Importance of Organophosphate/Carbamate (OP/C) Determination

The use of Organophosphate and Carbamate pesticides increased dramatically in the 1970s, following the ban on the use of organochlorine pesticides (such as DDT) on agricultural fields. OP/Cs are now among the most commonly used pesticides worldwide. Both Organophosphates and Carbamates operate through a similar mechanism, inhibiting the action of the enzyme acetylcholinesterase. Unfortunately, this enzyme is not unique to insects, making OP/Cs highly toxic to other animals, such as amphibians and birds and other mammals, including humans. Because of this high degree of toxicity, in addition to their use as insecticides, Organophosphates are also used as biological weapons (sarin, VX).

Exposure to Organophosphates and Carbamates can occur through ingestion of contaminated food or water, through contact with skin, and through inhalation. Symptoms of exposure to Organophosphates include headache; dizziness; increased nasal, ocular, and bronchial secretions; nausea; vomiting; diarrhea; seizures; decreased respiration, blood pressure, and heart rate; cardiac arrhythmia; and coma. Carbamates also decrease respiration and cause pulmonary edema. Due to the severe and potentially fatal effects of exposure, individuals providing assistance to OP poisoning victims are advised to wear protective clothing and to avoid contact with contaminated clothing and bodily fluids.

The OP/C plate assay allows for the analysis of 46 samples in duplicate determination. Less than 1 mL of sample is required. The test can be performed in less than 1 hour.

Performance Data

Limit of Detection Pattern (Sensitivity)

The limit of detection of the Organophosphate/Carbamate assay is estimated at 20% inhibition of color development. For compounds in 50% methanol:

Organophosphate	PPB
Azinphos methyl	0.3
Chlorpyrifos methyl	0.4
Chlorpyrifos ethyl	0.5
Diazinon	0.6
Dichlorvos	0.5
Dicrotophos	2.4
Disulfoton	40
Ethion	0.6
Malathion	1.2
Parathion	0.8
Phorate	1.0
Phosmet	1.2
Carbamate	**PPB**
Aldicarb	25
Carbaryl	206
Carbafuran	0.9

Precision

Three pools were spiked with an organophosphate pesticide at various levels and then assayed using the Abraxis Organophosphate/Carbamate Plate Assay. The following results were obtained when assayed in duplicate and run five times in each of 15 assays:

Between Assay Precision

Pool Number	1	2	3
Recovery	0.51 ppb	1.06 ppb	1.54 ppb
Standard Deviation	0.04 ppb	0.04 ppb	0.07 ppb
%CV	7.9%	3.4%	4.5%

Within Assay Precision

Pool Number	1	2	3
Recovery	0.53 ppb	1.07 ppb	1.53 ppb
Standard Deviation	0.02 ppb	0.03 ppb	0.05 ppb
%CV	3.9%	3.1%	3.5%

For ordering or technical assistance contact:

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

R091417

Organophosphate/Carbamate (Microtiter Plate)

Colorimetric Assay for the Determination
of Organophosphates and Carbamates in Contaminated Samples

Product No. 550055

1. General Description

The Abraxis Organophosphate/Carbamate (OP/C) plate assay is for the qualitative screening of a wide range of organophosphate (including thiophosphate) and carbamate pesticides. This test is suitable for the qualitative screening of water samples (drinking water, groundwater, surface water, and well water). The test can also be used for the testing of collected dislodgeable residues from surface wash as well as pesticide residues prepared from dried extracts (please contact Abraxis technical services for application bulletins and/or specific matrix validation guidelines). Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The positive control in the test kit contains a small amount of Diazinon. Avoid contact of kit reagents with skin and mucous membranes. If these reagents come in contact with skin, wash with water. Consult federal, state, and local regulations for proper disposal of all reagents.

3. Storage and Stability

The Organophosphate/Carbamate plate test kit should be stored in the refrigerator (4–8°C). 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.

4. Test Principle

The Abraxis Organophosphate/Carbamate plate assay is a qualitative colorimetric assay (modification of the Ellman method) for the detection of organophosphates and carbamates, based on a modification of their inhibition of the enzyme Acetylcholinesterase (Ach-E). Ach-E hydrolyzes acetylthiocholine (ATC), which reacts with 5,5'-Dithio-bis(2-Nitrobenzoic Acid) [DTNB] to produce a yellow color which is then read at 405 nm. If organophosphate or carbamate pesticides are present in a sample, they will inhibit Ach-E, reducing or eliminating color formation, depending on concentration.

