Human Peroxisome Proliferator-Activated Receptors (PPARs) ELISA

Cat No: K12-1644

Principle:

This is sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human PPARs in samples. Addition of standard or sample to microtiter well which is pre-coated with Human PPARs monoclonal antibody and addition of biotin labeled Human PPARs antibodies, followed by addition of HRP conjugate to form immune complex. Unbound HRP will get removed by washing step after incubation. Then addition of Substrate A and B, develops blue color during incubation period and reaction will get stop after addition of stop solution with development of yellow color. The concentration of the Human PPARs of sample is directly proportional to the yellow color developed in well and will be positively correlated.

Intended Use:

This kit is used to assay the level of PPARs in Human serum, blood, plasma, and other related tissue samples.

Materials provided in the kit:

1. Microtiter Coated Plate (96 wells)– 1 no
2. PPARs Ab Biotin Conjugate– 1 ml
3. Standard 96 ng/ml – 0.5 ml
4. HRP Conjugate– 6 ml
5. Wash Buffer (30X)– 20 ml
6. Standard Diluent– 3 ml
7. Substrate A – 6 ml
8. Substrate B– 6 ml
9. Stop Solution– 6 ml
10. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 µl to 1000 µl.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Semi-log graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.
9. Incubator

Storage Information:

1. All reagents should be stored at 2 ºC to 8 ºC.
2. All the reagents and wash solutions are stable until the expiration date of the kit.
3. 30 minutes prior before use, bring all components to room temperature (18-25 ºC). Store all the components of the kit at its appropriate storage condition after use.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all samples in accordance with NCCLS regulations.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. The kit cannot test samples which contain NaN_3, because NaN_3 inhibits HRP activity.
2. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
3. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
4. **Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 20-min at the 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
5. **Urine-** Collect urine in a sterile container, centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
6. **Cell Culture supernatant-** Detects secretory components. Collect sample in a sterile container. Centrifuge for 20 mins at 2000-3000 rpm. Remove the supernatant. Dilute cell suspension with PBS (pH 7.2-7.4), if cell concentration is greater than 1 million/ml. Repeated freeze-thaw cycles, can damage the cells and release intracellular components. If precipitation appeared, centrifuge again.
7. **Tissue samples-** After cutting the samples check the weight and add PBS (pH 7.2-7.4). Rapidly freeze with liquid nitrogen. Maintain samples at 2-8℃ after melting. Add PBS (pH 7.4). Homogenize by hand or grinder. Centrifuge for 20 mins at 2000-3000 rpm. Remove the supernatant.

Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Bring all reagents to Room Temperature prior to use.
2. To make 1X Wash Solution, add 10 ml of 30X Wash Buffer in 290 ml of DI water.

Procedural Notes:

1. After taking the kit out from 2-8˚C environment, the kit should be stabilized for 30 minutes at room temperature and then used. If the coated strips haven't been used up after opening, then the remaining strips should be stored in a sealed bag.
2. For each step, add sample with sample injector which should be calibrated frequently, in order to avoid unnecessary experimental tolerance.
3. The operation shall be carried out according to the instructions strictly. And test results must be based on the readings of the Microplate reader.
4. In order to avoid cross-contamination, it is forbidden to re-use the suction head and seal plate membrane in your hands.
5. All samples, washing buffer and each other component should be discarded according to infective material process.
6. Reagents not in used should be covered. Do not use reagent with different batches and use them before expiry date.
7. Substrate B is light-sensitive and so its prolonged exposure to light is forbidden.

Assay Procedure:

1) Bring all reagents to room temperature prior to use. It is strongly recommended that all Standards and Samples should be run in duplicates or triplicates. A standard curve is required for each assay.
2) Standards Dilution: Prepare the standards as per the table given below using the provided standard Concentration and standard diluent.

48 ng/ml	Standard No.5	120 µl Original Standard + 120 µl Standard diluent
24 ng/ml	Standard No.4	120 µl Standard No.5 + 120 µl Standard diluent
12 ng/ml	Standard No.3	120 µl Standard No.4 + 120 µl Standard diluent
6 ng/ml	Standard No.2	120 µl Standard No.3 + 120 µl Standard diluent
3 ng/ml	Standard No.1	120 µl Standard No.2 + 120 µl Standard diluent

3) The quantity of the plates depends on the quantities of samples and standards to be tested. It is suggested to remove the number of strips required for the assay.
4) Pipette out **50 µl** of **Standards** and **40 µl Samples** into the respective wells as mentioned in the work list. *Note do not add the sample, Biotin Conjugate and Streptavidin-HRP to the blank well.*
5) Pipette out **10 µl** of **Biotin Conjugate** into each sample well. *Do not pipette into the blank and standards wells.*
6) Pipette out **50 µl** of **HRP Conjugate** into each sample and standards well. *Do not pipette into the Blank well.*
7) Cover the plate and incubate for 1 hour at 37 °C in the incubator.
8) Aspirate and wash plate 4 times with **1X Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly. Then add **Substrate A 50 µl**, then **Substrate B 50 µl** to each well including Blank well. Gently mixed, incubate for 10 min at 37 ℃ in dark.
9) Pipette out **50 µl** of **Stop Solution**. Wells should turn from blue to yellow in colour.
10) Read the absorbance at 450 nm within 15 minutes after adding the Stop Solution blanking on the zero standards.

Calculation of Results:

Take the standard density as the horizontal axis, OD values in the vertical axis and draw the standard curve on graph paper. Find out the corresponding concentration according to the sample OD value from the sample curve (the result is the sample concentration) or calculate the straight line regression equation of the standard curve with the standard concentration and the OD value, with the sample OD value in the equation, calculate the sample concentration.

Precautions:

Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

Performance Characteristics:

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

Sensitivity:

Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD. 10 replicates of '0' standards were evaluated and the LOD was found to be **0.542 ng/ml**

Assay Range: 3 ng/ml to 48 ng/ml