



STRATEGIC DIAGNOSTICS INC.

EnviroGard™ Polynuclear Aromatic Hydrocarbons (PAH) in Water Test Kit

70620

Intended Use

The EnviroGard PAH in Water Test Kit is an enzyme immunoassay for the detection of a range of polycyclic aromatic hydrocarbons (PAHs) in water, to include the 16 compounds listed under EPA methods 525, 550 and 550.1. The EnviroGard PAH Test Kit allows for reliable and rapid semiquantitative screening for total PAH or benzo(a)pyrene at 2.0, 10, and 100 parts per billion (ppb) in water.

PAHs are a family of planar aromatic compounds having two or more fused rings, each of which contains 5 or 6 carbon atoms. These compounds are found in varying concentrations and combinations in petroleum products, creosote, coal and its residues, combustion residues, and other fossil fuel residues.

NOTE: Refer to the sections “Interpret the Results” and “Specificity” for more information on PAHs.

Test Principles

The EnviroGard PAH in Water Test Kit is based on the use of polyclonal antibodies that bind either PAH or PAH-enzyme conjugate. PAHs present in the sample and assay calibrators are bound during the first incubation by the anti-PAH antibodies, which are immobilized to the walls of the test tubes. After the sample is decanted and the test tubes are thoroughly washed, a PAH-enzyme conjugate is added.

NOTE: The amount of conjugate that is bound (by unoccupied anti-PAH antibody sites in the test tube) is inversely related to the amount of PAH that was originally present in sample.

After a second incubation, the unbound conjugate is decanted and the test tubes are thoroughly washed again. Finally, a clear solution of chromogenic substrate is then added to the test tube. In the presence of bound PAH-enzyme conjugate, the clear substrate is converted to a blue color.

Since there are the same number of antibody binding sites on every test tube and each test tube receives the same number of PAH-enzyme conjugate molecules, a sample

that contains a low concentration of PAH allows the antibody to bind many PAH-enzyme conjugate molecules. Therefore, a low concentration of PAH produces a dark blue color. Conversely, a high concentration of PAH will allow fewer PAH-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue color.

NOTE: Color development is inversely proportional to PAH concentration.

Darker color = Lower Concentration
Lighter color = Higher Concentration

The determination of the PAH level in an unknown sample is interpreted relative to the assay calibrator levels using visual comparison or by reading with a spectrophotometer.

Precautions

- Treat PAHs, solutions that contain PAHs, and potentially contaminated water samples as hazardous materials.
- Use gloves, proper protective clothing, and means to contain and handle hazardous material where appropriate.
- Store all test kit components at 4°C to 8°C (39 °F to 46°F) when not in use.
- Do not freeze test kit components or expose them to temperatures greater than 37°C (99°F).
- Do not store test kit components for more than 8 hours at ambient temperature.
- Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before beginning the test. This typically requires at least 30 minutes to warm from recommended storage conditions.
- Do not expose **substrate** to **direct sunlight**.
- Do not use test kit components after the expiration date.
- Do not use reagents or test tubes from one test kit with reagents or test tubes from a different test kit.

- Do not dilute or adulterate test reagents or use samples not called for in the test procedure; this may give inaccurate results.
- Use approved methodologies to confirm any positive results.
- Tightly recap the PAH calibrator vials to prevent evaporative loss.
- Some solutes and particulates found in untreated ground or surface waters may affect the sensitivity level of this test kit.

Materials Provided

- 20 PAH Antibody-Coated Test Tubes
- 1 vial of Negative Control
- 1 vial of 2.0 ppb PAH Calibrator (actual concentration is 0.3 ppb pyrene)
- 1 vial of 10 ppb PAH Calibrator (actual concentration is 1.5 ppb pyrene)
- 1 vial of 100 ppb PAH Calibrator (actual concentration is 15 ppb pyrene)
- 1 vial of PAH-Enzyme Conjugate
- 1 vial of Substrate
- 1 vial of Stop Solution
- 1 test tube rack
- 22 Pipette Tips, pink (for the Gilson M-25 Microman[®] positive displacement pipette)

