ENVIR LOGIX

OuantiPlate[™] Kit for Imidacloprid

Highlights:

- Assay range from 0.2 to 6 parts per billion
- *Results in under two hours*

Contents of Kit:

- 12 strips of 8 antibody-coated wells each, in plate frame
- 1 vial of Imidacloprid Negative Control
- 1 vial of 0.2 ppb Imidacloprid Calibrator
- 1 vial of 1 ppb Imidacloprid Calibrator
- 1 vial of 6 ppb Imidacloprid Calibrator
- 1 bottle of Imidacloprid-enzyme Conjugate
- 1 bottle of Substrate
- 1 bottle of Stop Solution

Precision

	Recovery (%CV)	OD (%CV)
Iı	ntra-Assay	n=7
0.5 ppb	6.1%	1.7%
3 ppb	1.9%	1.3%
In	ter-Assay	n=11
0.5 ppb	6.9%	n/a
3 ppb	5.7%	n/a

Catalog Number EP 006

Intended Use

The EnviroLogix QuantiPlate Kit for Imidacloprid is designed for the quantitative laboratory detection of Imidacloprid pesticide residues in ground and surface water samples, with an assay range from 0.2 to 6 parts per billion (ppb).

How the Test Works

This Kit is a competitive Enzyme-Linked ImmunoSorbent Assay (ELISA). In the test, Imidacloprid pesticide residues in the sample compete with enzyme (horseradish peroxidase)-labeled Imidacloprid 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

Limit of Detection

The Limit of Detection (LOD) of the EnviroLogix Imidacloprid Plate Kit is 0.07 ppb. The LOD was determined by interpolation at 91.5% B_0^* from a standard curve. 91.5% B_0 was determined to be 3 standard deviations from the mean of a population of negative water samples.

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

Limit of Quantification

The Limit of Quantification (LOQ) of the EnviroLogix Imidacloprid Plate Kit was validated at 0.3 ppb (quantification between the 0.2 ppb lowest calibrator and 0.3 ppb may be reliable, but has not been validated). The LOQ was determined by fortifying a population of negative water samples at 0.3 ppb. The mean recovery was 104% with a coefficient of variation [CV, (standard deviation/mean) x 100] of 7.4%.

Precision

Imidacloprid-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).

Fortification and Recovery

Six ground and surface water samples were fortified with Imidacloprid to a concentration of 2 ppb. The average recovery was 110%, with a CV of 7.9%.

Cross-Reactivity

The EnviroLogix Imidacloprid Plate Kit does not distinguish between Imidacloprid and certain other compounds, but detects their presence to differing

Cross Reactivity – non-reactive up to 1000 ppb (1 ppm)

Metalaxyl	Endosulfan I
Isofenphos	Endosulfan II
Bifenthrin	Endosulfan sulfate
Aldrin	Endrin aldehyde
α-BHC	Heptochlor epoxide
β-ΒΗC	Carbofuran
γ-BHC	Oxamyl
δ-BHC	Methomyl
p,p'-DDT	Aldicarb
p,p'-DDE	Aldicarb sulfone
p,p'-DDD	Aldicarb sulfoxide
Endrin	3-hydroxycarbofuran
Deltamethrin	Cyfluthrin

Cross Reactivity – non-reactive up to 100,000 ppb (100 ppm)

Humic acid



Remove unneeded strips

degrees. The following table shows the value for 50% B_0 and the value for 91.5% B_0 . for a list of compounds. Concentration is in ppb.

Compound	50% B ₀	91.5% B ₀
Imidacloprid	1.05	0.07
Imidacloprid Olefin	3.3	0.15
DesNitro Imidacloprid	1.75	0.09
Imidacloprid Urea	3.1	0.17
Thiamethoxam	2200	46
Thiacloprid	1.3	0.17
Clothianidin	1450	85
Acetamiprid	4.4	0.21

The compounds listed at left were found to be non-reactive up to 1000 ppb (1 part per million; ppm), and humic acid was non-reactive up to 100 ppm.

