STRATEGIC DIAGNOSTICS INC.

EnviroGard™ Lindane in Soil Test Kit

76300

Intended Use

The EnviroGard Lindane in Soil Test Kit is a semi-quantitative field test for the detection of Lindane in soil. The EnviroGard Lindane in Soil Test Kit allows reliable and rapid screening for Lindane at 0.4, 4.0, and 40.0 parts per million (ppm) in soil.

Test Principles

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

- A sample containing lindane is added to a test tube containing assay diluent. Lindane-enzyme conjugate is then added to the test tube. The lindane-enzyme conjugate competes with the lindane for the antibody binding sites.
- After the incubation, the unbound molecules are washed away.
- A clear solution of chromogenic substrate is then added to the test tube. In the presence of bound lindane-enzyme conjugate, the clear substrate is converted to a blue color. One enzyme molecule can convert many substrate molecules.

Since there are the same number of antibody binding sites on every test tube and each test tube receives the same number of lindane-enzyme conjugate molecules, a sample that contains a low concentration of lindane allows the antibody to bind many lindane-enzyme conjugate molecules. therefore, a low concentration of lindane produces a dark blue solution.

Conversely, a high concentration of lindane allows fewer lindane-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

Note: Color is inversely proportional to lindane concentration.

Darker color = Lower concentration
Lighter color = Higher concentration

Performance Characteristics

The EnviroGard Lindane in Soil Test Kit will not differentiate between Lindane and other structurally similar compounds, but will detect their presence to differing degrees. The following table shows a number of compounds and the approximate concentration of each required to yield a positive result (Lower Limit of Detection or LLD). It also shows the concentration required to inhibit one-half of the color developed by the Negative Control (IC50). Concentration is in parts per million (ppm) , or parts per billion (ppb) in soil.

Compound	LLD	IC50
Lindane*	0.4 ppm	4.0 ppm
Toxaphene	0.2 ppm	2.8 ppm
Endrin	6 ppb	22 ppb
Endosulfan I	6 ppb	36 ppb
Endosulfan II	6 ppb	28 ppb
Dieldrin	6 ppb	42 ppb
Heptachlor	6 ppb	34 ppb
Aldrin	20 ppb	116 ppb
Chlordane	14 ppb	100 ppb
Alpha- BHC	2 ppm	19 ppm
Delta-BHC	2 ppm	40 ppm

* Lindane is Gamma-BHC

Precautions

- Treat Lindane, solutions that contain Lindane and potentially contaminated soil samples as hazardous materials.
- Where appropriate, use gloves, proper protective clothing, and methods to contain and handle hazardous material.

- 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 expose **substrate** to **direct sunlight.**
- Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before beginning the test.
- 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.
- 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 Lindane calibrator vials to prevent evaporative loss.
- Distribution of Lindane in soils may be highly variable. The use of a composite sampling technique may be appropriate. Development of a sampling plan that assures adequate sample number and distribution is the responsibility of the analyst.

Materials Provided

EnviroGard Lindane in Soil Test Kit

This test kit contains the following items:

20 Antibody-Coated Test Tubes
1 vial of Assay Diluent
1 vial of Negative Control (methanol)
1 vial of 0.4 ppm Lindane Calibrator in methanol (actual concentration is 0.2 ppm)
1 vial of 4.0 ppm Lindane Calibrator in methanol (actual concentration is 2.0 ppm)
1 vial of 40.0 ppm Lindane Calibrator in methanol (actual concentration is 20.0 ppm)
1 vial of Lindane-Enzyme Conjugate
1 vial of Substrate
1 vial of Stop Solution
1 20-place Test Tube Rack
22 Pipette Tips, pink (for the Gilson M-25 Microman® Positive Displacement Pipettor)

NOTE: To determine the lindane concentration in soil, a dilution factor of **2** has been calculated in. This factor of **2** is derived from extraction of the 5 grams of soil with 10 mL of solvent.

Materials Required and Ordered Separately

See "Ordering Information" for the appropriate catalogue numbers.

