Importance of Imidacloprid Determination

Imidacloprid, Clothianidin, Thiamethoxam, etc., are insecticides belonging to the neonicotinoid class of insecticides. Imidacloprid is currently the most widely used insecticide in the world. These compounds act as agonists of the polysynaptic nicotinic acetylcholine receptors (nAChR). They exhibit low mammalian toxicity due to their selectivity fro insect nAChR over vertebrate nAChRs. The neonicotinoids are systemic insecticides and are very effective for the control of sucking and chewing insects such as aphid, whitefly, leafhoppers, and some beetles. Concerns over the use of these types of compounds arise over wind drift, contamination of surface and groundwater due to high water solubility, and toxicity to honey bees and other beneficial organisms. In late 2006 various reports started to appear on the drastic disappearance of western honey bee colonies in the USA, Europe, and Asian countries such as China and Japan. This phenomenon was called colony collapse disorder (CCD) and was first thought to be caused by a parasite, however, scientist are now convinced that neonicotinoids affect honey bees[1]. When worker bees collect nectar and pollen, they are directly exposed to these pesticides. They may also carry this contamination back to the hive and contaminate other bees.

The EFSA (European Food Safety Authority) concluded in January of 2013 that an unacceptably high risk was posed to bees by there neonicotinoid insecticides: Clothianidin, Imidacloprid, and Thiamethoxam have voted on a 2 year restriction on their use in flowering crops, staring in December 2013. According to EFSA, the risk arises from residues in pollen and nectar from dust treatments uses in maize, oilseeds, and cereals.

The monitoring of water sources and food products, such as meat, milk, and honey, for residues is necessary to ascertain that these compounds are not misused and do not present a danger to human or animal health.

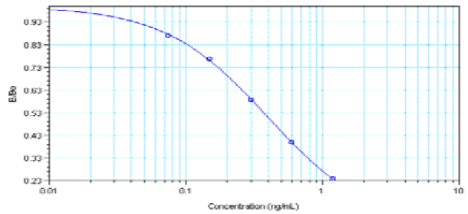
The Abraxis Imidacloprid ELISA allows the determination of 42 samples in duplicate determination. Only a few milliliters of sample are required. The test can be performed in 90 minutes.

References: [1]Whitehorn, P.R., O'Connor, S., Wackers, F.L., Goulson, D. Neonicotoid Pesticide Reduces Bumble Bee Colony Growth and Queen Production. Science 336 (6079); 351-352 (2012).

Performance Data

Test sensitivity: The limit of detection for Imidacloprid in water calculated as Xn +/- 3SD (n=20) or as 90% B/Bo is equal to 0.06 ng/mL. The concentration of residue necessary to cause 50% inhibition (50% B/B_0) is approximately 0.40 ng/mL. Determinations closer to the middle of the calibration range of the test yield the most accurate results.

The following is the LOQ in different matrixes: Water 0.30 ppb; Apple Juice 3.75 ppb; Grape Juice 7.5 ppb; Grapefruit and Orange juice 15.0 ppb.



Test reproducibility: Coefficients of variation (CVs) for standards: <10%; CVs for samples: <15%.

Selectivity: This ELISA recognizes Imidacloprid and related compounds with varying degrees:

Cross-reactivities:

Imidacloprid	100%
Clothianidin	121%
Thiacloprip	13%
Acetamiprid	4%
Thiamethoxam	<0.1%

Samples: To eliminate matrix effects in juices, etc., sample dilution is required. See Preparation of Samples section. For additional extraction procedures for various matrices please contact Abraxis LLC.

The monoclonal antibody and HRP conjugate included in this kit has been licensed from the Spanish National Research Council (CSIC) and the University of Valencia.

