PRINCIPLE OF THE TEST

PAH ELISA Kit is based on the use of monoclonal antibody anti-benzo(a)pyren (anti-B(a)P) that bind PAH or PAH-enzyme conjugate (PAH-Px). PAH present in the sample and assay calibrators are bound during the first incubation by the anti-B(a)P antibodies, which are immobilized to the wells. Second incubation is with PAH-Px. After a second incubation, the unbound PAH and PAH-Px is decanted and the wells are thoroughly washed. Finally, a clear solution of chromogenic substrate (TMB) is then added to the wells. In the presence of bound PAH-Px conjugate, the clear substrate is converted to a blue color. The reaction is stopped by adding of Stop solution and the blue substrate is converted to a yellow color.

INTENDED USE

PAH ELISA Kit is intended the detection of Polycyclic aromatic hydrocarbons (PAH) in samples.

GENERAL DESCRIPTION

Polycyclic aromatic hydrocarbons (PAHs), also known as poly-aromatic hydrocarbons or polynuclear aromatic hydrocarbons, are potent atmospheric pollutants that consist of fused aromatic rings and do not contain heteroatoms or carry substituents. Naphthalene is the simplest example of a PAH. PAHs occur in oil, coal, and tar deposits, and are produced as byproducts of fuel burning (whether fossil fuel or biomass). As a pollutant, they are of concern because some compounds have been identified as carcinogenic, mutagenic, and teratogenic. PAHs are also found in cooked foods. Studies have shown that high levels of PAHs are found, for example, in meat cooked at high temperatures such as grilling or barbecuing, and in smoked fish.

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MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled or deionised water for dilution of the Wash buffer concentrate.
2. Appropriate equipment for pipetting, liquid dispensing and washing.

STORAGE

1. Store the kit reagents at +2 to +10°C, in a dry place and protected from the light. Avoid freezing.
2. Store unused strips in the sealable pouch and keep the desiccant inside. Transport in thermo bags until 72 hours. Any damages of packaging of kit reagents advise to the producer without delay.
3. Do not store diluted samples and diluted PAH-Px conjugate. Always prepare fresh.

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**ASSAY PROCEDURE**

**Reagent And Sample Preparation**
1. Allow all kit components to reach room temperature.
2. Vortex samples, Diluting solution (DB) and TMB substrate in order to ensure homogeneity and mix all solution well prior to use.
3. Before use, dilute PAH-Px 1:10 with Diluting solution (final concentration is 10μg/mL).
4. For dilution of tested samples use Diluting solution (DB).
5. Prepare Wash buffer by diluting the concentrate 10 times with an appropriate volume of distilled or deionised water (100 mL of the concentrated Wash buffer + 900 mL of distilled water). If there are crystals of salt present in the concentrated Wash buffer, warm up the vial to +32 to +37°C in a water bath. Diluted Wash buffer is stable for one week if stored at +2 to +10°C.
6. Do not dilute TMB substrate and Stop solutions, they are ready to use.

**Assay Steps**
1. Allow the antibody coated strips to reach room temperature before opening in order to prevent water condensation within the wells. Withdraw an adequate number of antibody coated strips. Put the remaining strips back in the aluminium pouches and seal them if possible, keep the desiccant inside.
2. Wash and aspirate the wells three times with 250 μl/well of Wash buffer. Avoid crosscontamination between wells! If some liquid remains in the wells, invert the plate and tap it on an adsorbent paper to remove the last remaining drops.
3. Pipette 100 μL of Dilution buffer (DB) to the first well of the strip to determine the reaction background. Fill the next seven wells of the strip with Standard stock solution of PAH (STA, STB, ST..) and then fill the remaining wells with 100 μL diluted tested samples. If you want to exclude a possible laboratory error, two parallels may be employed for each sample.
4. Incubate for 120 (+/- 6) minutes at room temperature, 240 rpm.
5. Pipette 5 μL of PAH-Px with concentration 10 μg/mL in all wells. Final concentration of PAH-Px in well is 0.5 μg/mL.
6. Incubate for 60 (+/- 5) minutes at room temperature, 240 rpm.
7. Aspirate the liquid from the wells into a collecting bottle containing appropriate disinfectant. Wash and aspirate the wells five times with 250 μl/well of Wash buffer. Avoid crosscontamination between wells! If some liquid remains in the wells, invert the plate and tap it on an adsorbent paper to remove the last remaining drops.
8. Dispense 100 μL of the TMB substrate into each well. Pipette in a regular rhythm or use an appropriate dispensing instrument.
9. Incubate for 10 minutes (+/-5 seconds) at room temperature. The time measurement must be started at the beginning of TMB dispensing. Cover the strips with an aluminium foil or keep them in the dark during the incubation with TMB substrate.
10. Stop the reaction by adding 100 μL of Stop solution. Use the same pipetting rhythm as with the TMB substrate to ensure the same reaction time in all wells. Tap gently the microplate for a few times to ensure complete mixing of the reagents.
11. Read the absorbance at 450 nm with a microplate reader within 20 minutes. It is recommended to use reference reading at 690 nm.

**PROCESSING OF RESULTS**

Calculate the concentration for each sample:
1. Construct the calibration curve by plotting the absorbance (Y) of standards versus log of the known concentration (X) of standards.
2. Calculate the concentration of PAHs by equation of calibration curve. Use the value of sample absorbance (OD) instead of the value y. The values x represent the concentration of PAHs in samples. Note that you need to use the diluting factor in the calculation (i.e. if you dilute sample 100 times, you have to multiply the final concentration of PAHs by 100).

**ASSAY CHARACTERISTICS**

**Validity**
The results of the test are valid if:
1. The mean absorbance (OD) of PAH standard G (ST G) is more than 0.100.
2. The mean absorbance (OD) of PAH standard A (ST A) is more than 2,500.
3. The mean absorbencies (OD) of PAH standard can be lined up as follows:
   - ST G < ST F < ST E < ST D < ST C < ST B < ST A

**Precision**
Intraassay variability: 5.0% - 7.9%
Interassay variability: 3.7% - 9.9%

**REFERENCES**