Pyrethroids

• Intended Use

For detection of Permethrin and related pyrethroids (please refer to cross-reactivity table) in water (groundwater, surface water, well water). Please refer to the attached specific procedures for water (groundwater, surface water, well water). For soil, crop, and food use contact the company for application bulletins and/or specific matrix validation guidelines.

• Principle

The Abraxis Pyrethroid Assay applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of Permethrin and related pyrethroids. The sample to be tested is added, along with paramagnetic particles attached with antibodies specific to pyrethroids, to a disposable glass test tube, and incubated for 20 minutes. This is followed by the addition of an pyrethroid enzyme conjugate. Both the pyrethroids (which may be in the sample) and the enzyme labeled Permethrin analog (the enzyme conjugate) compete for antibody binding sites on the magnetic particles. At the end of a thirty minute (30) incubation period, a magnetic field is applied to hold the paramagnetic particles (with Pyrethroid and labeled Permethrin analog bound to the antibodies on the particles, in proportion to their original concentration) in the tube and allow the unbound reagents to be decanted. After decanting, the particles are washed with Washing Solution.

The presence of pyrethroids is detected by adding the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The enzyme-labeled Permethrin analog bound to the Pyrethroid antibody catalyzes the conversion of the substrate/ chromogen mixture to a colored product. After an incubation period of thirty (30) minutes, the reaction is stopped and stabilized by the addition of acid. Since the labeled Permethrin (conjugate) was in competition with the unlabeled Pyrethroids (sample) for the antibody sites, **the color developed is inversely proportional to the concentration of Pyrethroids in the sample**.

• Reagents

1. Pyrethroid Antibody Coupled Paramagnetic Particles
The Pyrethroid antibody (monoclonal anti-Permethrin) is bound to paramagnetic particles, which are suspended in buffered saline containing preservative and stabilizers.
100 test kit: one 60 mL vial

2. Pyrethroid Enzyme Conjugate
The horseradish peroxidase (HRP) labeled Permethrin analog is diluted in buffered saline containing preservative and stabilizers.
100 test kit: one 30 mL vial

3. Permethrin Standards
Five concentrations (0.75, 2.5, 5.0, 15.0 ppb) of Permethrin in a methanolic solution with preservative and stabilizers. Each vial contains 2.0 mL.

4. Control
A concentration (approximately 3.0 ppb) of Permethrin in a methanolic solution containing preservative and stabilizers. A 2.0 mL volume is supplied in one vial.

5. Diluent/Zero Standard
A methanolic solution containing preservative and stabilizers without any detectable Permethrin.
100 test kit: one 35 mL vial

6. Color Solution
A solution of hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine in an organic base.
100 test kit: one 65 mL vial

7. Stopping Solution
A solution of diluted sulfuric acid (0.5%).
100 test kit: one 60 mL vial

8. Washing Solution
Preserved deionized water.
100 test kit: one 250 mL vial

9. Test Tubes
Glass tubes (36) are packed in a box.
100 test kit: three 36 tube boxes

• Reagent Storage and Stability

Store all reagents at 2-8°C. Do not freeze. Reagents may be used until the expiration date on the box. *The test tubes require no special storage condition and may be stored separately from the reagents to conserve refrigerator space.*

Consult state, local and federal regulations for proper disposal of all reagents.

• Materials Required but Not Provided

In addition to the reagents provided, the following items are essential for the performance of the test:

Pipets*	Precision pipets capable of delivering 250 and 500 uL and a 1.0 mL repeating pipet.
Vortex Mixer*	Thermolyne Maxi Mix, Scientific Industries Vortex Genie, or equivalent

Magnetic Separation Rack*

Photometric Analyzer* capable of readings at 450 nm

Methanol (HPLC Grade or equivalent).

* These items are available from Abraxis.

• Sample Information

This procedure is recommended for use with water samples. Other samples may require modifications to the procedure and should be thoroughly validated.

> Water samples should be collected in glass vessels (teflon liners in the cap). **Immediately** upon collection, samples should be diluted with an equal volume (1:1) of methanol (HPLC grade) to prevent adsorptive losses to the glass containers.

After samples are diluted, those samples containing gross particulate matter should be filtered (e.g. 0.2 um Anotop™ 25 Plus, Whatman, Inc.) to remove particles.

If the Pyrethroid concentration of a sample exceeds 15.0 ppb, the sample is subject to repeat testing using a diluted sample. A ten-fold or greater dilution of the sample is recommended with an appropriate amount of Diluent/Zero Standard or Sample Diluent. For example, in a separate test tube make a ten-fold dilution by adding 100 uL of the sample to 900 uL of Diluent/Zero Standard. Mix thoroughly before assaying. Perform the assay according to the Assay Procedure and obtain final results by multiplying the value obtained by the dilution factor e.g. 10.