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Imidacloprid ELISA, Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Imidacloprid in Contaminated Samples

Product No. 500800

1. General Description

The Imidacloprid ELISA is an immunoassay for the detection of Imidacloprid and other neonicotinoids insecticides. This test is suitable for the quantitative and/or qualitative detection of Imidacloprid and other neonicotinoids in contaminated samples. Positive samples should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions in this test kit contain small amounts of Imidacloprid in solution. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of standard and stopping solutions with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

3. Storage and Stability

The Imidacloprid ELISA Kit should to be stored in the refrigerator (4–8°C) prior to use. The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box. Some reagents need to be stored frozen after reconstitution (Test Preparation, section C).

4. Test Principle

The test is a direct competitive ELISA based on the recognition of Imidacloprid by specific antibodies. Imidacloprid, when present in a sample and a Imidacloprid-enzyme conjugate compete for the binding sites of anti-Imidacloprid antibodies which are immobilized on the wells of the microtiter plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of Imidacloprid present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the Imidacloprid ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects can not be completely excluded.

The presence of the following substances up to 10,000 ppm were found to have no significant effect on the Imidacloprid Assay results: calcium sulfate, magnesium sulfate, magnesium chloride, sodium nitrate, phosphate, calcium chloride, copper chloride, manganese sulfate, aluminum oxide, ferric sulfate, zinc sulfate, sodium thiosulfate. Sodium Chloride up to 100,000 ppm. Humic acid up to 10 ppm. Methanol up to 10%.

Mistakes in handling the test can also cause errors. Possible sources for such errors can be:

Inadequate storage conditions of the test kit (or reagents), incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

The Abraxis Imidacloprid ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.), positive samples requiring some action should be confirmed by an alternative method.

Working Instructions

A. Materials Provided

1. Microtiter plate coated anti-Imidacloprid antibody, in a resealable foil pouch with desiccant.
2. Imidacloprid Standards (6): 0, 0.075, 0.15, 0.30, 0.60, and 1.2 ng/mL; Control at 0.50 ng/mL. Standard and Control vials supplied ready to use, 1 mL/vial.
3. Assay Buffer, 6 mL.
4. Sample Diluent, 25 mL. Use to dilute samples.
5. Imidacloprid-HRP Conjugate, 3 vials (lyophilized).
6. Conjugate Diluent, 1 bottle, 12 mL each.
7. Wash Solution (5X) Concentrate, 100 mL.
8. Color (Substrate) Solution (TMB), 16 mL.
9. Stop Solution, 12 mL.

B. Additional Materials (not included with the test kit)
1. Micro-pipettes with disposable plastic tips (10-200 and 200-1000 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with plastic tips (10-250 µL)
3. Microtiter plate reader (wave length 450 nm)
4. Timer
5. Tape or Parafilm
6. Glass vials with Teflon-lined caps
7. Distilled or deionized water
8. Vortex mixer

C. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the assay buffer, conjugate, substrate and stop solutions in order to equalize the incubations periods of the solutions on the entire microtiter plate. Please use only the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips should be stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standards, assay buffer, substrate, and stop solutions are ready to use and do not require any further dilutions.
4. The HRP conjugate provided is lyophilized (3 vials). Before each assay, calculate the volume of conjugate needed (when reconstituted, each vial will provide enough conjugate for 1/3 plate). Once reconstituted, the conjugate solution will only remain viable for 1 week refrigerated and 4 weeks if stored at –20 °C. If additional samples are to be analyzed greater than the listed shelf, a new vial of conjugate must be prepared. To reconstitute, add 3 mL of Conjugate Diluent to each vial of Conjugate required and vortex. If more than 1/3 of plate needs to be run, then combine the reconstituted HRP conjugate vials, vortex slowly and add to plate as described in the Assay procedure.
5. Dilute the wash buffer (5X) concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water.
6. The stop solution should be handled with care as it contains diluted H_2SO_4.

D. Preparation of Samples

Samples should be diluted in Sample Diluent or Deionized/Distilled water before analysis as follows:
NOTE: All samples and dilutions should be made in glass vials/tubes.

