

# OP/Carbamate

## • Intended Use

For the detection of a wide range of organophosphate (including thiophosphate), and carbamate pesticides in water, (drinking water, ground water, surface water and well water). This assay can also be used for testing these compounds collected as dislodgeable residues from a surface wash, as well as pesticide residues prepared as a dry extract (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 read at 405 nm. If OP or Carbamate pesticides are present in a sample, they will inhibit ACh-E and therefore the color formation will be reduced or absent 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) 20 Polystyrene test tubes (white caps), containing 500 ul of assay buffer. **ASSAY TUBES.**
- (2) 1 test tube (blue cap), used as **NEGATIVE (-) CONTROL** and as a substrate (ATC) diluent, 5 ml.
- (3) 1 test tube (green cap), used as diluent for the lyophilized ACh-E, 3ml.
- (4) Pesticide (+) **POSITIVE CONTROL** (amber vial), 1 ml.
- (5) **OXIDIZER** (ambervial), 1 ml.
- (6) Oxidizer Diluent (orange cap dropper bottle), 1.8 ml of assay buffer.
- (7) **NEUTRALIZER** (red cap dropper bottle), 2ml.
- (8) **ACh-E**, lyophilized (green cap dropper bottle)
- (9) **SUBSTRATE**, ATC, lyophilized (blue cap dropper bottle).
- (10) **CHROMOGEN** (DTNB) (yellow cap dropper bottle), 2ml.
- (11) **STOPPING SOLUTION** (purple cap dropper bottle), 2 ml.
- (12) 100 ul exact volume pipettes, 22 each.
- (13) 3 ml transfer pipets, 2 each.
- (14) Work station box (WSB).

## • 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:

Photometer\* capable of readings at 405-450 nm

\* Please contact Abraxis for supplier information.

## • Sample Information

This procedure is recommended for use with water samples. Other samples may require modifications to the procedure and should be thoroughly validated (contact Abraxis Technical support for information and guidance).

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

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.

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

## • Reagent Preparation

All reagents must be allowed to come to room temperature.

(1) **ACh-E** – Using a 3ml transfer pipet, remove 2 ml from the green cap test tube and add to the green cap dropper bottle by removing the green cap and dropper tip. After adding the 2 ml into the bottle put back the dropper tip and green cap on the bottle 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** – Using a 100 ul volume pipet, remove 200 ul (2 X 100 uL) of oxidizer from the amber vial and add to the orange cap dropper bottle by removing the orange cap and dropper tip. After adding the 200 ul into the bottle, put back the dropper tip and orange cap on the bottle and mix by shaking moderately. **This diluted oxidizer must be made fresh for each assay.** If additional diluted oxidizer needs to be prepared, dispense 2 ml of solution from the **negative control** tube using the transfer pipette, and 200 uL of the concentrated **Oxidizer** using the 100 uL exact volume pipettes (2 shots).

(3) **Substrate (ATC)** – Using a 3ml transfer pipet remove, 2 ml from the blue cap test tube, add to the blue cap dropper bottle by removing the blue cap and dropper tip. After adding the 2 ml into the bottle put back the dropper tip and blue cap on the bottle 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 tube in an identical manner.

**Proper usage of 100 ul exact volume pipette**—Squeeze the top bulb of the exact volume pipet and place the tip into the sample solution. Release the top bulb and the sample will be drawn into the pipet, any **overflow sample** will go into the middle bulb. Remove the pipet from the sample and transfer to assay tube by squeezing the top bulb to deliver the 100 ul sample (contained in the tip of the pipette). **Note:** Be careful not to allow the sample in the overflow bulb to be delivered with the 100 ul sample.

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. When using the dropper bottle, invert so the dropper tip is into the assay test tube as far as possible. Squeeze the bottle so that 2 drops of reagent fall into the bottom of assay tube. Avoid drops falling onto side of assay tube.

If performing 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.

Operators wearing heavy personal protection equipment such as heavy butyl gloves, etc. should use micropipette dispensers with disposable tips to dispense standards/samples and assay reagents (2 drops are equivalent to 80 uL).

## • Limitations

The Abraxis OP/Carbamate Assay will detect organophosphates 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. The positive is 5 ppb of Diazinon in DI water. 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. Label test tubes for controls, and samples. Place assay tubes into Work Station Box (WSB) and remove white caps, discard the caps.

Tube Number	Contents of Tube
1,2	Negative Control
3,4	Positive Control
5,6	Sample 1
7,8	Sample 2
9,10	Sample 3
11,12	Sample 4
13,14	Sample 5
15,16	Sample 6

2. Using the supplied pipettes, add 100 uL of the appropriate **control, or sample** into designated assay tubes, shake WSB to mix.
3. Add 2 drops of **Oxidizer** (orange cap dropper bottle) into assay tubes, shake WSB to mix. Incubate 5 minutes at 70° F +/- 20 degrees
4. Add 2 drops of **Neutralizer** (red cap dropper bottle) into assay tubes, shake WSB to mix.
5. Add 2 drops of **ACh-E** (green cap dropper bottle) into assay tubes, shake WSB to mix. Incubate 15-30 minutes at 70° F +/- 20 degrees
6. Add 2 drops of **Substrate-ATC** (blue cap dropper bottle) into assay tubes, shake WSB to mix.
7. Add 2 drops of **Chromogen** – DTNB (yellow cap dropper bottle) into assay tubes, shake WSB to mix. Incubate 15-30 minutes at 70° F +/- 20 degrees
8. Add 2 drops of **Stopping Solution** (purple cap dropper bottle) into assay tubes, shake WSB to mix. Read at 405 nm (optimum wavelength) or 450 nm.

## • 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 an 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% (IC 20) inhibition of color development.

**Compound                      Water**

<u>Organophosphate</u>	<u>PPB</u>
Azinphos methyl	0.8
Chlorpyrifos methyl	1.0
Chlorpyrifos ethyl	1.3
Diazinon	1.0
Dichlorvos	0.5
Dicrotophos	20
Disulfoton	25
Ethion	3.9
Malathion	1.4
Parathion	1.0
Phorate	4.0
Phosmet	0.7

**Limit of Detection Pattern**

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

<u>Carbamates</u>	<u>PPB</u>
Aldicarb	10
Carbaryl	160
Carbofuran	1.2

**• Ordering information**

Abraxis OP/Carbamate Assay Kit 20T                      PN 550051

**• Assistance**

For ordering or technical assistance contact:

Abraxis LLC  
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