PAH water ELISA Kit

Prod. No.: DEIA492
Unit Size: 1Kit/96T

INTENDED USE

PAH water ELISA Kit is intended the detection of Benzo(a)pyrene (B(a)P) (Polycyclic aromatic hydrocarbons, PAH) in water and seawater samples.

GENERAL DESCRIPTION

Polycyclic aromatic hydrocarbons (PAHs), also known as polyaromatic 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.

PRINCIPLE OF THE TEST

PAH water ELISA Kit is based on the use of rabbit polyclonal antibody anti-benzo(a)pyrene (anti-B(a)P) that bind B(a)P (PAH) or B(a)P-enzyme conjugate (B(a)P-Px). B(a)P (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. After a second incubation, the unbound B(a)P (PAH) and B(a)P-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 B(a)P-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. Since there is the same number of antibody binding sites on every well and each well receives the same number of B(a)P-Px conjugate molecules, a sample date that contains a low concentration of B(a)P (PAH) allows the antibody to bind many B(a)P-Px conjugate molecules. Therefore, a low concentration of B(a)P (PAH) produces a dark yellow color. Conversely, a high concentration of B(a)P (PAH) will allow fewer B(a)P-Px conjugate molecules to be bound by the antibodies, resulting in a lighter yellow color.

MATERIALS PROVIDED BUT NOT PROVIDED

1. 8-well strips coated with the specific rabbit polyclonal antibody: 12 strips
2. 1.3 mL B(a)P standard A (STA – concentration 0 ng/mL), ready to use: 1 glass vial
3. 1.3 mL B(a)P standard B (STB – concentration 1 ng/mL), ready to use: 1 glass vial
4. 1.3 mL B(a)P standard C (STC – concentration 5 ng/mL), ready to use: 1 glass vial
5. 1.3 mL B(a)P standard D (STD – concentration 25 ng/mL), ready to use: 1 glass vial
6. 0.240 mL Enzyme conjugate B(a)P-Px, 50 x concentrated: 1 plastic vial
7. 12 mL Buffer for conjugate dilution, ready to use: 1 glass vial
8. 125 mL Wash buffer concentrate, 10x concentrated: 1 plastic vial
9. 35 mL Assay buffer, ready to use: 1 glass vial
10. 12 mL Chromogenic substrate (TMB substrate), ready to use: 2 plastic vials
11. 6 mL Stop solution, ready to use: 1 plastic vial
12. Vial for conjugate B(a)P-Px dilution: 1 piece
13. Sealable pouch for unused strips: 1 piece
14. Instruction manual
15. Certificate of quality

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 B(a)H-Px conjugate. Always prepare fresh.
ASSAY PROCEDURE

Reagent And Sample Preparation
1. Allow all kit components to reach room temperature.
2. Vortex samples and mix all solution well prior use.
3. Just before use dilute B(a)P-Px 1:50 with Buffer for conjugate dilution (e.g. 120 µL of concentrated conjugate + 6 mL of buffer for conjugate dilution)
4. If needed, the samples may be diluted with distilled water. The grade of dilution is important to estimate the exact concentration of the probe.
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 standards, assay buffer, buffer for conjugate dilution, TMB substrate and Stop solutions, they are ready to use.
7. Water samples are analyzed without any extraction. Only the crude mechanical contaminations required the filtration using adequate filters.

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. Pipette 300 µLwell of Assay buffer into appropriate wells. After 20 seconds aspirate the liquid from the wells into a collecting bottle. Invert the plate and tap it on an adsorbent paper to remove the last remaining drops.
3. Pipette 100 µL of of B(a)P standards (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 5 minutes at room temperature
5. Pipette 100 µL of diluted B(a)P-Px in all wells.
6. Incubate for 120 (+/-5) minutes at room temperature
7. Aspirate the liquid from the wells into a collecting bottle containing appropriate disinfectant. Wash and aspirate the wells four times with 350 µL/well of Wash buffer. Avoid cross-contamination 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 200 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 50 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:
a. Construct the calibration curve by plotting the absorbance (Y) of standards versus log of the known concentration (X) of standards.
b. Calculate the concentration of B(a)P (PAH) by equation of calibration curve. Use the value of sample absorbance (OD) instead of the value y. The values x represent the concentration of B(a)P (PAH) in samples. Note that you need to use the diluting factor in the calculation (i.e. if you dilute sample 2 times, you have to multiply the final concentration of B(a)P (PAH) by 2).

REFERENCES