STRATEGIC DIAGNOSTICS INC.

EnviroGard™ Polynuclear Aromatic Hydrocarbons (PAH) in Soil Test Kit
7060600, EPA Method 4035

Intended Use

The EnviroGard PAH in Soil Test Kit is an enzyme immunoassay for the detection of a range of polycyclic aromatic hydrocarbons (PAHs) in soil, to include the 16 compounds listed under EPA SW-846 methods 8270, 8310 and 8100. The EnviroGard PAH Test Kit allows for reliable and rapid semiquantitative screening for total PAH at 1 or 10, and 100 parts per million (ppm) in soils. Other levels are possible using other dilution options. See "Limitations of Procedure" for discussion of sample heterogeneity issues. Samples can be screened with a 95% confidence of no false negatives at the specified action level.

Test Principles

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 section "Results Interpretation" and "Specificity" for more information on PAHs.

The EnviroGard PAH in Soil Test Kit employs an antibody against PAH that is coated onto 12 X 75 millimeter (mm) polystyrene test tubes. The method is based on the principles of competitive immunoassay, where the absorbance signal (optical density [OD]) of the final reaction mixture is inversely proportional to the concentration of analyte (PAH) present in the original sample. A soil sample that generates a signal greater than the signal of the PAH assay calibrator has a 95% probability of containing less PAH than the specified assay calibrator.

Test Principles

The EnviroGard PAH in Soil Test Kit is based on the use of polyclonal antibodies that bind either PAH or PAH-Enzyme Conjugate. These antibodies are immobilized on the walls of the test tubes. When PAH is present in the sample, it competes with the PAH-Enzyme Conjugate for a limited number of PAH binding sites on the immobilized antibodies.

- A sample containing PAH is added to a test tube. This is followed by the PAH-Enzyme Conjugate and the mixture is allowed to incubate 15 minutes. The PAH-Enzyme Conjugate competes with the PAH for the antibody binding sites.
- After this incubation, the unbound PAH-Enzyme Conjugate molecules are washed away.
- 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. One enzyme molecule can convert many Substrate molecules.

Since every test tube has the same number of antibody binding sites and 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 solution. Conversely, a high concentration of PAH allows fewer PAH-Enzyme Conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color development is inversely proportional to the 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.

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 Soil Test Kit is 1.0 ppm in soil. This is the lowest concentration of total PAH in soil 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 1 ppm decision level. These data are provided for illustrative purposes only and are 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. The calibrator levels are selected so that the levels of total PAH stated above will be detected with a maximum 5% false negative rate.

Compound	ppm Giving Positive Interpretation @ 1 ppm
Acenaphthene	3.7
Acenaphthylene	2.4
Anthracene	7.6
Benzo(a)anthracene	4.9
Benzo(b)fluoranthene	2.7
Benzo(k)fluoranthene	6.2
Benzo(ghi)perylene	5.3
Benzo(a)pyrene	0.8
Chrysene	4.3
Dibenz(ah)anthracene	356
Fluorene	3.4
Fluoranthene	0.3
Indeno(123cd)pyrene	6.5
Naphthalene	40
Phenanthrene	0.9
Pyrene	0.2
Other Compounds and Mixed Products	
1-methylnaphthalene	20
2-methylnaphthalene	33.3
Halowax 1001	10
Halowax 1013	10.5
Creosote	3.5
Diesel Fuel	75
Home Heating Oil	80
#2 Fuel Oil	150
#6 Fuel Oil	150
Bunker C Oil	125

Interfering Substances

The following substances were tested and found to have less than 0.5% weight-to-weight of the immunoreactivity of total PAH (more than 200 ppm required to give positive interpretation at 1 ppm).

Aroclor 1242
Aroclor 1248
Aroclor 1254
Aroclor 1260
biphenyl
gasoline
kerosene
pentachlorophenol (PCP)
crude oil
new motor oil
hydraulic oil
mineral oil
Halowax 1051
BTEX (benzene, toluene, ethylbenzene, xylenes)

