STRATEGIC DIAGNOSTICS INC.

EnviroGard™ Chlordane in Soil Test Kit
7311000, EPA Method 4041

Intended Use

The EnviroGard Chlordane in Soil Test Kit is a semi-quantitative enzyme immunoassay for the detection of Chlordane in soil. The Envirogard Chlordane in Soil Test Kit allows reliable and rapid screening for Chlordane at 20, 100 and 600 parts per billion (ppb) in soil. Samples can be screened with a 95% confidence of no false negatives at the specified action level.

Test Principles

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

- A sample containing Chlordane is added to a test tube containing Assay Diluent. Chlordane-Enzyme Conjugate is then added to the test tube. The Chlordane-Enzyme Conjugate competes with the Chlordane 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 Chlordane-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 Chlordane-Enzyme Conjugate molecules, a sample that contains a low concentration of Chlordane allows the antibody to bind many Chlordane-Enzyme Conjugate molecules. Therefore, a low concentration of Chlordane produces a dark blue solution. Conversely, a high concentration of Chlordane allows fewer Chlordane-Enzyme Conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color development is inversely proportional to the Chlordane concentration.

Darker color = lower concentration
Lighter color = higher concentration

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

Performance Characteristics

The EnviroGard Chlordane in Soil Test Kit will not differentiate between Chlordane 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 at the low calibrator (Method Detection Limit or MDL). It also shows the concentration required to inhibit one-half of the color developed by the Negative Control (IC50). Concentration is in parts per billion (ppb) in soil.

Compound	MDL	IC50
Chlordane	20 ppb	100 ppb
Endrin	4.4 ppb	22 ppb
Endosulfan I	7.2 ppb	36 ppb
Endosulfan II	5.6 ppb	28 ppb
Dieldrin	8.4 ppb	42 ppb
Heptachlor	6.8 ppb	34 ppb
Aldrin	23.2 ppb	116 ppb
Toxaphene	560 ppb	2,800 ppb
Gamma-BHC *	920 ppb	4,600 ppb
Alpha- BHC	3,800 ppb	19,000 ppb
Delta-BHC	8,000 ppb	40,000 ppb

*Gamma-BHC is Lindane

Precautions

- Treat Chlordane, solutions that contain Chlordane, 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 Chlordane 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 Chlordane 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 20 ppb Chlordane Calibrator in methanol (actual concentration is 10 ppb)
- 1 vial of 100 ppb Chlordane Calibrator in methanol (actual concentration is 50 ppb)
- 1 vial of 600 ppb Chlordane Calibrator in methanol (actual concentration is 300 ppb)
- 1 vial of Chlordane-Enzyme Conjugate
- 1 vial of Substrate
- 1 vial of Stop Solution
- 1 20-Place test tube rack
- 22 Pink (50-250 µL) Gilson Microman® positive displacement pipette tips

NOTE: To determine the Chlordane concentration in soil, a dilution factor of **2** has been calculated in. This factor of **2** is derived from the extraction of the 10 grams of soil with 20 mL of solvent.

Materials Required and Ordered Separately

See "Ordering Information" for the appropriate catalogue numbers.

SDI Sample Extraction Kit

Use this kit for the extraction of Chlordane 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 20 mL each of 90% 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 tip to dispense the same reagent each time, you can avoid reagent 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 Chlordane 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.

4. Store soil samples at 4°C (39°F).

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. **20 mL** of **90 % Methanol** will be used to extract Chlordane residues from a **10 g** soil sample.

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	20 ppb Calibrator
C2	100 ppb Calibrator
C3	600 ppb Calibrator
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 the 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 **1** and use the **12.5 mL** syringe to add **250 µL** of Assay Diluent to all test tubes.
4. Attach a clean pink pipette tip to the positive displacement pipet and adjust the dial to **"050"** to pipet **50 µL**.
5. Use the positive displacement pipettor to add the Negative Control (methanol), the Chlordane Calibrators, and the Sample extracts 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. Let tubes incubate for **15 minutes**.
7. Attach the **5.0 mL** Combitip labeled "Conjugate" to the Repeater pipettor and adjust the dial to **2** to add **200** m**L** of Chlordane-Enzyme Conjugate to each tube.
8. Gently shake the test tube rack to mix to 10 to 15 seconds. Leave the tubes undisturbed for **5 minutes.**
9. 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 absorbent paper.
10. Position the Repeater pipettor at Setting **2** and use a clean **12.5 mL** Combitip to add **500 µL** of Substrate to all test tubes. Briefly shake the test tube rack to mix, then incubate for **3 minutes**.
11. If a blue color does not develop in the negative control test tube within 3 minutes after you add the substrate solution, the test is invalid and you must repeat the entire test.
12. 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.**