Materials Required but Not Provided

You will also need several items:

- Methanol for each water sample
- Pipette, 200 microliters (μL) (Gilson M-25 Microman positive displacement pipette)
- Eppendorf[™] Repeater[®] Pipettor and 3 Combitips[®] (2 x 12.5 milliliter (mL) and 1 x 5.0 mL)
- Suitable glass containers with teflon caps for collection, dilution and storage of water samples

- Differential Photometer
- Empty 12x75 mm test tube for blanking photometer
- Indelible marker for labeling test tubes
- Watch or timer
- Clean running water or a wash bottle containing tap or deionized water (500 mL)
- Calculator (optional)

Materials Suggested but Not Required

- Protective clothing (e.g., latex gloves)
- Absorbent paper for blotting test tubes
- Liquid and solid waste containers

Suggestions for Pipettor Use

1. Practice using both pipettors (positive displacement and Repeater pipettor) with water and extra tips before you analyze your samples.
2. Use a new tip each time you use the Repeater pipettor to avoid reagent cross-contamination. Label two, 12.5 mL tips "Substrate" and "Stop," and one, 5.0 mL tip for "Conjugate".
3. Draw the desired reagent volume into the Repeater pipettor and dispense one portion of the reagent back into the container to properly engage the ratchet mechanism. If you do not do this, the first volume delivered may be inaccurate.
4. To add reagents using the Repeater pipettor, pipette down the side of the tube just below the rim.
5. To add samples and calibrators using the positive displacement pipette, pipette down to the bottom of the test tube.
6. The carryover volume of the positive displacement tips is minimal, but may affect results if you are going from a high to low PAH concentration. Use a new pipette tip each time you pipette a new unknown.

Assay Procedure

Collect And Prepare the Sample

The following steps explain how to properly collect and store your samples.

1. Collect water in appropriately-sized, glass containers with teflon cap liners.
2. Immediately upon collection, the water samples should be diluted with methanol to prevent any adsorptive losses of PAH to the glass. The samples should be diluted with 1 part methanol to 3 parts sample (e.g. 25 mL methanol to 75 mL water sample).
3. Cap container tightly, and shake vigorously to mix. Storage of water samples should follow the holding conditions recommended by the EPA.

Perform the Test

NOTE: Allow all reagents to reach room temperature before you begin the test (at least 30 minutes). Do not analyze more than 20 test tubes at a time.

1. Remove the test tubes from the plastic bag and label the test tubes. You do not have to perform the assay in duplicate, however doing so increases the precision of the test.
2. Place the test tubes in the test tube rack. Push down on each tube so that it is held firmly and does not fall out of the rack when shaken.
3. Attach a clean pipette tip to the positive displacement pipette and adjust the dial to “**200**”. Add 200 μL of the negative control and each calibrator to the appropriate test tube by placing the end of the pipette tip at the bottom of the tube and dispensing the volume. Use a new pipette tip for each calibrator.
4. Using a clean pipette tip for each sample, add 200 μL of each diluted sample to the appropriate sample test tube(s). *The time taken for addition of all calibrators, and samples to all tubes (from the start of step 3 to this point) should not exceed 5 minutes.*
5. Incubate the test tubes for 15 minutes.

CAUTION: Replace the cap(s) on the calibrator vials immediately after use to prevent evaporative loss.

6. Vigorously shake out the test tube contents into a sink or suitable container. Fill the test tubes to overflowing with cool tap or distilled water, then decant and vigorously shake out the remaining water.
7. Repeat this wash step three more times, being certain to shake out as much water as possible on each wash. After the final wash, remove as much water as possible by tapping the inverted tubes on absorbent paper.
8. Attach the **5.0 mL** Combitip labeled “Conjugate” to the Repeater pipettor and set the dial to **2**. Add 200 μL PAH Enzyme-Conjugate to each test tube.
9. Incubate the test tubes for 5 minutes.
10. Vigorously shake out the test tube contents into a sink or suitable container. Fill the test tubes to overflowing with cool tap or distilled water, then decant and vigorously shake out the remaining water.
11. Repeat this wash step three more times, being certain to shake out as much water as possible on each wash. After the final wash, remove as much water as possible by tapping the inverted tubes on absorbent paper.
12. Attach the **12.5 mL** Combitip labeled “Substrate” to the Repeater pipettor and set the dial to **2**. Add 500 μL of Substrate to each test tube. Briefly shake the test tube rack to mix.
13. Incubate the test tubes for 5 minutes.