Precautions

- Treat PAHs, solutions that contain PAHs, and potentially contaminated soil samples as hazardous materials.
- Use gloves, proper protective clothing, and methods 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. Storage at ambient temperature (18°C to 27°C or 64°F to 81°F) on the day of use is acceptable.
- Do not freeze test kit components or expose them to temperatures greater than 37°C (99°F).
- 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 1 hour to warm from recommended storage conditions.
- 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.
- Use approved methodologies to confirm any positive results.
- Soils obtained from areas adjacent to standing water, surface soils collected during or immediately after rain or snow, or any soils with relatively high amounts of water (= 30% by weight) should be dried before testing. Contact technical service for recommended methods.
- Distribution of PAHs in soils may be highly variable. This variability can be minimized through use of a composite sampling technique. Adequate sample number and distribution are the responsibility of the analyst.
- Portable spectrophotometer battery must be fully charged prior to use. It will not run directly off of AC current.
- Do not expose substrate to direct sunlight.
- Do not dilute or adulterate test reagents or use samples not called for in the test procedure; this may give inaccurate results.
- Tightly recap the PAH calibrator vials to prevent evaporative loss.

Materials Provided

- 20 Antibody coated test tubes, 12 X 75 mm
- 1 vial of Assay Diluent
- 1 vial of Negative Control (Methanol)
- 1 vial of PAH Calibrator 1 (actual concentration is 0.02 ppm pyrene in Methanol)
- 1 vial of PAH Calibrator 2 (actual concentration is 0.2 ppm pyrene in Methanol)
- 1 vial of PAH-Enzyme Conjugate
- 1 vial of Substrate
- 1 vial of Stop Solution
- 15 mL Methanol for dilution vials
- 20-Place test tube rack
- 16 empty Extract Dilution vials
- 36 Yellow (2-25 µL) Gilson Microman® positive displacement pipette tips
- 2 Pink (50-250 µL) Gilson Microman® positive displacement pipette tips

Materials Required and Ordered Separately

See "Ordering Information" for the appropriate catalogue numbers.

SDI Sample Extraction Kit

Use this kit for the extraction of PAH from soil samples. This kit contains enough devices to process 12 samples:

- 12 Extraction jars with screw caps, (each bottle contains 3 stainless steel mixing beads)
- 12 Filter modules (tops and bottoms)
- 12 Ampule crackers
- 12 Wooden spatulas
- 12 Weigh Canoes
- 12 Disposable Transfer Pipettes
- 12 Ampules containing 10 mL each of 100% Methanol

Ensys/Envirogard Field Soil Lab (Accessory Kit)

Accessory equipment may be rented or purchased from Strategic Diagnostics. See "Ordering Information" for the appropriate catalogue numbers.

The accessory kit contains the following items:

- Gilson M-25 Microman Positive Displacement Pipettor
- Eppendorf™ Repeater® Pipettor
- Electronic timer
- Polystyrene test tubes, 12 x 75 mm (for blanking spectrophotometer)
- Portable balance capable of weighing 10 g
- Wash bottle
- 5.0 mL Combitips® for the Repeater pipettor -for 0.1 mL to 0.5 mL dispensing volumes (3)
- 12.5 mL Combitips® for the Repeater pipettor -for 0.25 mL to 1.250 mL dispensing volumes (6)
- 50.0 mL Combitip® for the Repeater pipettor (with adapter)-for 1.0 mL to 5.0 mL dispensing volumes (1)
- Thirty position foam racks (2)
- Artel differential photometer - allows you to measure results in the form of optical density values. These values can be used for objective record keeping and quality assurance. It is included in the Ensys/Envirogard Field Soil Lab.

NOTE: Order replacement Combitips® and positive displacement tips separately. See the "Ordering Information" section.

Materials Required but Not Provided

- Protective clothing (e.g., latex gloves)
- Absorbent paper for blotting test tubes
- Liquid and solid waste containers
- Tap or distilled water for test tube washes
- Marking pen
- Calculator (optional)

Suggestions for Pipettor Use

- Practice using both pipettors (positive displacement and Repeater pipettor) with water and extra tips before you analyze your samples.
- Use a new tip each time you use the Repeater pipettor to pipette a different reagent to avoid reagent cross-contamination. Label three 12.5 mL tips "Diluent", "Substrate" and "Stop," and one 5.0 mL tip "Conjugate". Tips can be rinsed thoroughly in clean water and reused. By using the same tips to dispense the same reagent each time you can avoid cross contamination.
- 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.
- To add reagents using the Repeater pipettor, pipette down the side of the test tube just below the rim.
- When adding samples and calibrators using the positive displacement pipettor, always pipette below the liquid level. Pipet liquid up and down in tip to ensure complete volume transfer.
- 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 pipettor tip each time you pipette a new unknown.

