

Highlights:

- Quantitative detection of Paraquat pesticide residue
- Results in 1½ hours—faster and more cost effective than current methods

Contents of Kit:

- 12 strips of 8 antibody-coated wells each, in plate frame
- 1 vial of Assay Diluent
- 1 vial Negative Control
- 1 vial of 0.02 ppb Paraquat Calibrator (in deionized water)
- 1 vial of 0.04 ppb Paraquat Calibrator (in deionized water)
- 1 vial of 0.2 ppb Paraquat Calibrator (in deionized water)
- 1 vial of 0.4 ppb Paraquat Calibrator (in deionized water)
- 1 vial of 1.0 ppb Paraquat Calibrator (in deionized water)
- 1 bottle of Paraquat-enzyme Conjugate
- 1 bottle of Substrate
- 1 bottle of Stop Solution

Catalog Number EP 023

Intended Use

The EnviroLogix QuantiPlate Kit for Paraquat is designed for the quantitative laboratory detection of Paraquat pesticide residues in ground and surface water samples, with an assay quantitation range from 0.02 to 1.0 parts per billion (ppb).

How the Test Works

The EnviroLogix Paraquat Plate Kit is a competitive Enzyme-Linked ImmunoSorbent Assay (ELISA).

In the test, Paraquat pesticide residues in the sample compete with enzyme (horseradish peroxidase)-labeled Paraquat for a limited number of antibody binding sites on the inside surface of the test wells.

After a simple wash step, the outcome of the competition is visualized with a color development step.

As with all competitive immunoassays, sample concentration is inversely proportional to color development.

Darker color = Lower concentration
Lighter color = Higher concentration

Precision

Paraquat-fortified control solutions were repetitively analyzed both within a single assay, and in different assays on different days. The data is expressed as % CV for both the recovered concentration and for absorbance (OD).

	Recovery (% CV)	OD (% CV)
Intra-Assay n=7		
0.1 ppb	7.8%	2.2%
0.75 ppb	6.3%	4.1%
Inter-Assay n=11		
0.1 ppb	7.5%	n/a
0.75 ppb	6.4%	n/a

Fortification and Recovery

Six ground and surface water samples were fortified with Paraquat to a concentration of 0.3 ppb. The average recovery was 103%, with a CV of 7.4%.

Cross-Reactivity

The EnviroLogix Paraquat Plate Kit does not distinguish between Paraquat and certain other compounds, but detects their presence to differing degrees. The following table shows the value for 50% B₀ and the value for the 90.4% B₀ Limit of Detection for a list of compounds. Concentration is in ppb.

Items Not Provided

- *disposable tip adjustable air-displacement pipette which will measure 25, 75 and 100 µL*
- *marking pen (indelible)*
- *tape or Parafilm®*
- *timer (1 hour and 30 minutes)*
- *cool tap or distilled water for rinsing wells*
- *microtiter plate reader or strip reader*
- *microtiter plate washer (optional)*
- *multi-channel pipette that will measure 25, 75 and 100 µL (optional)*
- *racked dilution tubes for loading samples into the plate with a multi-channel pipette (optional)*
- *orbital plate shaker (optional)*



Remove unneeded strips



Add Calibrators and samples

Compound	50% B ₀	LOD 90.4% B ₀
Paraquat	0.2	0.01
Diquat	5000	300

100% B₀ equals the maximum amount of Paraquat-enzyme conjugate that is bound by the antibody in the absence of any Paraquat in the sample (i.e. negative control). %B₀ = (OD of Sample or Calibrator/OD of Negative Control) x 100.

The following compounds were not detected at 10 ppm:

Aldrin, α-BHC, β-BHC, γ-BHC, δ-BHC, p,p'-DDT, Endrin, Heptachlor p,p'-DDD, p,p'-DDE, Dieldrin, Endosulfan I, Endosulfan II, Endosulfan sulfate, Endrin aldehyde, Heptachlor epoxide (isomer B), Carbofuran, Oxamyl, Methomyl, Aldicarb, Aldicarb sulfone, Aldicarb sulfoxide, Hydroxycarbofuran, Methiocarb, Methylamine

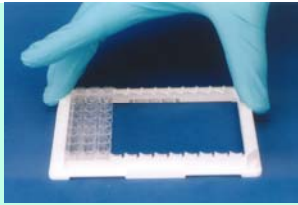
Humic Acid did not interfere in the assay up to a concentration of 100 ppm. Sodium thiosulfate did not interfere in the assay up to 0.0005N (the highest concentration tested).

