



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 80 µL of Negative Control (C), 80 µL of each calibrator (C1-C3), and 80 µL of each sample (S1 to S8) to their respective wells, as shown in step 1.

3. In the same order of addition, add 2 drops (80 µL) of Atrazine-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, & 10.

4. Move the strip holder in a rapid circular motion on the benchtop for about 1 minute to mix the contents of the wells thoroughly.

CAUTION: 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 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 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 Negative Control (C) and Calibrators (C1 to C3), and finishing with the Samples (S1 to S8).

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

NOTE: You MUST add substrate before the chromogen. However, if all 96 wells are to be used at once, you can pre-mix the substrate and chromogen by combining the contents of the 2 vials. Add 120 µL of this mixture to each well. 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 recommended 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 160 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, and calculate the %Bo as follows:

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

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 atrazine concentration on a semi-log scale.

3. Determine the atrazine 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).

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

## Ordering Information

Description	Catalog Number
EnviroGard Triazine Plate Kit	72110

## Technical Assistance

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