

### Prepare the Sample/Extract the Soil

1. Please follow the instructions from the Soil Extraction Kit to prepare the soil extract before the assay.
2. 10 mL of Methanol will be used to extract Toxaphene residue from a 5 gram soil sample.

### Perform the Test

1. Label the 12 mm X 75 mm test tubes (no more than 20 tubes/assay). 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 pressing down firmly on each tube so that they are secured.
3. Pipette 500  $\mu$ L of Assay Diluent to all test tubes.
4. Attach a clean pipette tip to the positive displacement pipette and adjust the dial to "200" to pipette 20  $\mu$ L.
5. Use the positive displacement pipettor to add the Negative Control (methanol), the toxaphene calibrators, and the Sample extracts and/or Sample extract dilutions to the appropriate test tubes. Replace the cap(s) to minimize evaporation.
6. Pipette 500  $\mu$ L of enzyme conjugate into each tube.
7. Shake the rack to mix the contents of the tubes and allow the tubes to incubate for 10 minutes.
8. Empty 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.
9. 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. Pipette 500  $\mu$ L of Substrate into each tube.
11. Allow the color development to proceed for 10 minutes.
12. Pipet 500  $\mu$ L of Stop Solution into each tube.

WARNING: Stop solution is 1.0 N hydrochloric acid. Handle carefully.

13. Add 1.0 mL of Stop Solution to the blank test tube and insert the tube into the Pocket Colorimeter<sup>TM</sup>II. Press the blue zero key. Dry the outside of each assay tube and measure the absorbance by placing each tube into the photometer. Press the green "check" key. Record the absorbance of each tube.

### RESULTS INTERPRETATION

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator tubes: Samples containing less color than a calibrator well have a concentration of Toxaphene greater than the concentration of the calibrator. Samples containing more color than a calibrator well have a concentration less than the concentration of the calibrator.
2. Quantitative interpretation requires graphing the absorbances of the calibrators (Y-axis) versus the log of the calibrator concentration (X-axis) on semi-log graph paper. A straight line is drawn through the calibrator points and the sample absorbances are located on the line. The corresponding point on the X-axis is the concentration of the sample. Samples

with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as <2 ppm or >50 ppm, respectively. A spreadsheet that will perform the curve fit and sample calculations can be provided upon request.

### QUALITY CONTROL

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.

### TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302.

### SAFETY

To receive complete safety information on this product, contact Beacon Analytical Systems, Inc. and request Material Safety Data Sheets. Stop Solution is 1N hydrochloric acid. Handle with care.

### General Limited Warranty

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## Toxaphene Tube Kit

Cat.# 20-0148

Instructional Booklet

READ COMPLETELY BEFORE USE.

### INTENDED USE

The Toxaphene in Soil Test Kit is an enzyme immunoassay for the detection of toxaphene in soil. The Toxaphene Test Kit permits reliable and rapid semi-quantitative screening for toxaphene at a range of 0 to 50 parts per million (ppm) in soils.

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## ASSAY PRINCIPLES

The Toxaphene in Soil Test Kit is a competitive enzyme-labeled immunoassay. The test kit uses polyclonal antibodies that bind either Toxaphene or Toxaphene-enzyme conjugate. When Toxaphene is present in the sample, it competes with the Toxaphene-enzyme conjugate for a limited number of toxaphene binding sites on the immobilized antibodies.

An assay diluent and a sample containing Toxaphene is added to a test tube. This is followed by the addition of Toxaphene-enzyme conjugate and the mixture is allowed to incubate for 10 minutes. The Toxaphene-enzyme conjugate competes with the Toxaphene for the antibody binding sites. In the assay procedure you will:

- Add a sample or calibrator containing Toxaphene to a test tube.
- Add Toxaphene enzyme conjugate. The conjugate competes with any Toxaphene in the sample for the same antibody binding sites.
- Wash away any unbound molecules, after you incubate this mixture for 10 minutes.
- Add clear substrate solution to each test tube. In the presence of bound Toxaphene -enzyme conjugate, the substrate is converted to a blue compound. One enzyme molecule can convert many substrate molecules.