How to Run the Assay

- Read all of these instructions before running the kit.
 - Allow all reagents to reach room temperature before beginning (at least 30 minutes with un-boxed strips and reagents at room temperature - do not remove strips from bag with desiccant until they have warmed up).
 - Organize all samples, reagents and pipettes so that steps 1 and 2 can be performed in 15 minutes or less.
 - If more than three strips are to be run at one time, the 15 minutes is likely to be exceeded, and the use of a multi-channel pipette is recommended (see "Note" below).
 - If three or fewer strips are to be run, use a disposable-tip air-displacement pipette and a clean pipette tip to add each Calibrator and sample to the wells. Conjugate, Substrate, and Stop Solution may be added in the same manner; alternatively, use a repeating pipette with a disposable tip on the end of the Combitip for the reagents.
 - If fewer than all twelve strips are used, reseal the unneeded strips and the desiccant in the plastic bag provided.
 - Use the well identification markings on the plate frame to guide you when adding the samples and reagents. Three strips may be used to run the Negative Control (NC), five Calibrators (C1-C5) and six samples, in duplicate. More samples require more strips. For an example plate layout see Figure 1.
1. Add **75 µL** of **Assay Diluent** to each well. Immediately add **25 µL** of **Negative Control (NC)**, **25 µL** of each **Calibrator (C1-C5)** and **25 µL** of each **sample (S1-S6)** to their respective wells, as shown in Figure 1. Follow this same order of addition for all reagents.

NOTE: In order to minimize setup time it is recommended that a multi-channel pipette be used in steps 1, 2, 6 and 8 when more than 3 strips are used.

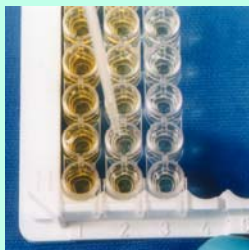
2. Immediately add **100 µL** of **Paraquat-enzyme Conjugate** to each well.
3. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. Be careful not to spill the contents!



Mix plate



Bottle Wash method



Complete protocol and add Stop Solution



Read plates in a Plate Reader within 30 minutes of the addition of Stop Solution.

- Cover the wells with tape or Parafilm to prevent evaporation and incubate at ambient temperature for 1 hour. If an orbital plate shaker is available shake plate at 200 rpm.
- After incubation, carefully remove the covering and vigorously shake the contents of the wells into a sink or other suitable container. Flood wells completely with cool tap water, then shake to empty. Repeat this wash step four times. Slap the plate on a paper towel to remove as much water as possible. Alternatively, use a microtiter plate washer for the wash step.
- Add **100 µL** of **Substrate** to each well.
- Thoroughly mix the contents of the wells, as in step 3. Cover the wells with new tape or Parafilm and incubate for 30 minutes at ambient temperature. Use orbital shaker if available.

Caution: Stop Solution is 1.0 N Hydrochloric acid. Handle carefully.

- Add **100 µL** of **Stop Solution** to each well and mix thoroughly. This will turn the well contents yellow.

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

How to Interpret the Results

Spectrophotometric Measurement and Interpretation

- Set the wavelength of your microtiter plate reader to 450 nanometers (nm). (If it has dual wavelength capability, use 600, 630 or 650 nm as the reference wavelength.)
- If the plate reader does not auto-zero on air, zero the instrument against 200 µL water in a blank well. Measure and record the optical density (OD) of each well's contents. Alternatively, measure and record the OD in every well, then subtract the OD of the water blank from each of the readings.
- A **semi-log** or **4-parameter** curve fit for the standard curve should be used if the microtiter plate reader you are using has data reduction capabilities. If not, calculate the results manually as described in the next section.

How to Calculate the Results

- After reading the wells, average the OD of each set of calibrators and samples, and calculate the %B₀ as follows:

$$\%B_0 = \frac{\text{average OD of Calibrator or sample} \times 100}{\text{average OD of Negative Control}}$$

The % B₀ calculation is used to equalize different runs of an assay. While the raw OD values of Negative Controls, Calibrators, and samples may differ from run to run, the % B₀ relationship of calibrators and samples to the Negative Control should remain fairly constant.