EnviroGard Soil Extraction Bottle Kit

Use this kit for the extraction of Lindane in soil samples. This kit contains enough devices to process 14 samples:

- 14 30 milliliter (mL) LDPE Bottles with screw caps (each bottle contains stainless steel mixing beads)
- 14 filtration caps
- 14 Millex® HV_{13} filters
- 18 Wooden Spatulas
- 1 Syringe with coupler
- 1 Syringe coupler
- 14 Screw Top Glass Vials, 4.0 mL
- 14 Stoppers
- 18 Weigh Boats

Methanol

ACS reagent grade Methanol is required for soil extraction, but is not included in the EnviroGard Soil Extraction Bottle Kit. You must order it separately. (See "Ordering Information.")

Prepare a 90% Methanol extraction solvent by mixing 180 ml of Methanol with 20 ml of laboratory grade water and mix thoroughly before use.

Materials Required but Not Provided

You will also need several other items, some of which are included in the EnviroGard Soil Field Lab. (See "Ordering Information" for the appropriate catalogue number).

- Gilson M-25 Microman Positive Displacement Pipettor
- Eppendorf™ Repeater® Pipettor and five Combitips® (3 x 12.5 mL, 1 x 5.0 mL, and 1 x 50 mL)
- Balance capable of accurately weighing 5 grams
- Differential Photometer or Enviro-Quant 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)

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 avoid reagent cross-contamination. Label three 12.5 mL tips "Diluent", "Substrate" and "Stop," and one 5.0 mL tip "Conjugate".
- 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.
- To add samples and calibrators using the positive displacement pipettor, pipette down the side of the test tube just above the liquid level.
- The carryover volume of the positive displacement tips is minimal, but may affect results if you are going from a high to low Lindane concentration. Use a new pipettor tip each time you pipette a new unknown.

Assay Procedure

Collect/Store the Sample

1. Collect soil in appropriately-sized and labeled containers.
2. Take care to remove excess twigs, organic matter and rocks or pebbles from the sample. For best results, wet soils should be air-dried overnight and thoroughly mixed before testing.
3. Store soil samples at 4°C (39°F).

Prepare the Sample/Extract the Soil

1. Please follow the instructions from the EnviroGard Soil Extraction Bottle Kit to prepare the soil extract before the assay.
2. **10 ml** of **90% Methanol** in water will be used to extract lindane residue from a 5 gram soil sample. As per instructions, attach a **50 mL** Combitip to the Repeater pipettor and set the dial to **5**. Deliver twice to add **10 mL** of **90% methanol** to the extraction vial, and cap tightly.

Perform the Test

NOTE: Allow all reagents and sample extracts to reach room temperature (approximately 60 minutes) before you begin the test.

Remove the test tubes from the plastic bag and label them as follows*:

Tube Label	Tube Contents
NC	Negative Control
C1	0.4 ppm Calibrator
C2	4.0 ppm Calibrator
C3	40.0 ppm Calibrator
S1	sample 1
S2	sample 2
etc.	

* You are not required to perform the assay in duplicate; however, doing so will increase the precision.

1. 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.

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

2. Attach the **12.5 mL** Combitip labeled "Diluent" to the Repeater pipettor and adjust the dial to **1.** Add **250 microliters (µL)** of Assay Diluent to each test tube.
3. Attach a clean pink pipette tip to the Microman pipettor and adjust the dial to "**050"**. Add **50 µL** of each calibrator (including negative control) to the corresponding test tube by placing the end of the pipette tip against the side of the tube (just above the level of the Assay Diluent) and dispensing the volume. Use a clean pipette tip each time.

CAUTION: Replace the caps on the calibrator vials immediately after use to minimize evaporation.

4. Using a clean tip for each sample, add **50 µL** of each sample extract to the appropriately-labeled test tube.
5. Let test tubes incubate for **15 minutes.**
6. Attach the **5.0 mL** Combitip labeled "Conjugate" to the Repeater pipettor and adjust the dial to **2.** Add **200 µL** of Lindane-Enzyme Conjugate to each test tube.

   Shake the test tube rack to mix for 10 to 15 seconds. Leave the test tubes undisturbed for **5 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.