Detection limits of the various OP/C pesticides differ depending on their ability to inhibit the enzyme (see Limit of Detection Pattern (Sensitivity) table). If it has been established that only a single OP/C is present, the test can be used in conjunction with appropriate standards for quantitative testing.

5. Limitations of the OP/C Plate Assay, Possible Test Interference

This test is recommended for use with samples in a matrix of 50% methanol. Other sample matrices may require modifications to the procedure and should be thoroughly validated (please contact Abraxis technical services for application bulletins and/or specific matrix validation guidelines).

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.

Pigmented samples may obscure color, potentially causing interferences, therefore a negative control should be prepared in a similar matrix and analyzed with the pigmented samples.

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, extreme temperatures (lower than 10°C or higher than 30°C) during the test performance, and performing the test in direct sunlight.

The Abraxis OP/C kit provides screening results. As with any analytical technique (GC/MS, HPLC, etc.), samples requiring regulatory action should be confirmed by an alternative method.

A. Reagents and Materials Provided

1. Microtiter Plate (12 X 8 strips)
2. Assay Buffer/Oxidizer Diluent (orange fluorescent dot), 10 mL
3. Negative Control, 50% methanol, 1 mL in an amber vial
4. Positive Control, 5 ppb Diazinon in 50% methanol, 1 mL in an amber vial
5. Oxidizer (orange fluorescent dot), 1 mL in an amber vial
6. Neutralizer (red dot), 3 mL
7. ACh-E (green dot), lyophilized enzyme, 1 vial
8. ACh-E Diluent (green dot), 4 mL (for reconstitution of lyophilized ACh-E)
9. ATC Substrate (blue dot), lyophilized, 1 vial
10. ATC, Diluent (blue dot), 4 mL (for reconstitution of lyophilized ATC Substrate)
11. Chromogen (yellow fluorescent dot), DTNB, 3 mL
12. Stopping Solution (purple dot), 3 mL

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

1. Micro-pipettes with disposable plastic tips (25-1000 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette (50-250 µL), or electronic repeating pipette with disposable plastic tips
3. Methanol (reagent grade)
4. Test tube or vial for dilution of Oxidizer
5. Timer
6. Tape or parafilm
7. Microtiter plate reader (wave length 405-450 nm)

C. Notes and Precautions

This procedure is for use with samples in a matrix of 50% methanol. Aqueous samples must therefore be diluted 1:1 with methanol before evaluation. Samples prepared as dry extracts (solvent evaporated residues) or as residues dislodged from surface washes must also be in 50% methanol for evaluation. Other sample matrices may require modifications to the procedure and should be thoroughly validated (please contact Abraxis technical services for application bulletins and/or specific matrix validation guidelines).

Pigmented samples may obscure color, potentially causing interferences, therefore a negative control should be prepared in a similar matrix and analyzed with the pigmented samples.

Diluted oxidizer must be made fresh for each assay. Use of previously diluted oxidizer may produce inaccurate results.

A high positive pesticide control (5 ppb Diazinon in 50% methanol) is provided with the OP/C test kit. It is recommended that the positive control be included in every run and treated in the same manner as unknown samples. Acceptable limits should be established by each laboratory.

If the negative control does not produce a yellow color when the assay procedure is complete, the test is invalid and should be repeated to obtain accurate results.

Micro-pipetting equipment and disposable pipette tips are necessary. The use of a multi-channel pipette, stepping pipette, or electronic repeating pipette is recommended for adding the assay buffer, diluted Oxidizer, Neutralizer, reconstituted ACh-E, Substrate (ATC), Chromogen (DTNB), and Stopping solutions in order to equalize the incubation periods on the entire microtiter plate. All pipette tips should be discarded after use and new tips obtained for subsequent testing.

To avoid drift and obtain accurate results, the addition of the diluted Oxidizer, Neutralizer, reconstituted ACh-E, Substrate (ATC), Chromogen (DTNB), and Stopping solutions should be performed in less than 2 minutes for each reagent. If additions to the entire microtiter plate cannot be completed in less than 2 minutes, run size should be decreased to the number of rows which can be pipetted in less than 2 minutes.