The %B₀ of each Calibrator should fall within these ranges:

Calibrator	%B ₀
0.02 ppb	80 - 90%
0.04 ppb	68 - 80%
0.2 ppb	38 - 50%
0.4 ppb	25 - 35%
1.0 ppb	12 - 20%

The CV for each pair of Calibrator and sample OD values should not exceed 15%.

Precautions and Notes

- Store all Plate Kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not expose Plate Kit components to temperatures greater than 37°C (99°F) or less than 2°C (36°F).
- Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before use.
- Do not use kit components after the expiration date.
- Paraquat in water will adhere to glass surfaces; samples and standards should be handled with plastics, preferably polypropylene or HDPE. To remove dissolved solids from surface water sample, use of a disposable 0.2 µm nylon filter in polypropylene or HDPE housing is recommended.
- If water samples have been treated with acid as a preservative, bring pH to 6 – 7.5 prior to analysis.
- 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 expose **Substrate** to **sunlight** during pipetting or while incubating in the test wells.
- Do not dilute or adulterate test reagents or use samples not called for in the test procedure.
- It is recommended that positive results be confirmed by an alternate method (such as liquid or gas chromatography).
- Observe any applicable regulations when disposing of samples and kit reagents.

2. Graph the %B₀ of each Calibrator against its Paraquat concentration on a semi-log scale (see Figure 3).
3. Determine the Paraquat concentration of each sample by finding its %B₀ value and the corresponding concentration level on the graph.
4. Interpolation of sample concentration is only possible if the %B₀ of the sample falls within the range of %B₀'s of the Calibrators.

If the %B₀ of a sample is higher than that of the lowest Calibrator, the sample must be reported as less than 0.04 ppb.

If the %B₀ of a sample is lower than that of the highest Calibrator, the sample must be reported as greater than 1.2 ppb. If a concentration must be determined for these high level samples, dilute the sample 1:25 in distilled water. Run this dilution in a repeat of the immunoassay. If the result now falls within the range of the %B₀'s of the Calibrators, you must then multiply the concentration measured in the diluted sample by a factor of 25.

Figure 1. Example of a typical plate setup.

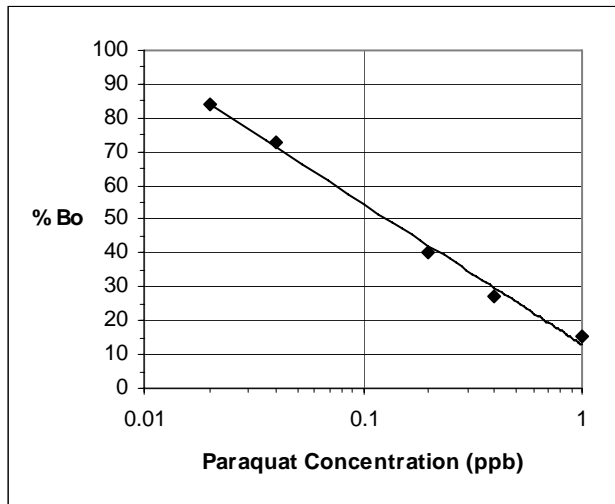
	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	C4	S3									
B	NC	C4	S3									
C	C1	C5	S4									
D	C1	C5	S4									
E	C2	S1	S5									
F	C2	S1	S5									
G	C3	S2	S6									
H	C3	S2	S6									

Figure 2. Illustrative calculations

Well contents	OD	Average OD ± sd	%CV	%B ₀	Paraquat Concentration (ppb)
Negative Control	1.688 1.718	1.703 ± 0.021	1.2	100	NA
0.02 ppb Calibrator	1.412 1.456	1.434 ± 0.031	2.2	84.2	NA
0.04 ppb Calibrator	1.250 1.223	1.237 ± 0.019	1.5	72.6	NA
0.2 ppb Calibrator	0.710 0.667	0.689 ± 0.030	4.4	40.5	NA
0.4 ppb Calibrator	0.452 0.485	0.469 ± 0.023	5.0	27.5	NA
1.0 ppb Calibrator	0.256 0.265	0.261 ± 0.006	2.4	15.3	NA
Sample	1.017 1.050	1.034 ± 0.023	2.3	60.7	0.07 ppb

Actual values may vary; this data is for demonstration purposes only.

Figure 3. Illustrative standard curve





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