Importance of Pyrethroids Determination

Pyrethroids are a group of compounds that are among the most commonly used insecticides in the world. Pyrethroids are synthetic analogues of the compound Pyrethrum, which is found naturally in chrysanthemums and contains active insecticidal compounds known as Pyrethrins. The synthetic Pyrethroids are more toxic and break down more slowly than the naturally occurring Pyrethrins; with times generally ranging from a few minutes to several weeks, although some of the most recently developed compounds may not degrade for several months.

Pyrethroids are used in agriculture, applied to crops as aerial sprays from planes or tractors, or by hand-held spray applicators. Pyrethroids are also used in large-scale mosquito spraying programs designed to kill adult mosquitoes which may carry West Nile Virus (WNV), Zika, and other mosquito-borne diseases. Household uses for Pyrethroids include their use in shampoo treatments for head lice and skin treatments for scabies, as mosquito repellents applied to clothes, as pet sprays, and as household insecticides, used as sprays and bombs. After the use of DDT was banned, Pyrethroids were seen as an alternative treatment method against bedbugs, but they are now developing resistance to Pyrethroids.

Humans may be exposed to Pyrethroids through eating fruits and vegetables sprayed with Pyrethroids, drinking contaminated water, breathing airborne Pyrethroids, either from drift from agricultural spraying or from fogging in mosquito control spraying programs, or through direct application to clothing, skin, or hair. Pyrethroids are commonly combined with other chemicals, including DEET, which may enhance their ability to enter the body. At lower doses, Pyrethroids are eye, skin, and upper respiratory irritants and may produce numbness, burning, or itching sensations with direct skin contact. Some people may become sensitized to Pyrethroids. Sensitive individuals and those who are allergic to Pyrethroids may experience allergic contact dermatitis or a worsening of asthma symptoms. Heavy skin exposure may produce numbness, burning, or itching sensations that can remain for several hours. Ingestion of large amounts may produce dizziness, nausea, and headache. Ingestion of extremely large amounts may cause convulsions. Very heavy or long-tern exposure may result in the accumulation of Pyrethroids in fatty tissues. Pyrethroids may function as endocrine disruptors at high doses and are possible carcinogens. In addition to the potential adverse effects in humans, Pyrethroids are toxic to bees, dragonflies, fish, and other aquatic animals. Because they are not removed in standard wastewater treatment processes, levels can be found in effluent which can be lethal to invertebrates. They are also highly toxic to cats, which lack a liver enzyme necessary to break down Pyrethroids.

Permethrin is listed on the EPA Safe Drinking Water Act (SDWA) Contaminant Candidate List 4 (CCL 4) and the EPA has established oral daily exposure limits for ten different Pyrethroids ranging from 0.005 mg per kilogram of body weight per day (mg/kg/day) to 0.05 mg/kg/day. The World Health Organization (WHO) has set a limit for Permethrin in drinking water of 20 µg/L.

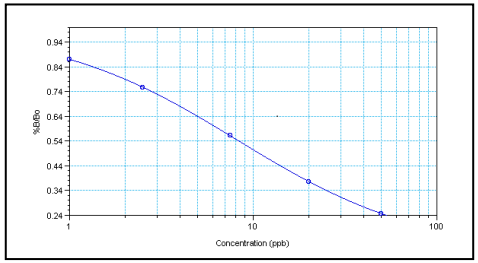
The ABRAXIS® Pyrethroids ELISA allows for the analysis of 26 samples in triplicate determination. Less than 1 mL of sample is required. The test can be performed in less than 2.5 hours.

Performance Data

Test sensitivity: The limit of quantitation for Pyrethroids (90% B/B_0 calculated from the average of 44 calibration curves) is approximately 0.714 ng/mL. The middle of the test (50% B/B_0 calculated from the average of 44 calibration curves) is approximately 10.3 ng/mL. Determinations closer to the middle of the calibration curve give the most accurate results.

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

Standard Curve:



For demonstration purposes only. Not for use in sample interpretation.

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For ordering or technical assistance contact:
Gold Standard Diagnostics
795 Horsham Road
Horsham, PA 19044
WEB: www.abraxiskits.com

Tel.: (215) 357-3911
Fax: (215) 357-5232
Ordering: info.abraxis@us.goldstandarddiagnostics.com
Technical Support: support.abraxis@us.goldstandarddiagnostics.com

Date this User Guide is effective: 05/15/2024

Version: 01

ABRAXIS® Pyrethroids ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Pyrethroids in Water and on Contaminated Surfaces

Product No. 500204

1. General Description

The ABRAXIS® Pyrethroids ELISA is an immunoassay for the quantitative and sensitive screening of Pyrethroids. This test is suitable for the quantitative and/or qualitative screening of Pyrethroids in water samples or on surfaces (see section C, Sample Preparation, Wipe Samples). Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

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

3. Storage and Stability

The ABRAXIS® Pyrethroids ELISA should be stored in the refrigerator (2-8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the last day of the month as indicated by the expiration date on the box.

