Pyrethroids
PN 500201

- 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 "Color Solution", which contains 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 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.

  2. Pyrethroid Enzyme Conjugate
     The horseradish peroxidase (HRP) labeled Permethrin analog is diluted in buffered saline containing preservative and stabilizers.

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

  4. Control
     A concentration (approximately 3.0 ppb) of Permethrin in a methanolic solution containing preservative and stabilizers.

  5. Diluent/Zero Standard
     A methanolic solution containing preservative and stabilizers without any detectable Permethrin.

  6. Color Solution
     A solution of hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine in an organic base.

  7. Stopping Solution
     A solution of diluted sulfuric acid (0.5%).

  8. Washing Solution
     Preserved deionized water.

  9. Test Tubes
     Glass tubes (36) are packed in a box.

- 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.

- 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 repipet pipet.

  Vortex Mixer* Thermolyne Maxi Mix, Scientific Industries

  Vortex Genie, or equivalent

  Magnetic Separation Rack*

  Photometric Analyzer* capable of readings at 450 nm

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

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

- 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.

- Tube Number
  Contents of Tube

  1.2 Diluent/Zero Standard, 0 ppb
  Standard 1, 0.75 ppb
  Standard 2, 2.5 ppb
  Standard 3, 5.0 ppb
  Standard 4, 15.0 ppb
  Control
  Sample 1
  Sample 2
  Sample 3

- 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.

- 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.

- Use reagents directly to the bottom of the tube while avoiding 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.

- Procedural Notes and Precautions
  The Magnetic Separation System consists of two parts: an upper rack which will securely hold the test tubes and a lower separator which contains the magnets used to lower 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.

- 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.
4. Mix the Pyrethroid Antibody Coupled Paramagnetic Particles thoroughly and add 500 µL to each tube.
5. Incubate for 1 to 2 seconds minimizing foaming.
6. Incubate for 20 minutes at room temperature.
7. Add 250 µL 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 µL 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 µL 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 Ln (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.

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

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.

- 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%

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 to automatically correct for the dilution factor.

**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:

<table>
<thead>
<tr>
<th>Control</th>
<th>Mean</th>
<th>S.D.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep.</td>
<td>%CV (between assay)</td>
<td>Days</td>
<td>n</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>7.1</td>
<td>5.6</td>
<td>4.0</td>
</tr>
<tr>
<td>3</td>
<td>9.7</td>
<td>8.2</td>
<td>10.1</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Amount of</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permethrin</td>
<td>Mean (ppb)</td>
</tr>
<tr>
<td>Added (ppb)</td>
<td>1.0</td>
</tr>
<tr>
<td>3.75</td>
<td>3.97</td>
</tr>
<tr>
<td>7.50</td>
<td>7.11</td>
</tr>
<tr>
<td>Average</td>
<td>98</td>
</tr>
</tbody>
</table>

**Specificity**
The cross-reactivity of the Abraxis Pyrethroid 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).

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, captafol, 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, Inc.
124 Railroad Drive
Warminster, PA 18974
(215) 357-3911 * Fax (215) 357-5232
Email: info@abraxiskits.com
WEB: www.abraxiskits.com

**Ordering Information**

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

**General Limited Warranty**
Abraxis, Inc. 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.