NOTE: If a blue color does not develop in the negative control test tube within 5 minutes after you add the substrate solution, the test is invalid and you must repeat the entire test.

14. Attach the **12.5 mL** Combitip labeled “Stop” to the Repeater pipettor and set the dial at **2**. Add 500 μL of Stop Solution to each test tube.

WARNING: Stop solution is 1.0 N hydro-chloric acid. Handle carefully.

15. Add 1.0 mL of Stop Solution to the blank test tube and insert the tube into the left well of the

differential photometer. Dry the outside of each assay tube and measure the absorbance [optical density (O.D.)] by placing each tube into the right well of the photometer. Record the absorbance of each tube.

Interpret the Results

The variability of individual PAH levels among environmental samples precludes quantitative interpretation of results from the EnviroGard PAH in Water Test Kit. However, the following table illustrates the semiquantitative interpretation of results for samples. Sample OD450 values are compared to the OD450 values obtained for the calibrators.

Actual OD values will vary. This data is for demonstration purposes only.

Tube	OD	Interpretation
Negative Control	1.381	
2.0 ppb Cal.*	1.063	
10 ppb Cal.	0.725	
100 ppb Cal.	0.299	
Sample 1	0.825	> 2 ppb, < 10 ppb
Sample 2	0.436	> 10 ppb, < 100 ppb
Sample 3	0.128	> 100 ppb

* Note that the sensitivity of the test does not reliably extend below 2 ppb.

Performance Characteristics

Sensitivity

The sensitivity is sufficient to perform the test at each calibrator level with 95% confidence. The minimum reliable detection limit for the EnviroGard PAH in Water Test Kit is 2.0 ppb in water. This is the lowest concentration of total PAH in water that is differentiated 95% of the time from zero.

Specificity

The test specificity is restricted to PAHs. The PAH antibody in this kit binds to different PAHs with different affinities. The following table gives the levels of pure compounds and various mixed products required to produce a positive response in the test at the 10 ppb decision level. This data is provided for illustrative purposes only and is not expected to be representative of real samples, which generally contain many of the compounds listed. The test response is a composite of individual responses to all of the PAHs present in that sample.

Compound	ppb Giving Positive Interpretation @ 10 ppb
Acenaphthene	>1000
Acenaphthylene	745
Anthracene	185
Benzo(a)anthracene	41
Benzo(b)fluoranthene	15
Benzo(k)fluoranthene	51
Benzo(ghi)perylene	36
Benzo(a)pyrene	4
Chrysene	41
Dibenz(ah)anthracene	>1000
Fluorene	337
Fluoranthene	5
Indeno(123cd)pyrene	7
Naphthalene	>1000
Phenanthrene	40
Pyrene	2

Limitations of the Procedure

The EnviroGard PAH in Water Test Kit is a screening test *only*. Actual quantitation of PAHs by the EnviroGard immunoassay is not possible because of variability in the relative amounts of the individual PAHs in environmental PAH samples.

Water sampling error may significantly affect testing. Split samples (e.g., for GC and immuno-assay) should always come from the same sample.

Ordering Information

Description	Catalogue Number
EnviroGard PAH in Water Test Kit	70620

Technical Assistance

To Place an Order or Receive Technical Assistance, please call Strategic Diagnostics Inc. at:

Call toll-free **800-544-8881**

Or 302-456-6789 Phone
 302-456-6782 Fax

General Limited Warranty

SDI's products are manufactured under strict quality control guidelines and are warranted to be free from defects in materials and workmanship. New instruments and related non-expendable items are warranted for one year from date of shipment against defective materials or workmanship under normal use and service.

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Safety

To receive complete safety information on this product, contact SDI Technical Support.

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