Version 10-09/22

User's Manual

Benzo(a)pyrene ELISA Kit

REF **DEIA3199**

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Benzo(a)Pyrene ELISA is an immunoassay for the quantitative and sensitive detection of Benzo(a)Pyrene in water samples.

General Description

Benzo(a)Pyrene (B(a)P) is an environmental pollutant emanating from both natural and anthropogenic sources. B(a)P is produced during incomplete combustion, pyrolysis of organic matter, during industrial processes and during household activities such as cooking, barbequing and smoking. Anthropogenic sources such as industrial production, transportation and waste incineration generate significant amount of this compound and other PAHs. Petroleum production, import and export of petroleum products also contribute to the contamination of marine samples. Other sources include crude oil contamination of ground and sea water (such as in oil spills), tobacco smoke, exhaust fumes and combustion of all forms of fossil fuels. Benzo(a)Pyrene being fat soluble tends to accumulate in marine organisms and contaminated coastal environments. B(a)P is a carcinogen known to induce tumors in a number of organs in animal models and humans. One of its metabolites is known to form DNA adducts which are responsible for mutagenesis and tumorigenesis. Due to the carcinogenic and mutagenic nature of PAHs, there has been increased concern in their detection and monitoring in recent years. Regulatory levels have been set for B(a)P in various countries.

Principles of Testing

The test is a direct competitive ELISA that allows the detection of Benzo(a)Pyrene. It is based on the recognition of enzo(a)Pyrene by specific antibodies. Benzo(a)Pyrene, when present in a sample, and a Benzo(a)Pyrene-HRP analogue compete for the binding sites of mouse anti-Benzo(a)Pyrene antibodies in solution. The Benzo(a)Pyrene antibodies are then bound by a second antibody (goat anti-mouse) immobilized in the plate. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of the Benzo(a)Pyrene present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

Reagents And Materials Provided

1. Microtiter plate coated with a second antibody (goat anti-mouse).
2. Standards (6): 0, 0.25, 0.75, 1.5, 2.5, 5.0 ng/mL.
3. Antibody solution (mouse anti-Benzo(a)Pyrene), 6 mL
4. Benzo(a)Pyrene-HRP, 6 mL
5. Diluent/zero, 25 mL. Use to dilute samples with concentration above 5.0 ppb.
6. Wash Solution 5X Concentrate, 100 mL
7. Color Solution (TMB), 12 mL

8. Stop Solution, 6 mL

Materials Required But Not Supplied

1. Micro-pipettes with disposable plastic tips (50-250 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with plastic tips (50-250 µL)
3. Reagent reservoir for multichannel pipettes
4. Microtiter plate washer (optional)
5. Microtiter plate reader (wave length 450 nm)
6. Shaker for microtiter plates (optional)
7. Methanol

Storage

The Benzo(a)Pyrene ELISA should be stored in the refrigerator (4–8°C).

Specimen Collection And Preparation

1. Collect 2.0 mL of water sample in a glass container.
2. To prevent loss of Benzo(a)Pyrene to the glass surface, immediately add 2.0 mL of Methanol, mix by hand.
3. Analyze preserved sample as samples (Assay Procedure step 1)

The Benzo(a)Pyrene concentration contained in the water sample is determined by multiplying the concentration of the diluted sample by a factor of 2. Highly contaminated samples outside the range of the curve should be diluted in Sample Diluent (50% methanol/water) and re-analyzed.

Assay Procedure

1. Add 50 µL of the standard solutions or samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add 50 µL of the enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add 50 µL of antibody solution to the individual wells successively using a multi- channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the bench top for about 30 seconds. Be careful not to spill contents. Incubate the strips for sixty (60) minutes at 4-8 °C.
4. After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips three times using the 1X washing buffer solution. Please use at least a volume of 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
5. Add 100 µL of substrate/color solution to the wells using a multi- channel pipette or a stepping pipette. The strips are incubated for 30 minutes at room temperature. Protect the strips from sunlight.
6. Add 50 µL of stop solution to the wells in the same sequence as for the substrate/color solution using a

multi- channel pipette or a stepping pipette.

7. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after stopping the reaction.

Interpretation Of Results

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-parameter, Logit/Log or alternatively point to point). For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/B0 for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the %B/B0 for each standard on a vertical linear (y) axis versus the corresponding Benzo(a)Pyrene concentration on the horizontal logarithmic (x) axis on graph paper. %B/B0 for controls and samples will then yield levels in ppb of Benzo(a)Pyrene by interpolation using the standard curve.

The concentrations of the samples are determined using the constructed standard curve (do not use a previously stored curve). Samples showing a lower concentration than 0.25 ppb of Benzo(a)Pyrene should be reported as < 0.25 ppb. Samples showing a higher concentration than standard 5 (5.0 ppb) must be diluted to obtain accurate results.

Detection Limit

0.30 ppb (ug/L).

Reproducibility

Coefficients of variation (CVs) for standards: <10%, for samples: < 20%.

Limitations

Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in water samples, test interferences caused by matrix effects can't be completely excluded. The presence of the following substances up to 10,000 ppm were found to have no significant effect on the Benzo(a)Pyrene Assay results: aluminum oxide, calcium chloride, calcium sulfate, manganese sulfate, magnesium sulfate, magnesium chloride, sodium chloride, phosphate, sodium thiosulfate, sodium nitrate. Copper chloride, zinc sulfate, ferric sulfate, sodium fluroride up to 1,000 ppm. Humic acid up to 10 ppm.