Assay Procedure

Collect/Store the Sample

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

1. Collect soil in appropriately sized and labeled containers.
2. Take care to remove excess twigs, organic matter, and rocks or pebbles from the soil sample to be tested.
3. Soils obtained from areas adjacent to standing water, surface soils collected during or immediately after rain or snow, or any soils with relatively high amounts of water (= 30% by weight) should be dried before testing. Contact Technical Services for recommended methods.

Prepare the Sample/Extract the Soil

1. Please follow the instructions from the SDI Sample Extraction Kit to prepare the soil extract before the assay.
2. **10 mL** of **Methanol** will be used to extract PAH residue from a **10g** soil sample.

NOTE: When extracting clay samples, it is possible that the sample will soak up all of the methanol, leaving little or no excess liquid to filter. You should add an additional 10.0 mL of methanol

to the sample and shake vigorously for an additional 1–2 minutes. Make sure to factor the dilution into the calculations. See the "Results Interpretation" section.

Dilute the Extracts

NOTE: If you are testing only at 1 ppm, no dilutions are necessary. If you are testing at 10 and/or 100 ppm, use only the first dilution (steps 1-3 below). If you are testing at higher levels, then use the second dilution described in step 4. If you wish to test at other levels, see "Other Dilution Options" in the section "Results Interpretation".

1. Label the extract dilution vials or arrange in rack corresponding to the samples to be run.
2. With a positive displacement pipet and a clean tip, add 1 mL of methanol into the required number of dilution vials. This can be done in one of two ways:
   a. With the Gilson pipet: Use a pink tip and adjust the setting to "**250**" to dispense 250 µL four times for a total of 1 mL.
   b. With the Eppendorf Repeater Pipettor: Use a clean 12.5 mL pipet tip and adjust the dial to "**4**" to dispense 1 mL into each vial. Don't forget to dispense one time back into the Methanol bottle to prime the pipet.
3. Attach a clean yellow pipette tip to the positive displacement pipet and adjust the dial to **"100"** to pipet 10 µL. Remove 10 µL from the filtration unit, place it in a dilution vial. Fill and empty pipet tip 3 times to rinse. Cap and mix to give a 1:100 dilution.
4. Using the same pipet setting, remove 10 µL from the 1:100 dilution vial, place it in a new dilution vial, cap and mix to give a 1:10,000 dilution. If extra dilution vials from the kit are not available, use a clean glass test tube or vial to make the dilution.

Perform the Test

NOTE: Allow all test kit components to come to ambient temperature (at least 1 hour) before use.

1. Remove the Antibody coated test tubes from the foil pouch and label as follows (no more than 20 tubes/assay):

Tube Label	Tube Contents
NC	Negative Control
C1	Calibrator #1
C2	Calibrator #2
S1	Sample 1
S2	Sample 2
Etc.	

* To conserve reagents not all calibrators need to be run but you should always use the negative control and relevant calibrators for your action level. You do not have to perform the assay in duplicate; however, doing so increases the accuracy of the test.

2. Place the test tubes in the test tube rack pressing down firmly on each tube so that they are secured.

CAUTION: Do not "snap" the test tubes into the rack as this may result in a cracked tube.

3. Position the Repeater pipettor at Setting **2** and use the **12.5 mL** syringe to add **500 µL** of Assay Diluent to all test tubes.
4. Attach a clean yellow pipette tip to the positive displacement pipet and adjust the dial to **"100"** to pipet **10 µL**.
5. Use the positive displacement pipettor to add the Negative Control (methanol), the PAH Calibrators, and the Sample extracts and/or Sample extract dilutions to the appropriate test tubes. **Use a clean pipette tip for each addition.**

CAUTION: Replace the cap(s) on the calibrator vials immediately after use to minimize evaporation.

6. Position the Repeater pipettor at Setting **2** and use the **5 mL** syringe to add **200 µL** of the PAH enzyme-conjugate to all test tubes. **The time taken for addition of all calibrators, samples, and PAH enzyme-conjugate to all tubes (from the start of step 5 to this point) should not exceed 5 minutes.** Briefly shake the test tube rack to mix, then incubate for **15 minutes**.
7. 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.
8. 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.

9. Position the Repeater pipettor at Setting **2** and use a clean **12.5 mL** syringe to add **500 µL** of Substrate to all test tubes. Briefly shake the test tube rack to mix, then incubate for **5 minutes**.

