

# STRATEGIC DIAGNOSTICS INC.

## EnviroGard® Chlorpyrifos Plate Kit

7250000

### Intended Use

The EnviroGard Chlorpyrifos Plate Kit is a quantitative laboratory test for the detection of chlorpyrifos-ethyl in water. The kit can be calibrated with chlorpyrifos-ethyl.

\*%B<sub>0</sub> equals the average optical density (OD) of the calibrator or sample, divided by the average OD of the negative control, multiplied by 100 (see "Calculate the Results").

### Test Principles

The EnviroGard Chlorpyrifos Plate Kit uses polyclonal antibodies, which bind either chlorpyrifos or a chlorpyrifos-enzyme conjugate. These antibodies are immobilized to the walls of the test wells. Chlorpyrifos in the sample competes with the chlorpyrifos-enzyme conjugate for a limited number of antibody binding sites.

Since the same number of antibody binding sites are available on every test well, and each test well receives the same number of chlorpyrifos-enzyme conjugate molecules, a sample which contains a low concentration of chlorpyrifos allows the antibody to bind many chlorpyrifos-enzyme conjugate molecules. The result is a dark blue solution.

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

**NOTE:** Color is inversely proportional to chlorpyrifos concentration.

**Darker color = lower concentration.**

**Lighter color = higher concentration.**

### Performance Characteristics

The EnviroGard Chlorpyrifos Plate Kit does not differentiate between chlorpyrifos and other closely related compounds, but detects their presence to differing degrees. The following chart shows the concentration yielding 50% B<sub>0</sub>\* and the approximate concentration of the lower limit of detection (LLD) for a number of compounds. Concentration is in parts per billion (ppb).

Compound	50% B <sub>0</sub>	LLD
Chlorpyrifos-Ethyl	0.3	0.05
Chlorpyrifos-Methyl	0.05	0.02
3,5,6-Trichloro-2-Pyridinol	>1000	100
Azinphos	293	10
Azinphos-Methyl	715	82
Bromophos	3	0.5
Bromophos-Methyl	0.4	0.07
Fenchlorphos	0.6	0.01
Fenitrothion	450	40
Parathion-Methyl	522	50
Picloram	>1000	1000
Quinalfos	200	20
Triazophos	30	2
Triclopyr	48	3

## Precautions

- Store all plate kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not store test kit components for more than 8 hours at ambient temperatures (20°C to 37°C or 68°F to 99°F).
- Do not freeze plate 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.
- Do not use plate kit components after the expiration date.
- Do not use reagents or test well strips from one plate kit with reagents or test well strips from a different plate kit.
- Do not dilute or adulterate test reagents or use samples not called for in the test procedure.
- Aqueous solutions of chlorpyrifos may stick to plastics and are destroyed by alkaline conditions. Therefore, collect all samples and prepare all calibrators in glassware that has been rinsed free of all alkaline detergent residues.
- Store chlorpyrifos samples and solutions in glass containers. Do not store them in plastic.
- Tightly recap the Chlorpyrifos Stock Solutions to prevent evaporative loss.
- Use approved methodologies to confirm results.
- Do not expose **substrate** to **direct sunlight**.

## Materials Provided

The EnviroGard Chlorpyrifos Plate Kit contains the following:

- 8 antibody-coated strips (12 wells each), in strip holder
- 1 vial of 1.0 part per million (ppm) Chlorpyrifos-ethyl Stock Solution
- 1 vial of Chlorpyrifos-Enzyme Conjugate
- 1 vial of Substrate
- 1 vial of Stop Solution (Caution! 1.0N Hydrochloric Acid)

## Materials You Provide

You also need these items:

- 100 milliliter (mL) glass volumetric flask
- Positive-displacement pipette and tips that will measure 10 microliters (µL)
- Disposable-tip pipette and disposable tips that will measure 100 µL
- Pipettes that will measure 0.5 mL, 2.0 mL, 3.0 mL, 5.0 mL, 7.0 mL, 8.0 mL, 9.5 mL and 10 mL.
- Deionized water (or equivalent) for calibrator preparation
- **Glass** tubes or vials for calibrator preparation.  
**Caution: Do not use plastic.**
- marking pen (indelible)

- tape or Parafilm®
- timer (1 hour and 30 minutes)
- tap or distilled water for rinsing wells
- microtiter plate reader or strip reader
- a multi-channel pipette (optional)
- microtiter plate washer (optional)
- orbital shaker (optional)
- calculator (optional)

## Sample Collection and Storage

Chlorpyrifos breaks down under alkaline conditions. If your samples are not stored frozen or analyzed immediately after collection the samples should be buffered to a neutral or slightly acidic pH.

## Prepare the Calibrators

The EnviroGard Chlorpyrifos Plate Kit contains a 1.0 ppm Stock Solution of chlorpyrifos-ethyl in methanol/acetic acid. The kit can be calibrated with chlorpyrifos-ethyl.

**CAUTION:** Do not use these Stock Solutions directly in the assay.

The Stock Solutions must be diluted in laboratory-grade water. Use deionized water (or the equivalent) as a Negative Control in each assay.