Items Not Provided

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

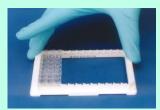
How to Run the Assay

- 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 loading time will most likely exceed 10 minutes, 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 for these three reagents.
- If fewer than all twelve strips are used, reseal the remaining strips and the desiccant in the foil pouch, and refrigerate.
- Use the well identification markings on the plate frame as a guide when adding the samples and reagents. Two strips may be used to run the Negative Control (NC), three Calibrators (C1-C3) and four samples, in duplicate. More samples require more strips. Refer to Figure 1 for a quantitative assay example plate layout.

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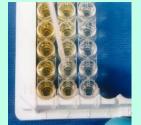
Add Calibrators and samples



Mix plate



Bottle Wash method



Complete protocol and add Stop Solution

1. Add 100 μ L of Negative Control (NC), 100 μ L of each Calibrator (C1-C3) and 100 μ L of each sample (S1-S8) 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 three strips are used.

- 2. Immediately add 100 µL of Imidacloprid-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!
- 4. 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.
- 5. After incubation, carefully remove the covering and vigorously shake the contents of the wells into a sink or other suitable container. Flood the 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.
- 6. Add 100 µL of Substrate to each well.
- 7. Thoroughly mix the contents of the wells, as in step 3. Cover the wells with <u>new</u> 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.

8. Add $100 \ \mu$ L 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

- 1. 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.)
- 2. If the plate reader does not auto-zero on air, zero the instrument against 200 μ L 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.
- 3. A **semi-log** 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.

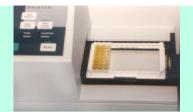
How to Calculate the Results

1. After reading the wells, average the OD of each set of calibrators and samples, and calculate the B_0 as follows:

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

The $\[Member B_0\]$ calculation is used to equalize different runs of an assay. While the raw OD values of Negative Controls, Calibrators, and samples may differ

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Read strips or plate in a Plate Reader within 30 minutes of the addition of Stop Solution



from run to run, the $\[mathcal{B}_0\]$ relationship of calibrators and samples to the Negative Control should remain fairly constant.

The $\[Membra B_0\]$ of each Calibrator should fall within these ranges:

Calibrator	<u>% B</u> 0
0.2 ppb	75 - 86%
1 ppb	40 - 57%
6 ppb	14 - 24%

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

- 2. Graph the B_0 of each Calibrator against its Imidacloprid concentration on a semi-log scale (see Figure 3).
- 3. Determine the Imidacloprid concentration of each sample by finding its B_0 value and the corresponding concentration level on the graph.
- 4. Interpolation of sample concentration is only possible if the B_0 of the sample falls within the range of B_0 's of the Calibrators.

If the B_0 of a sample is <u>higher</u> than that of the <u>lowest</u> Calibrator, the sample must be reported as less than 0.2 ppb.

If the $\%B_0$ of a sample is <u>lower</u> than that of the <u>highest</u> Calibrator, the sample must be reported as greater than 6 ppb. If a concentration must be determined for these high level samples, dilute the sample 1:20 in distilled water. Run this dilution in a repeat of the immunoassay. If the result now falls within the range of the $\%B_0$'s of the Calibrators, you must then multiply the concentration measured in the diluted sample by a factor of 20.

Figure 1. Example of a typical plate setup:

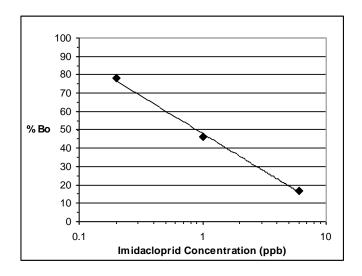
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В	C1	C1										
С	C2	C2										
D	C3	C3										
Е	S 1	S 1										
F	S2	S2										
G	S 3	S 3										
Н	S 4	S4										

Figure 2. Illustrative calculations:

Well contents	OD	Average OD ± sd	%CV	%B0	Imidacloprid Concentration
Negative Control	1.992 2.008	2.000 ± 0.011	0.6	100	NA
0.2 ppb Calibrator	1.541 1.584	1.563 ± 0.004	0.4	78	NA
1 ppb Calibrator	0.909 0.915	0.912 ± 0.004	0.4	46	NA
6 ppb Calibrator	0.334 0.331	0.333 ± 0.002	0.6	17	NA
Sample	0.519 0.495	0.507 ± 0.017	3.3	25.3	3.49 ppb

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

Figure 3. Illustrative standard curve



Precautions and Notes

- Store all QuantiPlate Kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not expose QuantiPlate 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.
- Do not use reagents or test well strips from one QuantiPlate Kit with reagents or test well strips from a different QuantiPlate Kit.
- Do not expose Substrate to sunlight during pipetting or while incubating in the test wells.
- As with all tests, it is recommended that results be confirmed by an alternate method when necessary.
- The assay has been optimized to be used with the protocol provided in the kit. Deviation from this protocol may invalidate the results of the test.
- Observe any applicable regulations when disposing of samples and kit reagents.







