STRATEGIC DIAGNOSTICS INC.
EnviroGard® Diazinon Plate Kit
7270000

Intended Use
The EnviroGard Diazinon Plate Kit is a quantitative laboratory test for the detection of diazinon residues in water.

Test Principles
The EnviroGard Diazinon Plate Kit is based on the use of polyclonal antibodies which bind either diazinon or a Diazinon-Enzyme Conjugate. These antibodies are immobilized to the walls of the test wells. When diazinon is present in the sample, it competes with Diazinon-Enzyme Conjugate for a limited number of antibody binding sites.

Since there are the same number of antibody binding sites on every test well, and each test well receives the same number of Diazinon-Enzyme Conjugate molecules, a sample which contains a low concentration of diazinon allows the antibody to bind many Diazinon-Enzyme Conjugate molecules. Therefore, a low concentration of diazinon will produce a dark blue solution. Conversely, a high concentration of diazinon will allow fewer Diazinon-Enzyme Conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

Note: Color is inversely proportional to diazinon concentration.

Darker color = lower concentration.
Lighter color = higher concentration.

Performance Characteristics
The EnviroGard Diazinon Plate Kit is very specific for diazinon and is essentially non-reactive, with the exception of pirimiphos-ethyl and pirimiphos-methyl, to closely-related organophosphate compounds and other pesticides. The following chart shows the concentration yielding 50% B_O* and the approximate concentration yielding 85% B_O, which is the Lower Limit of Detection (LLD), for a number of compounds. Concentrations are in parts per trillion (ppt) or parts per billion (ppb).

Compound	50% B_O*	LLD (85% B_O)
Diazinon	100 ppt	22 ppt
Diazoxon	900 ppt	200 ppt
Pirimiphos-ethyl	700 ppb	125 ppb
Pirimiphos-methyl	= 5000 ppb	900 ppb

* % Bo = 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".)

The following compounds are not detected at 1000 ppb:

Etrimfos	Fenitrothion	DDT
Fensulfothion	Fenchlorphos	Diuron
Bromophos	Bromophos-Methyl	
Tetrachlorvinphos	Parathion	
Methyl-Parathion	Paraoxon	
Chlorpyrifos	Chlorpyrifos-Methyl	
Azinphos-Methyl	Fenamiphos	
Methidathion	Dicapthon	
Temephos	Cythioate	
Atrazine	Simazine	
Chlorthal	Dieldrin	
Molinate	Diazinon Hydroxypyrimidine Metabolite	

Precautions
• Store all plate kit components at 4°C to 8°C (39°F to 46°F) when not in use.
• 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 expose substrate to direct sunlight.
• 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.
• Tightly re-cap the Diazinon Stock Solution after use to avoid evaporative losses.
• Use approved methodologies to confirm any positive results.
• Some solutes and particulates found in un-treated ground or surface waters may affect the sensitivity level of this plate kit.
• Aqueous solutions of diazinon are affected by acidic conditions. Collect all samples and prepare all calibrators in glassware that has been rinsed free of all acidic detergent residues.

Materials Provided
You should have the following items in your plate kit:

8 antibody-coated strips (12 wells each), in strip holder
1 vial of 100 nanogram/milliliter (ng/mL) Diazinon Stock Solution
1 vial of Diazinon-Enzyme Conjugate
1 vial of Substrate
1 vial of Stop Solution

Materials Required - Not Provided
You will also need these other items:
- 10 mL volumetric flask
- positive-displacement pipette which will measure 50 microliters (µL)
- disposable-tip pipette which will measure 100 µL
- pipette(s) which will measure 0.3, 1.0, 4.0 and 4.7 mL
- deionized water for calibrator preparation
- glass tubes or vials for calibrator preparation
- marking pen
- tape or Parafilm®
- timer (1 hour and 30 minutes)
- tap or distilled water for rinsing wells
- orbital shaker (optional)
- microtiter plate reader or strip reader
- calculator which performs linear regression (optional)
- microtiter plate washer (optional)

- a multi-channel pipette (optional)
- a repeater pipette (optional)

Prepare The Calibrators

The EnviroGard Diazinon Plate Kit contains a 100 ng/mL (100 ppb) Stock Solution of Diazinon in methanol. ***Do not use the stock solution directly in the assay.*** This Stock Solution **must** be diluted in laboratory grade (deionized) water in order to prepare 30, 100 and 500 ppt calibrators.

Note: Accurate pipetting of the Stock Solution and thorough mixing of the calibrator solutions are critical to the performance of this assay.

1. Be certain that the 100 ng/mL Diazinon Stock Solution is at room temperature. Gently swirl the vial to mix before pipetting.

2. Prepare the 500 ppt calibrator by pipetting 50 µL Diazinon Stock Solution into a 10 mL volumetric flask (use a positive-displacement pipette to measure the Stock Solution). Bring it to volume with deionized water (or the equivalent).

3. Prepare the 100 ppt calibrator by mixing 1.0 mL of the 500 ppt calibrator with 4.0 mL water.

4. Prepare the 30 ppt calibrator by mixing 0.3 mL of the 500 ppt calibrator with 4.7 mL water.

5. Deionized water (or the equivalent) alone will be used as the Negative Control.

Note: These aqueous calibrators may be unstable and should be prepared fresh just prior to use.

Assay Procedure

The raised markings on the strip holder identify the well location while you add the reagents and samples. To add the calibrators, samples, Conjugate, Substrate, and Stop Solution, a 100 µL pipette must be used.

1. Two strips may be used to run the Negative Control , three calibrators , and eight samples in duplicate. For example:

   Negative Control (C)
   Calibrator 1 (C1) = 30 ppt
   Calibrator 2 (C2) = 100 ppt
   Calibrator 3 (C3) = 500 ppt
   Samples (S1, S2, S3, etc.)

	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 (39°F to 46°F) in the re-sealable plastic bag (with desiccant) provided.

2. Add 100 µL of Negative Control (C) and each calibrator (C1 to C3), and 100 µL of each sample (S1 to S8) to their respective wells, as shown above.

3. Using the same order of addition, add 100 µL of Diazinon-Enzyme Conjugate to each well.

Note: If you are running more than three strips, it is recommended that a multi-channel or repeater pipette be used 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 rpm is preferable, 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 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. Mix the contents of the wells, as in step 4. 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 1 N hydrochloric acid.

9. Add 100 µL of Stop Solution to each well and shake to **mix thoroughly**. This will turn the solution yellow.

Note: Read the plate as soon as possible. The color is unstable beyond 30 minutes.

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 the instrument against 200 µL water in a blank well, then measure and record the optical density (OD) of each well's contents. Or, measure and 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.

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Calculate the Results

1. After the wells have been read, average the OD of each set of calibrators and samples, and calculate the %Bo as follows:

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

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.

NOTE: To ensure accurate results, you should meet the following guidelines for each of the three calibrators you are testing. The %CV [Coefficient of Variation = (standard deviation/mean) x 100] for the calibrator OD values should not exceed 15%.

2. Graph the %Bo of each calibrator against its diazinon concentration on a semi-log scale.

3. Determine the diazinon concentration of each sample by finding its %Bo value and the corresponding concentration level on the graph.

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 with laboratory grade water so it falls on the standard curve when you run the assay again.

Ordering Information

Description	Catalog Number
EnviroGard Diazinon Plate Kit	7270000

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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