   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.

10. Position the Repeater pipettor at Setting **2** and use a **12.5 mL** syringe to add **500 µL** of Stop Solution to all test tubes. This will turn the color from blue to yellow.

WARNING: **Stop solution is 1.0 N Hydrochloric acid. Handle carefully.**

11 1.0 mL of deionized water or Stop Solution to a blank test tube (from the accessory kit) and insert the tube into the left well of the spectrophotometer. Dry the outside of each assay tube and measure the absorbance by placing each tube into the right well of the spectrophotometer. Record the absorbance of each tube. Results should be read within 30 minutes of adding stop solution.

NOTE: Be careful not to mix plastic blanking tubes with the antibody coated tubes from the foil pouch in the test kit.

Results Interpretation

The variability of individual PAH levels among environmental samples precludes quantitative interpretation of results from the EnviroGard PAH in Soil Test Kit. However, the following chart illustrates the semiquantitative interpretation of results for the standard dilutions described in the "Dilute the Extracts" section above. Additional dilution options are given for testing at other levels. Sample OD_{450} values are compared to the OD_{450} values obtained for the calibrators. If sample was extracted with 20 mL of methanol rather than 10 mL, all ppm values below must be multiplied by 2 (e.g. <2.0 ppm interpretation for an otherwise undiluted sample between Calibrators 1 and 2).

Interpretation of Results for Standard Dilutions from "Dilute the Extracts" Section

Sample Dilution	OD_{450} above Cal. 1	OD_{450} between Cal. 1 & 2	OD_{450} below Cal. 2
Undiluted	*	<1.0	>1.0
1:100	<10	>10,<100	>100
1:10,000	<1000	>1000<10,000	>10,000

* Below detection level of the test kit. The sensitivity of the test does not reliably extend below 1 ppm

Interpretation of Results for Other Dilution Options

Sample Dilution (µL added to 1 mL methanol)	OD_{450} above Cal. 1	OD_{450} between Cal. 1 & 2	OD_{450} below Cal. 2
1:10 (111)	<1.0	>1, <10	>10
1:1000 (10 of 1:10 dilution)	<100	>100,<1000	>1000
1:50 (20)	<5	>5, <50	>50

Limitations of the Procedure

The EnviroGard PAH in Soil 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.

Soil sampling error may significantly affect testing reliability. The distribution of PAHs in different soils can be extremely heterogeneous. You should homogenize soils thoroughly before analysis by any method. Split samples (e.g., for GC and immunoassay) should always come from the same homogenate.

To ensure accurate and reliable results, you should make every effort to perform the EnviroGard PAH in Soil test at temperatures between 15°C (59°F) and 30°C (86°F).

Ordering Information

Description	Catalogue Number
EnviroGard PAH in Soil Test Kit	7060600
SDI Sample Extraction Kit (with methanol in ampules or bulk)	70606EA or 70606EB
Ensys/Envirogard Field Soil Lab (Accessory Kit) **	6050400
Differential Photometer (110V)	6000001
Differential Photometer (220V)	6000002
5 mL Combitip for Repeating Pipette (1 each)	6005200
12.5 mL Combitip for Repeating Pipette (1 each)	A00009
50 mL Combitip for Repeating Pipette (1 each)	6005600
Gilson Microman Positive Displacement Pipette Tips- yellow (200/bag)	6030500
Gilson Microman Positive Displacement Pipette Tips – pink (200/bag)	6030600
Ensys/Envirogard Field Soil Lab (Accessory Kit) Rental	6997020
**** To obtain part numbers and pricing for individual items in the Field Soil Lab contact SDI at the number below.**	

Ordering/Technical Assistance

Should you have any questions regarding this procedure prior to analysis contact Technical Service to avoid costly mistakes.

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
Web site: www.sdix.com
E-mail: techservice@sdix.com

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.
Warranty obligation is limited to repair or replacement of the defective product or to refund of the purchase price, at the discretion of SDI. Other warranties, express or implied, are disclaimed. SDI's liability under any warranty claim shall not exceed the refund of the purchase price paid by the customer. Under no circumstances shall SDI be liable for special, indirect or consequential damages.

Safety

To receive complete safety information on this product, visit our web site at www.sdix.com.

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Operation of the Repeater Pipet

To Set or Adjust Volume

To determine the pipetting volume, the dial setting (1-5) is multiplied by the minimum pipetting volume of the tip (indicated on the side of the Combitip, e.g. 1~100 uL.)