4. Test Principle

The test is a direct competitive ELISA based on the recognition of Pyrethroids by specific antibodies. Pyrethroids, when present in a sample and a Pyrethroids-HRP analogue compete for the binding sites of the mouse anti-Pyrethroids antibodies in solution. The Pyrethroids antibodies are then bound by a second antibody (anti-mouse) immobilized on the wells of the microtiter plate. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of Pyrethroids present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader at 450 nm. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the ABRAXIS® Pyrethroids ELISA, Possible Test Interference

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

The presence of the following substances were found to have no significant effect on the Pyrethroids assay results: aluminum oxide, calcium chloride, calcium sulfate, magnesium chloride, magnesium sulfate, manganese sulfate, potassium phosphate, sodium chloride, sodium fluoride, and sodium nitrate up to 10,000 ppm; copper chloride, and zinc sulfate up to 100 ppm; and ferric sulfate and humic acid up to 10 ppm. No matrix effects have been observed with samples that have been treated with sodium thiosulfate at concentrations ≤ 10 mg/mL or ascorbic acid at concentrations ≤ 1 mg/mL.

Mistakes in handling the test can cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, inappropriate temperatures (less than 4°C or higher than 8°C) during the incubation periods, and use of room temperature Wash Buffer during washing steps. The ABRAXIS® Pyrethroids ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.), samples requiring some regulatory action should be confirmed by an alternative method.

6. Working Instructions

A. Reagents and Materials Provided

1. Microtiter plate (12 X 8 strips) coated with a secondary antibody, in a resealable aluminum pouch
2. Pyrethroids Assay Buffer, 6 mL
3. Pyrethroids Calibrators/Standards (6): 0, 1.0, 2.5, 7.5, 20.0, 50.0 ng/mL (ppb) Permethrin, 1 mL each
4. Antibody Solution (mouse anti-Pyrethroids), 6 mL
5. Pyrethroids-HRP Conjugate Solution, 12 mL
6. ABRAXIS® Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (section D)

7. Sample Diluent, 25 mL
8. Substrate (Color) Solution (TMB), 12 mL
9. Stop Solution, 6 mL (handle with care)

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (50-200 µL)
2. Multi-channel pipette (50-250 µL), stepper pipette (50-250 µL), or electronic repeating pipette with disposable plastic tips
3. Microtiter plate reader (wave length 450 nm)
4. Container with 500 mL capacity (for 1X diluted Wash Buffer, see Test Preparation, section D)
5. Deionized or distilled water
6. Methanol (pesticide grade or equivalent)
7. 70% isopropyl alcohol (pesticide grade of equivalent)
8. Glass vials with Teflon lined caps (4 mL, 20 mL, and 40 or 60 mL)
9. Paper towels or equivalent absorbent material
10. Timer
11. Refrigerator (2-8°C)
12. Tape or parafilm
13. 3" x 3" cotton gauze pads (see Sample Preparation, section C)
14. Antiseptic alcohol swabsticks (Dynarex PN 1203 or equivalent)
15. Glass serological pipettes (10 mL)
16. Protective gloves

C. Sample Preparation

Water Samples (2.0 ppb – 100.0 ppb detection range)

Water samples should be collected in glass sample containers. Immediately upon collection, water samples should be preserved with methanol at a ratio of 1:1. For example, a 10 mL water sample added to a sample container should have 10 mL of methanol added (cap tightly and mix thoroughly).

The Pyrethroids concentration contained in water samples is determined by multiplying the ELISA result by the dilution factor of 2. Highly contaminated samples (those outside of the calibration range of the assay) must be diluted further in sample diluent and re-analyzed.

Wipe Samples (10,000 ppb – 500,000 ppb or 10 µg/wipe – 500 µg/wipe detection range)

Note: Protective gloves should be worn during the sample collection procedure. Gloves should be changed between wipes to avoid cross-contamination. Avoid inhaling solvent fumes. Additional sample diluent for the dilution of sample extracts can be prepared, if necessary, by combining equal amounts of methanol and deionized or distilled water. For example, in a 100 mL capacity glass bottle with a Teflon lined cap, add 50 mL of methanol and 50 mL of deionized or distilled water. Mix thoroughly and allow to cool to room temperature before use.

Wipe Sampling Procedure I (Methanol Procedure)

This procedure is intended for use only with cotton gauze wipes (packaged individually as sterile gauze pads). Other materials should be thoroughly validated before use with this procedure.

1. Saturate a wipe with 1-2 mL of methanol.
2. Thoroughly wipe a 10 cm x 10 cm (100 cm^2) area with the saturated wipe.
3. Transfer the wipe to a clean, appropriately labeled 40 mL glass vial.
4. Proceed to wipe extraction procedure, below.

Wipe Sampling Procedure II (Alcohol Swab Procedure)

This procedure is intended for use only with alcohol swabs (packaged individually as antiseptic alcohol swabs). Other materials should be thoroughly validated before use with this procedure.