Samples containing gross particulate matter should be filtered (e.g. 0.2 µm Anotop™ 25 Plus, Whatman, Inc.) to remove particles.

If performing the assay outdoors, avoid direct sunlight.

Please only use the reagents from one package lot in one test, as they have been adjusted in combination.

D. Test Preparation

1. Allow the reagents and samples to reach room temperature before use.
2. The negative control, positive control, neutralizer, chromogen, and stopping solutions are ready to use and do not require any further dilutions.
3. Reconstitute the lyophilized ACh-E by adding 3 mL of the ACh-E diluent to the lyophilized enzyme (matching green dots), cap tightly and mix by shaking moderately. Allow at least 5 minutes for the ACh-E to go into solution before use in the assay.
4. Determine the amount of diluted oxidizer needed for the assay. Dilute the oxidizer at a ratio of 1:9 in oxidizer diluent (matching orange fluorescent dots). For example, to prepare 1 mL of diluted oxidizer, add 100 µL of oxidizer to 900 µL of oxidizer diluent in a glass vial or test tube. Mix by shaking moderately. **Please note that the diluted oxidizer must be made fresh for each assay. Use of previously diluted oxidizer may produce inaccurate results.**
5. Reconstitute the lyophilized substrate by adding 3 mL of the ATC diluent to the lyophilized ATC substrate (matching blue dots), cap tightly, and mix by shaking moderately.

E. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test.

Neg.: Negative Control
Pos.: Positive Control

Samp1, Samp2, etc.: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Neg.	etc.										
B	Neg.	etc.										
C	Pos.											
D	Pos.											
E	Samp1											
F	Samp1											
G	Samp2											
H	Samp2											

F. Assay Procedure

1. Add 50 µL of the **assay buffer** (orange fluorescent dot) to the wells to be used in the assay.
2. Add 25 µL of the appropriate **control or sample** to appropriate wells according to the working scheme given. Analysis in duplicate or triplicate is recommended. Mix the contents by moving the strip holder in a circular motion on the benchtop for 15 seconds. Be careful not to spill the contents.
3. Add 25 µL of the **diluted Oxidizer** to each well. Mix the contents by moving the strip holder in a circular motion on the benchtop for 15 seconds. Be careful not to spill the contents. Incubate for 5 minutes at 70°F ± 20°.
4. Add 25 µL of **Neutralizer** (red dot) to each well. Mix the contents by moving the strip holder in a circular motion on the benchtop for 15 seconds. Be careful not to spill the contents.
5. Add 25 µL of the **reconstituted ACh-E** (green dot) to each well. Mix the contents by moving the strip holder in a circular motion on the benchtop for 15 seconds. Be careful not to spill the contents. Incubate for 15 minutes at 70°F ± 20°.
6. Add 25 µL of **reconstituted Substrate** (blue dot) to each well. Mix the contents by moving the strip holder in a circular motion on the benchtop for 15 seconds. Be careful not to spill the contents.
7. Add 25 µL of the **Chromogen** (yellow fluorescent dot) to each well. Mix the contents by moving the strip holder in a circular motion on the benchtop for 15 seconds. Be careful not to spill the contents. Incubate for 30 minutes at 70°F ± 20°.
8. Add 25 µL of **Stopping Solution** (purple dot) to each well. Mix the contents by moving the strip holder in a circular motion on the benchtop for 15 seconds. Be careful not to spill the contents.
9. Read the absorbance at 405 nm (optimum wavelength) or 450 nm using a microplate ELISA photometer. Be sure no bubbles are visible in the wells before reading, as bubbles may cause erroneous readings.

G. Evaluation

The negative control and any sample that has no detectable organophosphate or carbamate will develop a dark yellow color. **If the negative control does not result in a yellow color, the test is invalid and should be repeated.** Any sample with a detectable organophosphate or carbamate residue will have a reduced color development compared to the negative control. A 20% inhibition of color indicates the presence of an organophosphate or carbamate at or above the limit of detection (please see Limit of Detection Pattern (Sensitivity) table).