Results Interpretation

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. 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 Chlordane at a concentration *lower* than the calibrator.
- If a sample test tube contains *less* color than the calibrator test tube, the sample may contain Chlordane at a concentration *greater* than the calibrator.
- If the sample test tube contains color that is between the calibrator test tubes, the sample contains Chlordane 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 Chlordane at a concentration *approximately equal* to the calibrator.
- If the sample test tube contains less color than the 600 ppb 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 "Photometric Interpretation" for further details.)

Photometric Interpretation

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

1. Dry the outside of all assay tubes prior to photometric analysis.
2. Place a blank test tube (from the EnSys/EnviroGard Field Accessory Kit) containing 1.5 mL of deionized water or equivalent in the left (reference) well of the differential photometer.

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

3. Place the Negative Control test tube into the right (sample) well. Record the optical density (OD) of the Negative Control.
4. Remove the Negative Control test tube and replace it with the 20 ppb 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.

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 Chlordane at a concentration *approximately equal* to the calibrator.
- If a sample OD is *greater* than a calibrator OD, the sample contains *less* Chlordane than the calibrator.
- If a sample OD is *lower* than a calibrator OD, the sample may contain *more* Chlordane than that calibrator.
- If an assay result indicates that a soil sample contains greater than 600 ppb total Chlordane, 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.

NOTE: If you know in advance that the "action level" of interest is greater than 600 ppb total Chlordane in soil, the assay may be modified to pinpoint that particular concentration.

Example Data

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

Tube	OD	Interpretation
NC	0.90	
C1 (20 ppb)	0.65	
C2 (100 ppb)	0.49	
C3 (600 ppb)	0.35	
S1	0.58	>20 ppb < 100 ppb
S2	0.16	> 600 ppb

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 Chlordane in Soil Test Kit	7311000
SDI Sample Extraction Kit (with methanol in ampules)	73110EA
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 and 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

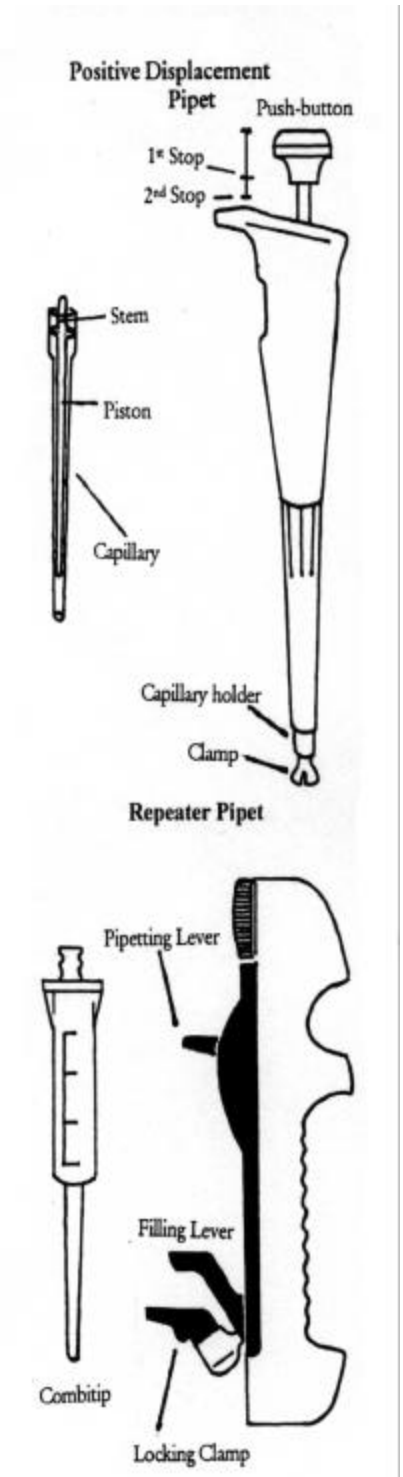
With tip mounted in position on pipet, immerse end of tip into solution. Slide filling lever upward slowly. Combitip will fill with liquid.

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.)