   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 absorbant paper.
8. 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. Leave the test tubes undisturbed for **3 minutes.**

   NOTE: If a blue color does not develop in the Negative Control test tube within 3 minutes after adding the Substrate, the test is invalid and you must repeat it.

Interpret the Results

You can either interpret the results visually within 3 minutes after adding the Substrate to each test tube, or you can perform a more precise analysis with a photometer after you add the Stop Solution.

Visual Interpretation

After you add the Substrate, wait 3 minutes then mix the test tubes by shaking them for a few seconds until they are a uniform blue color. Compare the sample test tube to the calibrator test tubes against a white background. The test tube rack in the kit is well-suited for this purpose.

- If a sample test tube contains *more* color than the calibrator test tube, the sample contains Lindane at a concentration *lower* than the calibrator.
- If a sample test tube contains *less* color than the calibrator test tube, the sample may contain Lindane at a concentration *greater* than the calibrator.
- If the sample test tube contains color that is between the calibrator test tubes, the sample contains Lindane at a concentration between the calibrator concentrations.
- If a sample test tube contains *approximately the same* amount of color as the calibrator test tube, the sample contains Lindane at a concentration *approximately equal* to the calibrator.
- If the sample test tube contains less color than the 40 ppm Calibrator test tube, you may dilute a fraction of the soil extract in 90% methanol (for example, 1:10) and perform the assay again. To determine the concentration of the diluted extract multiply the result by the dilution factor. (Go to "Semi-Quantitative Interpretation" for further details.)

Photometric Interpretation

After you add the Substrate, wait 3 minutes, then add the Stop Solution to each test tube.

WARNING: Stop solution is 1N Hydrochloric acid. Handle carefully.

Attach the **12.5 mL** Combitip labeled "Stop" to the Repeater pipettor and set the dial to **2**. Add 500 µL of Stop Solution to each test tube. This converts the blue color in the test tubes to yellow.

NOTE: After you add Stop Solution to the test tubes, results should be read within 30 minutes.

Differential Photometer

1. Place a water blank test tube containing 1.5 mL of Milli-RO® or Milli-Q® water, or equivalent in the left (reference) well.
2. Place the Negative Control test tube into the right (sample) well. Record the optical density (OD) of the Negative Control.
3. Remove the Negative Control test tube and replace it with the 0.4 ppm Calibrator test tube to reactivate the photometer. Record the result. Repeat this step to determine the OD for each of the remaining calibrators and for each sample.

Semi-quantitative Interpretation

Compare the OD of each sample to the OD of each calibrator:

- If a sample OD is *equal* to the OD of a calibrator, the sample contains Lindane at a concentration *approximately equal* to the calibrator.
- If a sample OD is *greater* than a calibrator OD, the sample contains *less* Lindane than the calibrator.
- If a sample OD is *lower* than a calibrator OD, the sample may contain *more* Lindane than that calibrator.
- If an assay result indicates that a soil sample contains greater than 40 ppm total Lindane, but you need more specific information, the soil extract may be diluted 1:10 in 90% methanol, and assayed again. You must then multiply the results of the re-assay by 10 to determine the approximate sample extract concentration.

Example Data

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

Tube	OD	Interpretation
NC	1.07	
C1 (0.4 ppm)	0.80	
C2 (4.0 ppm)	0.57	
C3 (40.0 ppm)	0.32	
S1	0.64	>0.4 ppm < 4 ppm
S2	0.16	> 40 ppm

NOTE: The EnviroQuant Photometer is also available. This dual wavelength instrument measures the OD at 450 nanometers (nm) minus 600 nm of all samples and calibrators, and provides a printout of results. See "Ordering Information" for the appropriate catalogue number.

Limitations of the Procedure

Soil sampling error may significantly affect testing reliability. The distribution of pesticides in different soils can be extremely heterogeneous. Soils should be dried and homogenized before analysis by any method. Split samples (i.e. for GC and immunoassay) should always derive from the same homogenate.

Ordering Information

Description	Catalogue Number
EnviroGard Lindane in Soil Test Kit	76300
EnviroGard Soil Extraction Bottle Kit	72010

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

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

3099510, Rev 6/10/97