Since the same number of antibody binding sites are available in every test tube, and each tube receives the same number of Toxaphene-enzyme conjugate molecules, a sample containing a low concentration of Toxaphene allows the antibody to bind many Toxaphene -enzyme conjugate molecules. The result is a dark blue solution. Conversely, a high concentration of Toxaphene allows fewer Toxaphene-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color is inversely proportional to Toxaphene concentration.

Darker color = Lower concentration

Lighter color = Higher concentration

Soil sampling error may significantly affect testing reliability. The distribution of Toxaphenes 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 Toxaphene in Soil test at temperatures between 15°C (59°F) and 30°C (86°F).

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## 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 Toxaphene is 2 ppm in soil.

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## SPECIFICITY

The Beacon Toxaphene Tube Kit is specific for Toxaphene and closely related compounds. The following table shows the % cross-reactivity versus toxaphene.

Compound	% Cross Reactivity
Toxaphene	100%
Heptachlor	< 1%
Chlordane	< 1 %
Dieldrin	< 1%
Endin	< 1%
Endosulfan	< 1%
Lindane	60%

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## PRECAUTIONS

1. Each reagent is optimized for use in the Toxaphene Tube Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Toxaphene Tube Kits with different lot numbers.
2. Dilution or adulteration of reagents or samples not called for in the procedure may produce inaccurate results.
3. Do not use reagents after expiration date.
4. Store all test kit components at 4 degrees Celsius (°C) to 8°C (39 degrees Fahrenheit (°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.
5. Reagents should be brought to room temperature, 20 – 28°C (62 – 82°F) prior to use. Avoid prolonged (> 24 hours) storage at room temperature. Do not freeze test kit components or expose them to temperatures greater than 37°C (99°F).
6. The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.
7. Treat Toxaphene, solutions that contain Toxaphene, and potentially contaminated soil samples as hazardous materials. Use gloves, proper protective clothing, and means to contain and handle hazardous material where appropriate.
8. Use approved methodologies to confirm any positive results.
9. 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.
10. Distribution of Toxaphene 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.

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## REAGENTS AND MATERIALS PROVIDED

The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 – 8°C.

- 40 Toxaphene antibody-coated, 12 mm X 75 mm polystyrene test tubes in 2 foil bags.
- 1 vial containing 3 mL of Negative Control (Methanol) .
- 3 vials containing 2 mL of Toxaphene calibrators at 2, 10 and 50ppm. (Note: Because of the 1:2 dilution of the soil sample in the extraction step, the calibrators actually contain 1/2 of the stated value. No further correction back to the concentration in the original soil sample is required.)
- 1 bottle Toxaphene-enzyme conjugate
- 1 bottle of Assay Diluent
- 1 bottle of Substrate.
- 1 bottle of Stop Solution. (Caution! 1N HCl. Handle with care.)

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## MATERIALS REQUIRED BUT NOT PROVIDED

- Photometer for reading absorbance at 450nm in 12mm x 75mm tubes. The photometer allows you to measure results in the form of optical density values. These values can be used for objective record keeping, quality assurance, or semi-quantitative determination of sample concentrations.
- Soil Extraction Kit- The soil extraction kit provides all additional materials required for the extraction of soil samples.
- Methanol (10 mL per sample) is required for soil extractions.
- A pipette capable of delivering 10 mL for addition of methanol to soil samples for extraction.
- Clean running water or a wash bottle containing tap or deionized water.
- Pipet with disposable tips capable of dispensing 500 µL.
- Positive displacement pipet with disposable tips capable of delivering 20 µL.
- Paper towels or equivalent absorbent material.
- Timer
- A repeating pipette capable of delivering variable volumes is recommended for running more than 10 tubes in one assay.

**TEST PROCEDURE** (Note: Running calibrators and samples in duplicate will improve assay precision and accuracy.)

### Collect/Store the Sample

1. Collect soil in appropriately sized and labeled containers. Take care to remove excess twigs, organic matter, and rocks or pebbles from the soil sample to be tested.
2. 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.

NOTE: Contact technical service for recommended methods.

3. Storage of soil samples should follow the holding conditions recommended for EPA methods 8100 and 8310 (respectively