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	3 Details of the supplier of the safety data she Manufacturer/Supplier:	EnviroLog	ix Inc., 500 Riverside Industri	ial Pkwy.				1000-000-000-000			
		Phone: (20	(7) 797-0300								
	4 Emergency telephone number:	(207) 797-	0300 Technical Service						Causes Serious Eye Damage	Н	318
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15.2 Chemical Safety assessment	Not carried out		
Canada – WHMIS Canadian Ingredient Disclosure List	WHMIS classification of E, D2A. CAS# 7647-01-0 is listed on the Canadian Ingredient Disclosure List.		
Canada – DSL/NDSL	CAS# 7647-01-0: 1		
REACH No:	A registration number is not available for this substance as the substance uses are exempted from registration, the annual tonnage does not require registration or the registration is erwisaged for a later registration deadlini		
European/International Regulations	are listed.		
US State Regulations	CAS# 7647-01-0: can be found on the following state right to know lists NJ, PA, MN, MA. CA Prop 05: no Significant Risk Level – none of the chemicals in this pro		
OSHA	CAS# 7647-01-0: is considered highly hazardous by OSHA.		
Substances) Clean Air Act Clean Water Act	CAS# 7647-01-0: is listed as a hazardous air pollutant (HAP). CAS# 7647-01-0: is listed as a hazardous Substance under the CWA.		
SARA Section 302 (Extremely Hazardous Substances)	CAS# 7647-01-0: 5000 lb final RQ; 2270 Kg final RQ; CAS# 7647-01-0: 500 lb TPQ.		
Chemical Test Rule CERCLA	None under a Chemical Test Rule. CAS# 7647-01-0: 5000 lb final RQ; 2270 Kg final RQ.		
TSCA Health and Safety Reporting List	CAS# 7647-01-0 is not listed on the TSCA inventory. None listed.		
substance or mixture US Federal Regulations			
15.1 Safety, health and environmental regulations/legislation specific for the			
SECTION 15. Regulatory information			
and the IBC code:	No information available.		
14.6 Special precautions for user : 14.7 Transport in bulk according to Annex II of			
14.5 Environmental hazards 14.6 Special precautions for user :	Not hazardous to the environment.		
14.4 Packing group (DOT, ADR, IMDG, IATA):	m		
14.2 UN proper snipping name DOT, ADR, ADP 14.3 Transport hazard class(es) DOT, ADR, AD			
 HOADER TRANSport Information UN-Number DOT, ADR, ADN, IMDG, IAT. UN proper shipping name DOT, ADR, ADN 	A : UN1789 J. IMDG. IATA : HYDROCHLORIC ACID SOLUTION		
ECTION 14. Transport information			
	Disposal of surplus or waste solutions must be in accordance with applicable local, and national laws and regulations.		Continue rinsing.
SECTION 13. Disposal considerations Waste treatment methods:	Contact a licensed professional waste disposal service to dispose of this material.	H318 Causes Serious Eye Damage	P305+ P351+P338 IF IN EYES: Rinse cautiously with water for seven minutes. Remove contact lenses if present and easy
		H290 May be Corrosive to Metals H315 Causes Skin Irritation	P281 Use Personnel Protective equipment as Requir P302 + P352 IF ON SKIN: Wash with plenty of scap and water
12.6 Other adverse effects:	No Data Available	EnviroLogix Inc. Codes:	
12.5 Results of PBT and vPvB assessment:	Not available as a chemical safety assessment, not required/not conducted.	EHS Department	
12.4 Mobility in soil :	No Data Available	use. This document shall not constitute a guarante relationship	we for any specific product features and shall not establish a legally valid contractu
12.3 Bio accumulative potential:	No Data Available	This information is true based on our present know	wledge. However, EnviroLogix makes no representation of its accuracy or complete their independent judgment in determining the product's safety and suitability for it