## Importance of Benzo(a)Pyrene Determination

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.

## Performance Data

Test sensitivity: The detection limit for this assay in water samples is 0.30 ppb (ug/L).

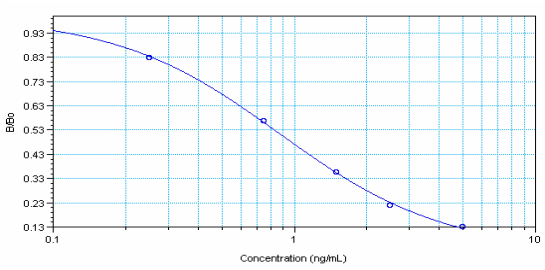
Test reproducibility: Coefficients of variation (CVs) for standards: <10%, for samples: < 20%.

Selectivity: The assay exhibits very good cross-reactivity with Benzo(a)Pyrene but not with other non-related compounds tested.

Cross-reactivities:

| | |
|---|---|
| Benzo(a)Pyrene | 100% (per definition) |
| Chrysene | 77.6% |
| Indeno{1,2,3-cd} Pyrene | 49.7% |
| Benzo(b) Fluoranthene | 20.2% |
| Benzo(a)Anthracene | 11.7% |
| Pyrene | 10.9% |
| Fluoranthene | 8.6% |
| Benzo(k) fluoranthene | 5.0% |

The following PAHs exhibited <= 1% cross-reactivity: naphthalene, acenaphthylene, acenaphthene, fluorine, phenanthrene, anthracene, diben(ah)anthracene, benzo(gh)perylene.

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ABRAXIS

# Benzo(a)Pyrene ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of Benzo(a)Pyrene in Water Samples

Product No. 530039

## 1. General Description

The Abraxis Benzo(a)Pyrene ELISA is an immunoassay for the quantitative and sensitive detection of Benzo(a)Pyrene in water samples. Sample concentration is not required prior to analysis for LOD of 0.3 ppb, if lower LOD are required a simple concentration step is necessary. If necessary, positive samples can be confirmed by HPLC or other conventional methods.

## 2. Safety Instructions

The substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. Avoid contact of the TMB and stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

## 3. Storage and Stability

The Benzo(a)Pyrene ELISA should be stored in the refrigerator (4–8°C). Solutions should be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box. Consult state, local and federal regulations for proper disposal of all reagents.

## 4. Test Principle

The test is a direct competitive ELISA that allows the detection of Benzo(a)Pyrene. It is based on the recognition of Benzo(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.

## 5. Limitations of the Benzo(a)Pyrene ELISA, Possible Test Interference

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.

Mistakes in handling the test can also cause errors. Possible sources for such errors can be:
Inadequate storage conditions of the test kit, wrong pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction. The assay procedure should be performed away from direct sun light.

As with any analytical technique (GC, HPLC, etc…..) positive results requiring some action should be confirmed by an alternative method.

## A. 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

## B. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the antibody, enzyme conjugate, substrate solution, and the stop solution in order to equalize the incubations periods of the standard solutions and the samples on the entire microtiter plate. Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination. Read and understand the instructions and precautions given in this insert before proceeding.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standard, control, antibody solution, enzyme conjugate, substrate and stop solutions are ready to use and do not require any further dilutions.
4. The wash solution is a 5X concentrated solution and needs to be diluted with deionized water. In a 1L container dilute the 5X solution 1:5 (i.e. 100 mL of the 5X wash solution plus 400 mL of deionized water). The diluted solution is used to wash the microtiter wells.
5. The stop solution has to be handled with care as it contains diluted $H_2SO_4$.

## C. 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.

## D. Evaluation

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 $\%B/B_0$ 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 $\%B/B_0$ 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. $\%B/B_0$ 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.

## E. Additional Materials (not delivered with the test kit)

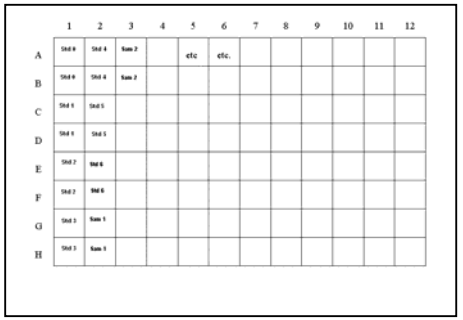
1. Micro-pipettes with disposable plastic tips (50-250 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with plastic tips (50-250 µL)
3. Reagent reservoir for multichannel pipettes
4. Microtiter plate washer (optional)
5. Microtiter plate reader (wave length 450 nm)
6. Shaker for microtiter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

## F. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std 5: Standards

Sam1, Sam2, Sam3, etc.: Samples




## G. Surface and Sea Water

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.

For other sample matrices, please contact Abraxis for technical bulletins or validation guidance.