The presence of the following substances up to 10,000 ppm were found to have no significant effect on the Pyrethroid Assay results: copper, manganese, calcium, magnesium, sodium, phosphate, sulfate, thiosulfate, and nitrate. Sodium Fluoride up to 1,000 ppm. Copper and $FeSO_4$ up to 100 ppm, and Humic acid up to 10 ppm was found to have no significant effect. In addition Sodium Chloride up to 1M was found to have no significant effect.

• Reagent Preparation

All reagents must be allowed to come to room temperature and the antibody coupled paramagnetic particles should be mixed thoroughly before use.

• Procedural Notes and Precautions

Prepare water samples as described above. Follow the assay procedure as described in the Assay Procedure section of this package insert.

As with all immunoassays, a consistent technique is the key to optimal performance. To obtain the greatest precision, be sure to treat each tube in an identical manner.

Add reagents directly to the bottom of the tube while **avoiding contact between the reagents and the pipet tip**. This will help assure consistent quantities of reagent in the test mixture.

Avoid cross-contaminations and carryover of reagents by using clean pipets for each sample addition and by avoiding contact between reagent droplets on the tubes and pipet tips.

Avoid foam formation during vortexing.

The magnetic separation rack consists of two parts: an upper rack which will securely hold the test tubes and a lower separator which contains the magnets used to attract the antibody coupled paramagnetic particles. During incubations the upper rack is removed from the lower separator so that the paramagnetic particles remain suspended during the incubation. **For separation steps, the rack and the separator are combined to pull the paramagnetic particles to the sides of the tubes**.

To obtain optimum assay precision, it is important to perform the separation steps carefully and consistently. Decant the rack by slowly inverting away from the operator using a smooth turning action so the liquid flows consistently along only one side of the test tube. While still inverted, place the rack on an absorbent pad and allow to drain. Lifting the rack and replacing gently onto the pad several times will ensure complete removal of the liquid from the rim of the tube.

Mix the antibody coupled paramagnetic particles just prior to pipetting.

> Standard and Control vials should remain capped when not in use, to prevent evaporation.

Do not use any reagents beyond their stated shelf life.

Avoid contact of Stopping Solution (sulfuric acid) with skin and mucous membranes. If this reagent comes in contact with skin, wash with water.

• Limitations

The Abraxis Pyrethroid Assay will detect Pyrethroids to different degrees. Refer to specificity table for data on various Pyrethroids. The Abraxis Pyrethroid Assay kit provides screening results. As with any analytical technique (GC, HPLC, etc...) positive results requiring some action should be confirmed by an alternative method.

The total time required for pipetting the magnetic particles should be kept to two (2) minutes or less, therefore the total number of tubes that can be assayed in a run should be adjusted accordingly.

• Quality Control

A control solution at approximately 3.0 ppb of Permethrin is provided with the Abraxis Pyrethroid Assay kit. It is recommended that it be included in every run and treated in the same manner as unknown samples. Acceptable limits should be established by each laboratory.

• Assay Procedure

Read Reagent Preparation, Procedural Notes and Precautions before proceeding.

1. Perform the appropriate sample preparation according to the attached water or soil procedure. For any other sample matrices refer to specific procedures available from Abraxis.

2. Label **glass** test tubes for standards, control, and samples.

Tube Number	Contents of Tube
1,2	Diluent/Zero Standard, 0 ppb
3,4	Standard 1, 0.75 ppb
5,6	Standard 2, 2.5 ppb
7,8	Standard 3, 5.0 ppb
9,10	Standard 4, 15.0 ppb
11	Control
12	Sample 1
13	Sample 2
14	Sample 3

3. Add 250 uL of the appropriate standard, control, or sample to the test tube.
4. Mix the Pyrethroid Antibody Coupled Paramagnetic Particles thoroughly and add 500 uL to each tube.
5. Vortex for 1 to 2 seconds minimizing foaming.
6. Incubate for 20 minutes at room temperature.
7. Add 250 uL of Pyrethroid Enzyme Conjugate to each tube.
8. Vortex for 1 to 2 seconds minimizing foaming.
9. Incubate for 30 minutes at room temperature.
10. Separate in the Magnetic Separation Rack for **two (2) minutes**.
11. Decant and **gently** blot all tubes briefly in a consistent manner.
12. Add 1 mL of Washing Solution to each tube and vortex tubes for 1-2 seconds. Return tubes and allow to remain in the magnetic separation unit for **two (2) minutes**.
13. Decant and **gently** blot all tubes briefly in a consistent manner.
14. Repeat Steps 12 and 13 an additional time.
15. Remove the rack from the separator and add 500 uL of Color Solution to each tube.
16. Vortex for 1 to 2 seconds minimizing foaming.
17. Incubate for 30 minutes at room temperature.
18. Add 500 uL of Stopping Solution to each tube.
19. Add 1 mL Washing Solution to a clean test tube. Use as blank in Step 20.
20. Read results at 450 nm within 15 minutes after adding the Stopping Solution.