1. Thoroughly wipe a 10 cm x 10 cm (100 cm^2) area with the alcohol swab.
2. Transfer the alcohol swab to a clean, appropriately labeled 60 mL glass vial.
3. Proceed to wipe extraction procedure, below.

Wipe Extraction Procedure

Note: Either methanol or 70% isopropyl alcohol can be used for extraction (step 1 below), but only 50% methanol Sample Diluent can be used for sample extract dilutions (steps 4 – 7 below).

1. Add 20 mL of methanol or 70% isopropyl alcohol to the vial containing the wipe or swab.
2. Shake vigorously for 60 seconds.
3. Allow the sample to settle.
4. Add 5 mL of Sample Diluent to a clean, appropriately labelled 20 mL glass vial.
5. Add 50 µL of sample extract to the diluent in the 20 mL vial. Vortex thoroughly.
6. Add 1.6 mL of Sample Diluent to a clean, appropriately labelled 4 mL glass vial.
7. Add 400 µL of diluted sample extract (from step 5) to the diluent in the 4 mL vial. Vortex thoroughly.

The sample is then ready to analyze (see Assay Procedure, section F, step 1).

The Pyrethroids concentration contained in the wipe sample is determined by multiplying the ELISA result by the extraction/dilution factor of 10,000. Highly contaminated samples (those outside of the calibration range of the assay) must be diluted further in sample diluent and re-analyzed.

D. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and samples are necessary. In order to equalize the incubation periods on the entire microtiter plate, a multi-channel pipette, stepping pipette, or electronic repeating pipette is recommended for adding the enzyme conjugate, antibody, substrate, and stop solutions. Please use only the reagents and standards from one package lot in one test, as they have been adjusted in combination.

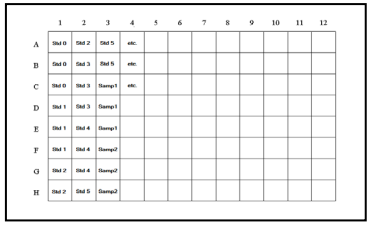
1. Allow the microtiter plate, reagents, and samples to reach room temperature before use.
2. Remove the number of microtiter plate strips required from the aluminum pouch. The remaining strips are stored in the aluminum pouch with the desiccant (tightly sealed) in the refrigerator (2-8°C).
3. The assay buffer, standard solutions, conjugate, antibody, substrate and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the ABRAXIS® 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 and mix thoroughly. **Diluted Wash Buffer should be stored refrigerated (2-8°C) and must be used cold (do not warm to room temperature before use for wash steps).** Use of room temperature Wash Buffer may produce inaccurate results.
5. The stop solution must be handled with care as it contains diluted H_2SO_4.

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; 1.0; 2.5; 7.5; 20.0; 50.0 ppb)

Samp1, Samp2, etc.: Samples



F. Assay Procedure

1. **Add 25 µL of the assay buffer** to the wells of the test strips using a multi-channel, stepping, or electronic repeating pipette.
2. **Add 75 µL of the calibrator/standard solutions or samples** into the wells of the test strips according to the working scheme given. Analysis in triplicate is recommended.
3. **Add 50 µL of antibody 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. Be careful not to spill the contents. **Incubate the strips for 90 minutes in a refrigerator at 2–8°C**.
4. Remove the covering and decant the contents of the wells into a sink. **Wash the strips three times using the cold diluted washing buffer**. Please use at least a volume of 250 µL of cold washing buffer for each well in 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.
5. **Add 100 µL of conjugate solution** to the individual wells successively using a multi-channel, stepping, or 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. Be careful not to spill the contents. **Incubate the strips for 30 minutes in a refrigerator at 2–8°C**.
6. Remove the covering and decant the contents of the wells into a sink. **Wash the strips three times using the cold diluted washing buffer.** Please use at least a volume of 250 µL of cold washing buffer for each well in 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 100 µL of substrate (color) solution** to the 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. Be careful not to spill the contents. **Incubate the strips for 20 minutes in a refrigerator at 2–8°C**. Protect the strips from direct sunlight.
8. **Add 50 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 stopping solution.

G. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as 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 Pyrethroids concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppb (or ng/mL) of Pyrethroids by interpolation using the standard curve. Results for water samples are determined by multiplying the concentration determined from the curve by the dilution factor of 2; results for wipe samples are determined by multiplying the concentration determined from the curve by the extraction/dilution factor of 10,000. Samples showing a lower concentration of Pyrethroids than standard 1 (1.0 ppb) should be reported as containing < 2.0 ppb of Pyrethroids for water samples or < 10,000 ppb (< 10 ppm or < 10 µg/wipe) for wipe samples. Samples showing a higher concentration than standard 5 (50 ppb) should be reported as containing > 100 ppb for water samples or > 500,000 ppb (> 500 ppm or >500 µg/wipe) for wipe samples. If a quantitative result is necessary, samples must be diluted further with sample diluent and re-analyzed.