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User's Manual

Imidacloprid ELISA Kit

REF **DEIABL-QB52**

Σ **96T**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Imidacloprid ELISA Kit is suitable for the quantitative and/or qualitative detection of Imidacloprid and other neonicotinoids in contaminated samples.

General Description

The Imidacloprid ELISA Kit 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 GC/MS, HPLC, or other conventional methods.

Principles of Testing

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.

Reagents And Materials Provided

1. Microtiter plate coated anti-Imidacloprid antibody
2. Imidacloprid Standards (6): 0, 0.075, 0.15, 0.30, 0.60, and 1.2 ng/mL, 1 mL each
3. Control: 0.50 ng/mL, 1 mL
4. Assay Buffer, 6 mL
5. Sample Diluent, 25 mL, use to dilute samples
6. Imidacloprid-HRP Conjugate, 3 vials (lyophilized)
7. Conjugate Diluent, 12 mL
8. Wash Buffer (5X) Concentrate, 100 mL
9. TMB, 16 mL
10. Stop Solution, 12 mL

Materials Required But Not Supplied

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 (50-250 µL), or electronic repeating pipette with disposable plastic tips

3. Container with 500 mL capacity
4. Microtiter plate reader (wave length 450 nm)
5. Timer
6. Tape or Parafilm
7. Glass vials with Teflon-lined caps
8. Distilled or deionized water
9. Vortex mixer

Storage

Store at 4-8°C

Specimen Collection And Preparation

Water (Dilution 1:4)

1. Filter water sample using a 0.45 µm polyethersulfone (PES) filter.
2. Add 250 µL of filtered water sample to a glass vial/tube and 750 µL of Sample Diluent or distilled/deionized water.
3. Vortex to mix and analyze as sample (Assay Procedure, step1).

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 µL 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).

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 µL 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).

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 µL 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).

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 µL 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).

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%

Assay Procedure

1. Add 50 µL of assay buffer solution to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette.
2. Add 50 µL of the standards, control, samples or sample extracts into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
3. Add 50 µL of reconstituted enzyme conjugate to the individual wells successively using a multi-channel, stepping, or electronic repeating 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. Remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. Wash the strips three times using the diluted wash buffer. Please use at least a volume of 250 µL of 1X wash buffer for each well and each washing step. Blot the inverted plate after each wash step on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
7. Add 150 µL of substrate (color) solution to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette. 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 (color) solution using a multichannel, stepping, or electronic repeating pipette.
9. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

Sensitivity

Water 0.30 ppb; Apple Juice 3.75 ppb; Grape Juice 7.5 ppb; Grapefruit and Orange juice 15.0 ppb.

Specificity

Imidacloprid: 100%

Clothianidin: 121%

Thiacloprip: 13%

Acetamiprid: 4%

Thiamethoxam <0.1%

Reproducibility

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