Limit of Detection Pattern-Carbamates
Limit of Detection of the ABRAXIS® OP/Carbamate Test is estimated at 20% (IC 20)inhibition of color development.

Carbamates	PPB
Aldicarb	10
Carbaryl	160
Carbofuran	1.2

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ABRAXIS® OP/Carbamate ELISA Tube Particle

Product No. 550051

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 dryextract (please contact Gold Standard Diagnostics technical support for information).

Storage and Stability
Store all reagents at 2-8 °C. Reagents may be useduntil the last day of the month as indicated by the expiration date on the box. Consult state, local and federal regulations for proper disposal of all reagents.

Assay 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 OPor Carbamate pesticides are present in a sample, theywill 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 (referto 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.

Limitations of the ABRAXIS® OP/Carbamate ELISA Tube Particle
The ABRAXIS® OP/Carbamate ELISA Tube Particle Kit will detectorgano-phosphates and carbamates to different degrees. Refer to specificity table for data. The ABRAXIS® OP/Carbamate ELISA Tube Particle Kit provides screening results. As with any analytical technique (GC, HPLC, etc...) positive results requiring some action should be confirmed by an alternative method.

Procedural Notes and Precautions
As with all assays, a consistent technique is the key tooptimal 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 pipet tip. This will help assure consistent quantitiesof 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 thedropper bottle, invert so the dropper tip is into the assay test tube as far as possible. Squeeze the bottleso 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 shelflife.
- 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 todispense standards/samples and assay reagents (2

drops are equivalent to 80 uL).

Working Instructions

Materials Provided

1. 20 Polystyrene test tubes (white caps), containing 500 uL of assay buffer. ASSAYTUBES.
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 forthe lyophilized ACh-E, 3 mL.
4. Pesticide (+) POSITIVE CONTROL (ambervial), 1 mL.
5. OXIDIZER (amber vial), 1 mL.
6. Oxidizer Diluent (orange cap dropperbottle), 1.8 mL of assay buffer.
7. NEUTRALIZER (red cap dropper bottle),2 mL.
8. Ach-E, lyophilized (green cap dropperbottle)
9. SUBSTRATE, ATC, lyophilized (blue capdropper bottle).
10. CHROMOGEN (DTNB) (yellow cap dropperbottle), 2 mL.
11. STOPPING SOLUTION (purple cap dropperbottle), 2 mL.
12. 100 uL exact volume pipettes, 22 each.
13. 3 mL transfer pipets, 2 each.
14. Workstation box (WSB).

Materials Required (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

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 Gold Standard Diagnostics 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 AssayProcedure). 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 roomtemperature.

1. ACh-E – Using a 3 mL 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 andmix 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 byshaking moderately. This diluted oxidizer must be made fresh for each assay.

   NOTE: 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 volumepipettes (2 shots).
3. Substrate (ATC) – Using a 3 mL 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 bottleput back the dropper tip and blue cap on the bottle and mix by shaking moderately.

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 andPrecautions before proceeding.

1. Label test tubes for controls, and samples.Place assay tubes into Work Station Box (WSB) and remove white caps, discard thecaps.

Tube Number	Contents of Tube	Tube Number	Contents of Tube
1,2	Negative Control	9,10	Sample 3
3,4	Positive Control	11,12	Sample 4
5,6	Sample 1	13,14	Sample 5
7,8	Sample 2	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 dropperbottle) into assay tubes, shake WSB to mix. **Incubate 5 minutes at 70º F +/- 20 degrees**
4. **Add 2 drops of Neutralizer** (red cap dropperbottle) 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 dropperbottle) into assay tubes, shake WSB to mix.
7. **Add 2 drops of Chromogen – DTNB** (yellow capdropper bottle) into assay tubes, shake WSB to mix
8. **Incubate 15-30 minutes at 70° F +/- 20 degrees**
9. **Add 2 drops of Stopping Solution** (purple cap dropper bottle) into assay tubes, shake WSB to mix
10. Read at 405 nm (optimum wavelength) or450 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 tothe negative control. A 20% inhibition of color indicates the presence of organophosphate or carbamate at or above the limit of detection (pleaserefer to sensitivity table)

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

Limit of Detection Pattern (Sensitivity)-OP

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

Compound Organophosphate	Water PPB
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