## Chlorpyrifos-ethyl Calibrators

1. Make sure the 1.0 ppm Chlorpyrifos-ethyl Stock Solution is at room temperature. Gently swirl the vial to mix before pipetting.
2. Prepare a 1.0 ppb calibrator by using a positive-displacement pipette to pipette 10 µL Chlorpyrifos-ethyl Stock Solution into 10 mL deionized water (or the equivalent).
3. Prepare a 0.3 ppb calibrator by mixing 3.0 mL of the 1.0 ppb calibrator with 7.0 mL of water.
4. Prepare a 0.05 ppb calibrator by mixing 0.5 mL of the 1.0 ppb calibrator with 9.5 mL of water.

**CAUTION:** Accurate pipetting of the Chlorpyrifos-ethyl Stock Solution and thorough mixing of the calibrator solutions are critical to the performance of this assay. These aqueous calibrators are unstable and should be prepared immediately before use.

## Assay Procedure for the Detection of Chlorpyrifos in Water

The raised markings on the strip holder identify the well location as you add the reagents and samples.

1. Two strips may be used to run the Negative Control, three Calibrators and eight samples in duplicate. For example (using Chlorpyrifos-ethyl calibrators):

- Negative Control (C) = deionized water
- Calibrator 1 (C1) = 0.05 ppb calibrator
- Calibrator 2 (C2) = 0.3 ppb calibrator
- Calibrator 3 (C3) = 1.0 ppb calibrator
- Samples (S1, S2, S3, S4, S5, S6, S7 and S8)

	1	2	3	4	5	6	7	8	9	10	11	12
A	C	C	C1	C1	C2	C2	C3	C3	S1	S1	S2	S2
B	S3	S3	S4	S4	S5	S5	S6	S6	S7	S7	S8	S8
C												
D												
E												
F												
G												
H												

NOTE: When you use fewer than eight strips, remove the unneeded strips and store them at 4°C to 8°C in the re-sealable plastic bag (with desiccant) provided.

2. Add 100 µL of Negative Control (C), each calibrator (C1 to C3), and each sample (S1 to S8) to their respective wells, as shown above.
3. Add 100 µL of Chlorpyrifos Enzyme Conjugate to each well in the same order of addition as calibrators and samples.

NOTE: If you are running more than three strips, you should use a multi-channel pipette in steps 2, 3, 7 and 9.

4. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill the contents.
5. Cover the wells with tape or Parafilm to prevent evaporation and incubate at ambient temperature for 1 hour. During incubation, orbital mixing at 200 revolutions per minute (rpm) is recommended, but not mandatory.
6. After incubation, carefully remove the covering and vigorously shake the contents of the wells into a sink. Flood the wells completely with cool running tap or distilled water, then shake to empty. Repeat this wash step five times. Invert the plate and tap out as much water as possible. Alternatively, use a microtiter plate washer for the wash steps.
7. Add 100 µL of Substrate to each well, beginning with the Negative Control (C) and calibrators (C1 to C3), then the samples (S1 to S8).
8. Cover the wells with new tape or Parafilm and incubate at ambient temperature for 30 minutes. During incubation, orbital mixing at 200 rpm is preferable, but not mandatory.

**WARNING:** Stop Solution is 1N Hydrochloric acid.

9. Add 100 µL of Stop Solution to each well and mix thoroughly. The solution will turn yellow.

NOTE: Read the plate within 30 minutes of adding the Stop Solution.

**INTERPRET THE RESULTS**

Spectrophotometric Measurement and Analysis

1. Adjust the wavelength of your microtiter plate reader to 450 nanometers (nm). (If it has dual wavelength capability, use 600 or 650 nm as the "reference" wavelength.)
2. If the plate reader does not auto-zero on air, zero it against 200 µL water in a blank well, then measure & record the optical density (OD) of each well's contents. Or, measure & record the OD in every well, then subtract the OD of the water blank from each of the readings.
3. If the microtiter plate reader you are using has data reduction capabilities, use a semi-log curve fit for the standard curve. You can also calculate the results manually as described in the next section.

#### Calculate the Results

1. After you read all of the wells, average the OD of each set of calibrators and samples, and calculate the %B° as follows:

$$\%B^{\circ} = \frac{\text{average OD of calibrator or sample}}{\text{average OD of negative control}} \times 100$$

The %Bo calculation is used as a means of equalizing different runs of an assay. While the raw OD readings of negative controls, calibrators, and samples are likely to differ from run to run, the %Bo relationship of calibrators and samples to the Negative Control should remain fairly constant.

2. Graph the %Bo of each Calibrator against its Chlorpyrifos concentration on a semi-log scale (see "Sample Calculations").
3. Determine the Chlorpyrifos concentration of each sample by finding its %Bo value and the corresponding concentration level on the graph.
4. Interpolation of sample concentration is only valid if the %Bo of the sample falls within the range of the %Bo's set by the calibrators. If the %Bo of a sample is lower than that of the highest calibrator, dilute that sample so it falls on the standard curve when you run the assay again, then multiply by the dilution factor.

#### Ordering Information

Description	Catalog Number
EnviroGard Chlorpyrifos Plate Kit	7250000

#### 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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