OP/Carbamate

• Intended Use

For the detection of a wide range of organophosphates including thiophosphate, and carbamate pesticides in water, (drinking water, ground water, surface water and well water). This plate assay can also be used for testing collected dislodgeable residues from a surface wash, as well as pesticide residues prepared from dried extracts (please contact Abraxis technical support for information).

• Principle

The test 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 Acetyl Cholinesterase (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 OP or Carbamate pesticides are present in a sample, they will inhibit ACh-E reducing or eliminating color formation depending on their concentration.

Detection limits of the various OP/C pesticides differ depending on their ability to inhibit the enzyme (refer to 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.

• Reagents

The Abraxis OP/Carbamate Kit contains the following items:

(1) 1 Microtiter plate with removable 8 strips of 12 wells and a stripholder.
(2) 1 vial (blue dot), used as diluent for the substate (ATC), 5 ml.
(3) 1 vial (green dot), used as diluent for the lyophilized ACh-E, 3 ml.
(4) 1 Amber vial with 1 ml of positive pesticide control-5 ppb Diazinon in 50% MEOH
(5) 1 Amber vial (orange fluorescent dot), 1 ml of OXIDIZER .
(6) Assay Buffer and Oxidizer Diluent (orange fluorescent dot), 10 ml.
(7) 1 vial (red dot), 3 ml.-NEUTRALIZER
(8) 1 vial (green dot) ACh-E, lyophilized
(9) 1 vial (blue dot), SUBSTRATE, ATC, lyophilized
(10) 1 vial (yellow fluorescent dot), CHROMOGEN (DTNB) 3 ml.
(11) 1 vial (purple dot),STOPPING SOLUTION, 3 ml.
(12) 1 Amber vial with 1ml of negative pesticide control-50% MEOH.

• Reagent Storage and Stability

Store all reagents at 2-8°C. Reagents may be used until the expiration date on the box.

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:
Precision pipets capable of delivering 25-1000 ul and tips
Microplate* or strip reader capable of reading absorbance between 405-450 nm
Test tube or vial to use for diluting of Oxidizer

* Please contact Abraxis for supplier information.

• Sample Information

(1) This procedure is recommended for use with samples in a matrix of 50% MEOH. Other sample matrices may require modifications to the procedure and should be thoroughly validated (contact Abraxis Technical support for information and guidance). If testing water samples, the samples must be diluted with and equal volume of methanol upon collection and before testing.

(2) Samples may be prepared as dry extracts (solvent evaporated residues) or as residues dislodged from surface washes (see Sample Preparation under Assay Procedure). Other samples may require modifications to the procedure and should be validated.All samples to be analyzed should be in a 50% MEOH matrix.

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

Pigmented samples may obscure color and cause some interferences, therefore a negative control should be prepared in a similar matrix.

• Reagent Preparation

All reagents must be allowed to come to room temperature.

(1) **ACh-E** – Add the 3 ml of the ACh-E diluent to the lyophilized enzyme, cap vial and mix by shaking moderately. Allow at least 5 minutes for the ACh-E to go into solution before use in the assay.

(2) **Oxidizer** –Determine the amount of diluted oxidizer needed for the assay. Dilute the oxidizer (1 part oxidizer to 9 parts oxidizer diluent) and mix by shaking moderately. **This diluted oxidizer must be made fresh for each assay**.

(3) **Substrate** (ATC) – Add 3 ml of ATC diluent to the lyophilized ATC, cap the vial and mix by shaking moderately.

• Procedural Notes and Precautions

As with all assays, a consistent technique is the key to optimal performance. To obtain the greatest precision, be sure to treat each plate to mix the contents well in an identical manner. Swirl by moving the strip holder in a circular motion on the benchtop. Be careful not to spill contents of the wells.

Add reagents directly to the bottom of the well 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 pipet tips for each sample addition and by avoiding contact between reagent droplets on the wells and pipet tips.

If performing the assay outdoors, avoid direct sunlight.

Do not use any reagents beyond their stated shelf life.

Avoid contact of reagents with skin and mucous membranes. If a reagent comes in contact with skin, wash with water.

• Limitations

The Abraxis OP/Carbamate Assay will detect organo-phosphates and carbamates to different degrees. Refer to specificity table for data . The Abraxis OP/Carbamate 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.

• Quality Control

A high positive pesticide control is provided with the Abraxis OP/Carbamate Assay kit (5 ppb of Diazinon in 50% MEOH). 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. Add 50 ul of assay buffer (orange fluorescent dot) to microwells to be used in the assay.
2. Add 25 uL of the appropriate **control or sample** to designated assay wells, swirl plate to mix -15 seconds.

Well Number	Contents of Tube
A1,2	Negative Control
A3,4	Positive Control
A5,6	Sample 1
A7,8	Sample 2
B1,2	Sample 3
B3,4	Sample 4
B5,6	Sample 5
B7,8	Sample 6

3. Add 25 ul of **diluted Oxidizer** into assay each wells, swirl plate to mix-15 seconds. Incubate for 5 minutes at 70º F +/- 20 degrees
4. Add 25 ul of **Neutralizer** (red dot) into each assay well, swirl plate to mix-15 seconds.
5 Add 25 ul of **ACh-E** (green dot) into each assay well, swirl to mix plate-15 seconds. Incubate 15 minutes at 70° F +/- 20 degrees.
6. Add 25 ul of **Substrate**-ATC (blue dot) into assay each well, swirl plate to mix-15 seconds.
7. Add 25 ul of **Chromogen** – DTNB (yellow fluorescent dot) into each assay well, swirl plate to mix-15 seconds. Incubate 30 minutes at 70° F +/- 20 degrees
8. Add 25 ul of **Stopping Solution** (purple dot) into each assay well, swirl plate to mix-15 seconds. Read at 405 nm (optimum wavelength) or 450 nm. Be sure no bubbles are viscible in any well. The bubbles cause erroneous readings.

• Results

The negative control and any sample that has no detectable organophosphate or carbamate will develop a dark yellow color. 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 a organophosphate or carbamate at or above the limit of detection (please refer to sensitivity table).

NOTE: If the negative control does not result in a yellow color, the test is invalid and should be repeated.

Limit of Detection Pattern (Sensitivity)

Limit of Detection of the Abraxis OP/Carbamate Test is estimated at 20% inhibition (IC 20) of color development

Compound in 50% MEOH

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

Carbamates	PPB
Aldicarb	25
Carbaryl	206
Carbafuran	0.9

• Performance Data

Precision

Three pools were spiked with an organophosphate pesticide at various levels and then assayed using the Abraxis OP/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 1 (ppb)	Pool 2 (ppb)	Pool 3 (ppb)
X	0.51	1.06	1.54
SD	0.04	0.035	0.07
CV%	7.86	3.35	4.48

Within Assay Precision

	Pool 1 (ppb)	Pool 2 (ppb)	Pool 3 (ppb)
X	0.53	1.07	1.53
SD	0.02	0.03	0.05
CV%	3.9	3.1	3.5

• Ordering information

Abraxis OP/Carbamate Assay Kit 96 Tests PN 550055

• Assistance

For ordering or technical assistance contact:

Abraxis LLC
Sales Department
54 Steamwhistle Drive
Warminster, Pennsylvania, 18974

Phone : (215) 357-3911
Fax: (215) 357-5232
Email: info@abraxiskits.com
WEB: www.abraxiskits.com

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