358	

CALCULATION OF RESULTS

Calculate the average OD value of the standard and sample, then subtract the OD value of the zero concentration standard.

With the standard product concentration as the horizontal coordinate and OD value as the vertical coordinate, the standard curve is formed by using the nonlinear four parameter logistic(4-PL) and the sample concentration is calculated by the curve.Please refer to our official website for the specific fitting method(https://www.feiyuebio.cn/index/e31/65.html).

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

SENSITIVITY

The minimum detection concentration of the Human PPAR-δ detected by this kit is usually less than the 0.1 ng/mL. The sensitivity was determined by adding two standard deviations to the mean OD value of twenty zero standard replicates and calculating the corresponding concentration.

PRECISION

Mean coefficient of variation for Intra-Assay and Inter-Assay: 3 samples with low, middle and high level concentration were tested for repeat multiple times, respectively.The results showed that the coefficient of variation of the kits was less than 10%, which met the precision quality control standard.

SPECIFICITY

This method has high sensitivity and specificity for the detection of Human PPAR-δ. No significant cross-reactivity was observed between the Human PPAR-δ and its analogues.

RECOVERY

The known concentration of Human PPAR-δ was added to different samples respectively, and the recovery experiment was conducted. The results showed that the recovery range and average recovery rate of the kit were 80-120%, which met the recovery quality control standard.

LINEARITY

Dilute linear experiments were performed on samples with Human PPAR-δ to evaluate the linearity of the kit. The results showed that the kit linear range (%) was 80-120%, which met the linear quality control standard.

DECLARATION

1. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis of all the raw material provided. There might be some qualitative and technical risks for users using the kit.
2. This assay is designed to eliminate interference by factors present in biological samples. Until all factors have been tested in the ELISA immunoassay, the possibility of interference cannot be excluded.
3. The final experimental results will be closely related to the validity of products, operational skills of the operators, the experimental environments and so on We are only responsible for the kit itself, but not for the samples consumed during the assay. The users should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
4. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions.
5. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Micro plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.
6. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
7. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipment, and so on. Intra assay variance among kits from different batches might arise from the above reasons too.
8. Kits from different manufacturers or other methods for testing the same analyte could bring out inconsistent results, since we haven’t compared our products with those from other manufacturers.
9. The kit is designed for research use only, we will not be responsible for any issues if the kit is applied in clinical diagnosis or any other related procedures.
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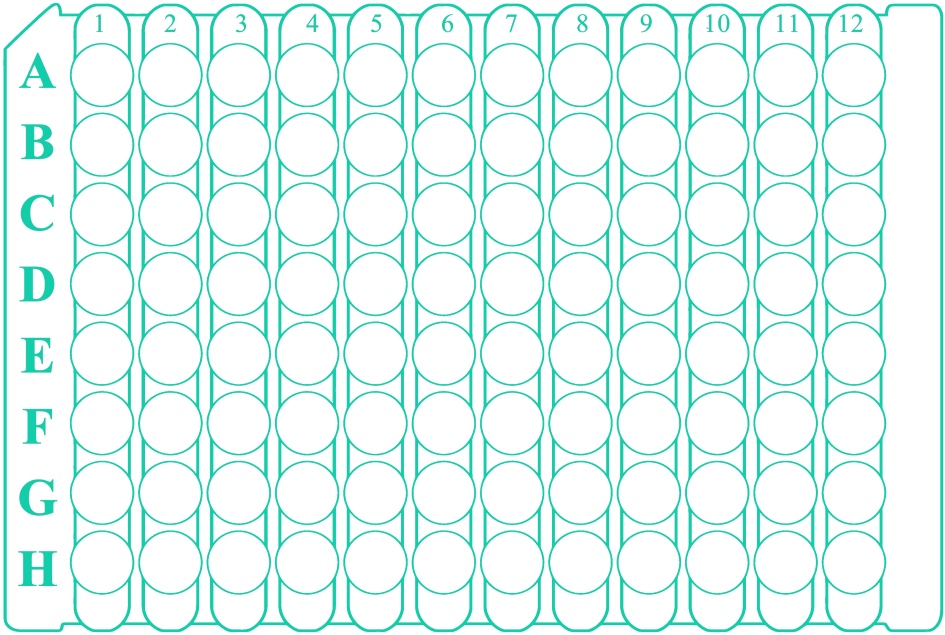
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



If you have any questions, please contact us.

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