

STRATEGIC DIAGNOSTICS INC.

EnviroGard® Isoproturon Plate Kit

74500

Intended Use

The EnviroGard Isoproturon Plate Kit is a quantitative laboratory test for the detection of isoproturon residues in water.

Test Principles

The EnviroGard Isoproturon Plate Kit uses polyclonal antibodies which bind both isoproturon compounds and an isoproturon-HRP enzyme conjugate. Isoproturon in the sample competes with isoproturon-HRP enzyme conjugate for a limited number of antibody binding sites. Antibodies which bind isoproturon are immobilized to the inside of the test wells.

Since the same number of antibody binding sites are available in every well, and each well receives the same number of isoproturon-HRP enzyme conjugate molecules, a sample containing a low concentration of isoproturon allows the antibody to bind many isoproturon-HRP enzyme conjugate molecules. The result is a dark blue solution.

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

NOTE: Color is inversely proportional to isoproturon concentration.

Darker color = Lower concentration

Lighter color = Higher concentration

Performance Characteristics

The EnviroGard Isoproturon Plate Kit does not differentiate between isoproturon and closely related compounds, but detects their presence to differing degrees. The following table shows the value for 50% Bo* and the approximate value for 85% Bo which is the Lower Limit of Detection (LLD). All concentrations are in parts per billion (ppb).

Compound	Interpolated LLD (85% B ₀)	Interpolated 50% B ₀ Dose
Isoproturon	0.02	0.13
Chlorbromuron	85	1000
Chlorotoluron	6	165
Chloroxuron	>100	>1000
Diuron	7	475
Fenuron	>100	>1000
Fluometuron	>100	>1000
Linuron	46	2249
Metobromuron	3	46
Metoxuron	47	>1000
Monolinuron	41	442
Monuron	5	104
Neburon	6	586
Siduron	>100	>1000
4-Isopropylaniline	30	306

* % B₀ = the average optical density (OD) of the calibrator or sample divided by the average OD of the 0 ppb Calibrator multiplied by 100.

Precautions

• Store all 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 and samples to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before you begin the test.
- Do not store plate kit components for more than 8 hours at ambient temperature.
- Do not use kit components after the expiration date.
- Do not mix reagents or test well strips from plate kits with different lot numbers.
- 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.
- Some solutes and particulates found in untreated ground or surface waters may affect the sensitivity level of this kit.
- Tightly recap the calibrator vials immediately after use to avoid evaporative losses.

Materials Provided in the EnviroGard Isoproturon Plate Kit

This test kit contains the following items:

- 8 strips of 12 wells each, antibody-coated, in strip holder
- 1 vial of 0.0 ppb Isoproturon Calibrator (Negative Control)
- 1 vial each of 0.05 ppb, 0.20 ppb, and 0.50 ppb Isoproturon Calibrator
- 1 vial of Isoproturon-HRP Enzyme Conjugate
- 1 vial of Substrate
- 1 vial of Chromogen
- 1 vial of Stop Solution

Materials You Provide

You also need these items:

- Marking pen
- Disposable-tip pipette which will measure 80 microliters (μL), (120 μL optional)
- Microtiter plate reader
- Tape or Parafilm®
- Watch or timer
- Clean running water or a wash bottle containing tap or deionized water [500 milliliters (mL)]
- Calculator (optional)
- Orbital shaker (optional)
- Microtiter plate washing device (optional)

Assay Procedure

Have your plate kit materials available and follow these steps:

NOTE: The raised markings on the strip holder help keep the format in the correct order while you add the reagents and sample. When adding reagents, hold the dropper vial upright over the wells and allow the drops to fall freely into the wells. Do not touch the dropper tip to the sides of the wells. To add samples, a pipette must be used.

1. Plan the strip format allowing for the placement of the 4 calibrators (C-C3), and the samples S1-4) in triplicate.

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

NOTE: To set up an assay using 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 2 drops (80 µL) of each calibrator (C-C3), and 80 µL of each sample (S1 to S4) to their respective wells, as shown above.

3. Using the same order of addition, add 2 drops (80 µL) of Isoproturon-HRP Enzyme Conjugate to each well.

NOTE: If you are running more than three strips, it is recommended that a multi-channel pipette be used in steps 2, 3, 7, 8, and 10.

4. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for about 1 minute. 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. Orbital mixing at 200 rpm during incubation 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 2 drops (80 µL) of Substrate to each well, beginning with the Calibrators (C to C3), and finishing with the Samples (S1 to S4).

8. Add 2 drops (40 µL)* of Chromogen to each well in the same order as the Substrate. The Chromogen solution contains methanol, and therefore each drop equals 20 µL rather than 40 µL.

NOTE: The Substrate MUST be added BEFORE the chromogen. However, if you are using more than three strips at once, you should premix two volumes of Substrate with one volume of Chromogen in an amount sufficient to fill the number of wells used. Add 120 µL of this mixture to each well with a multi-channel pipette. Mix immediately before use and do not retain any of the unused mixture.

9. 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. Orbital mixing at 200 rpm is preferable, but not mandatory.

WARNING: Stop Solution is 2.5 N sulfuric acid.

10. Add 1 drop (40 µL) of Stop Solution to each well to arrest the blue color development and turn the reaction solution yellow. Mix thoroughly, without spilling, until all of the blue has converted to yellow.

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 120 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.

Calculate the Results

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

$$\%B^{\circ} = \frac{\text{average OD of calibrator or sample} \times 100}{\text{average OD of 0 ppb calibrator}}$$

The %Bo calculation is used as a means of equalizing different runs of an assay. While the raw OD readings of calibrators and samples are likely to differ from run to run, the %Bo relationship of calibrators and samples to the 0 ppb calibrator should remain fairly constant.

2. Graph the %Bo of each non-zero calibrator against its isoproturon concentration on a semi-log scale (see "Sample Calculations").

3. Determine the isoproturon concentration of each sample by finding its %Bo value and the corresponding concentration level on the graph (or plug the %Bo values into the equation of the line calculated by linear regression).

Ordering Information

Description	Catalog Number
EnviroGard Isoproturon Plate Kit	74500

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