Water (Dilution 1:4)

Prior to analysis, each sample must be filtered using a 0.45 um polyethersulfone (PES) filter and diluted with Sample Diluent as follows:
1. Add 250 uL of water sample to a glass vial/tube and 750 uL of Sample Diluent or distilled/deionized water.
2. Vortex to mix and analyze as sample (Assay Procedure, step1).
3. The Imidacloprid concentration contained in the water samples is then determined by multiplying the ELISA result by the dilution factor of 4. Recoveries obtained were 80-120%

Apple Juice (Dilution 1:50)
1. Add 100 uL of apple juice sample to a glass vial/tube and 4.9 mL of Sample Diluent or distilled/deionized water.
2. Vortex to mix and analyze as sample (Assay Procedure, step1).
3. The Imidacloprid concentration contained in the apple juice samples is then determined by multiplying the ELISA result by the dilution factor of 50. Recoveries obtained were 87-101%

Grape Juice (Dilution 1:100)
1. Add 50 uL of apple juice sample to a glass vial/tube and 4.95 mL of Sample Diluent or distilled/deionized water.
2. Vortex to mix and analyze as sample (Assay Procedure, step1).
3. The Imidacloprid concentration contained in the grape juice samples is then determined by multiplying the ELISA result by the dilution factor of 100. Recoveries obtained were 90-101%

Grapefruit Juice (Dilution 1:200)
1. Add 50 uL of apple juice sample to a glass vial/tube and 9.95 mL of Sample Diluent or distilled/deionized water.
2. Vortex to mix and analyze as sample (Assay Procedure, step1).
3. The Imidacloprid concentration contained in the grapefruit juice samples is then determined by multiplying the ELISA result by the dilution factor of 200. Recoveries obtained were 90-120%

Orange Juice (Dilution 1:200)
1. Add 50 uL of apple juice sample to a glass vial/tube and 9.95 mL of Sample Diluent or distilled/deionized water.
2. Vortex to mix and analyze as sample (Assay Procedure, step1).
3. The Imidacloprid concentration contained in the orange juice samples is then determined by multiplying the ELISA result by the dilution factor of 200. Recoveries obtained were 90-106%

NOTE: Highly contaminated samples (those outside of the calibration range of the assay) must be diluted further in Sample Diluent or deionized/distilled water and re-analyzed. Samples with values below the first standard should not be multiplied and reported as < 0.075 ppb.

E. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std 5: Standards
0; 0.075; 0.15; 0.30; 0.60 ; 1.2 ppb

Control

Samp1, Samp2, etc.: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4	Samp 2									
B	Std 0	Std 4	Samp 2									
C	Std 1	Std 5	Samp 3									
D	Std 1	Std 5	Samp 3									
E	Std 2	Control	etc.									
F	Std 2	Control	etc.									
G	Std 3	Samp 1										
H	Std 3	Samp 1										

F. Assay Procedure
1. Add 50 µL of assay buffer solution to the individual wells successively using a multi-channel pipette or a stepping pipette.
2. Add 50 µL of the standard solutions and samples or sample extracts into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
3. Add 50 µL of enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette.
4. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill contents.
5. Incubate the strips for 60 minutes at room temperature.
6. After incubation, remove the covering and vigorously shake the contents of these wells into a sink. Wash the strips three times using the 1X washing buffer solution. Use 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
7. Add 150 µL of substrate (color) solution to the wells. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Incubate the strips for 20-25 minutes at room temperature. Protect the strips from direct sunlight.
8. Add 100 µL of stop solution to the wells in the same sequence as for the substrate solution.
9. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

G. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs [4-Parameter (preferred) or Logit/Log]. For manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on the vertical linear (y) axis versus the corresponding Imidacloprid concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppb of Imidacloprid by interpolation using the standard curve. Samples showing lower concentrations of Imidacloprid compared to Standard 1 (0.075 ng/mL) should be reported as containing < 0.075 ng/mL. Samples showing a higher concentration than Standard 5 (1.2 ng/mL) must be diluted further to obtain accurate results.

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