To Assemble Pipet Tip

Slide filling lever down until it stops. Then raise the locking clamp and insert the tip until it clicks into position. Be sure the tip plunger is fully inserted into the barrel before lowering the locking clamp to affix the tip in place.

To Fill Tip

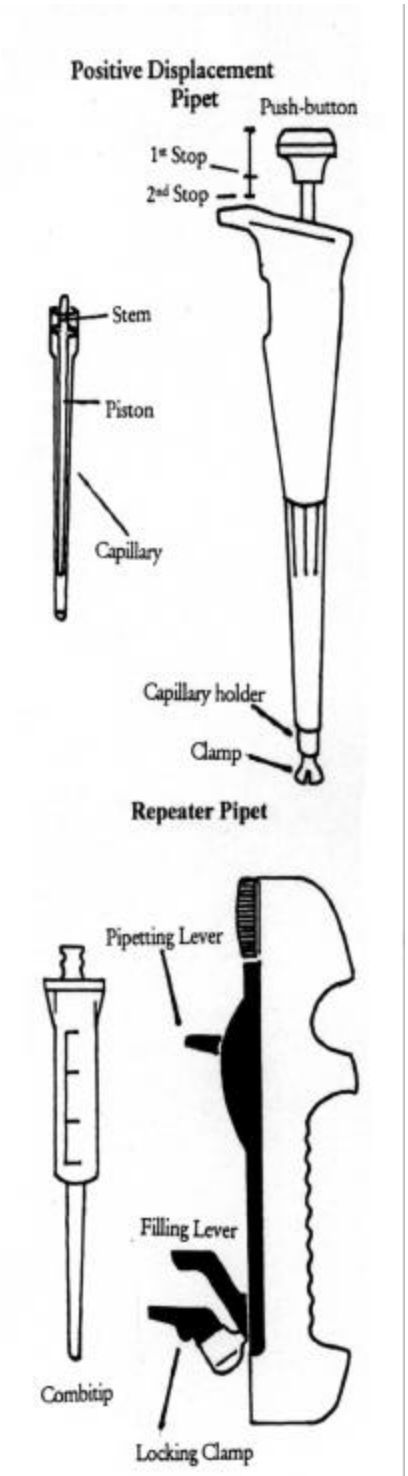
With tip mounted in position on pipet, immerse end of tip into solution. Slide filling lever upward slowly. Combitip will fill with liquid.

To Dispense Sample

Check the volume selection dial to ensure pipetting volume. Place tip inside test tube so that tip touches the inner wall of tube. Completely depress the pipetting lever to deliver sample. NOTE: Dispense one portion of reagent back into the container to engage the ratchet mechanism and ensure accuracy.

To Eject Tip

Empty tip of any remaining solution into appropriate container by pushing filling lever down. Raise locking clamp upward, and remove the Combitip.



Operation of the Positive Displacement Pipet

To Set or Adjust Volume

Turn lower part of push-button to adjust volume up or down. See kit instructions for appropriate setting.

To Assemble Pipet Tip

Press push button to 2nd stop to open clamp (see diagram, this is as far as push button will go down.) Select piston and slide stem fully into clamp. Slide mounted piston into capillary. Gently push capillary until it snaps onto capillary holder.

To Withdraw Sample

With tip mounted in position on pipet, press push-button to 1st stop and hold it. (If you push beyond the 1st stop tip will eject.) Place tip at bottom of liquid sample and slowly release push-button to withdraw measured sample. Ensure that no air bubbles exist in the pipette tip. If bubbles exist, dispense sample and re-withdraw.

To Dispense Sample

Wipe any liquid from outside of capillary taking care not to touch orifice. Place tip into dispensing vessel (immersing end of the tip if vessel contains liquid) and slowly press push-button to 1st stop. Pipet liquid up and down in tip to ensure complete transfer. Hold push-button at 1st stop when removing tip from vessel.

To Eject Tip

Press push-button to second stop. Tip (capillary and piston) is ejected.

NOTE: When using yellow tips on the positive displacement pipet, pipetting volumes range from 5-25 uL. (i.e. Pipet set on 2-5-0 will pipet 25 uL.)
When using pink tips on the positive displacement pipet, pipetting volumes range from 50-250 uL. (i.e. Pipet set on 2-5-0 will pipet 250 uL.)