• Results

Manual Calculations

1. Calculate the mean absorbance value for each of the standards.
2. Calculate the %B/Bo for each standard by dividing the mean absorbance value for the standard by the mean absorbance value for the Diluent/Zero Standard.
3. Construct a standard curve by plotting the %B/Bo for each standard on vertical Logit (Y) axis versus the corresponding Pyrethroid concentration on horizontal Ln (X) axis on the graph paper provided.
4. %B/Bo for controls and samples will then yield levels in ppb of Pyrethroid by interpolation using the standard curve.

(Contact Abraxis for detailed application information on specific photometers.)

Photometric Analyzer

Some instrument manufacturers make available photometers allowing for calibration curves to be automatically calculated and stored. Refer to the instrument operating manual for detailed instructions. To obtain results from the Abraxis Pyrethroid Assay on instruments allowing data transformation, the parameter settings given below are recommended.

Multiply the sample results by a factor of 2 to account for the initial 1:1 dilution of sample with methanol or alternatively program the Photometric Analyzer as listed below to automatically correct for the dilution factor.

Data Reduct	:	Lin. Regression
Xformation	:	Ln/Logit
Read Mode	:	Absorbance
Wavelength	:	450 nm
Units	:	PPB
# Rgt Blk	:	0
Calibrators:		
# of Cals	:	5
# of Reps	:	2
Concentrations:		
#1:	0.0	PPB
#2:	0.75	PPB
#3:	2.5	PPB
#4:	5.0	PPB
#5:	15.0	PPB
Range	:	0.75 – 15.0
Correlation	:	0.990
Rep. %CV	:	10%

• Expected Results

In a study with soil extracts, the Abraxis Pyrethroid Assay was shown to correlate well with GC.

• Performance Data

Precision

The following results were obtained:

Control	1	2	3
Replicates	5	5	5
Days	5	5	5
n	25	25	25
Mean (ppb)	0.95	3.7	7.9
% CV (within assay)	7.1	5.6	4.0
% CV (between assay)	9.7	8.2	10.1

Sensitivity

The Abraxis Pyrethroid Assay has an estimated minimum detectable concentration, based on a 90% B/Bo of 0.75 ppb.

Recovery

Five (5) groundwater samples, were spiked with various levels of Permethrin and then assayed using the Abraxis Pyrethroid Assay. The following results were obtained:

Amount of Permethrin Added (ppb)	Recovery Mean (ppb)	Recovery S.D. (ppb)	Recovery %
1.0	0.92	0.13	92
3.75	3.97	0.21	106
7.50	7.11	0.32	95
Average			98

Specificity

The cross-reactivity of the Abraxis Pyrethoid Assay for various Pyrethroid analogues can be expressed as the least detectable dose (LDD) which is estimated at 90% B/Bo, or as the dose required for 50% absorbance inhibition (50% B/Bo).

Compound	LDD (ppb)	50% B/Bo (ppb)
Permethrin	0.750	4.25
Cypermethrin	4.75	100
Lambda (λ) Cyhalothrin	9.2	89.5
Bifenthrin	13.5	150
Resmethrin	200	2,400
Cyfluthrin	220	3,400
Tetramethrin	> 1,000	> 10,000
3, PBA	170	1,700

The following compounds demonstrated no reactivity in the Abraxis Pyrethroid Assay at concentrations up to 1000 ppb: aldicarb, aldicarb sulfoxide, aldicarb sulfone, alachlor, atrazine, benomyl, butachlor, butylate, captan, carbaryl, carbendazim, carbofuran, 2,4-D, 1,3-dichloropropene, dinoseb, MCPA, metolachlor, metribuzin, pentachlorophenol, picloram, propachlor, terbufos, thiabendazole, and thiophanate-methyl.

• Assistance

For ordering or technical assistance contact:

Abraxis LLC
Sales Department
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• Ordering Information

Abraxis Pyrethroid Assay Kit, 100T	PN	500201
Pyrethroid Sample Diluent	PN	500202

• General Limited Warranty

Abraxis LLC warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

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