Organophosphate/Carbamate Detection Assay

Catalog Number: EZ2015

For Research Use Only. Not for use in Diagnostic Procedures.

1. Background

The kit uses a spectrophotometric kinetic assay to detect organophosphate and carbamate (OPaC) pesticides directly from samples. The unique features of the kit are:

- High sensitivity
- Rapid
- Robust
- High reproducibility
- Flexible format
- Proprietary enzyme designed for higher sensitivity and specificity of OPaC.

2. Kit Contents (96 determinations)

Component Name	Volumes	Storage
Substrate Solution	10 mL	2-8°C
Chromogen Solution	2 mL	2-8°C
AChE Solution	2 mL	2-8°C
Reaction Buffer	11 mL	2-8°C
Oxonation Reagent 1	1 mL	-15 to -25°C
Oxonation Reagent 2	1 mL	-15 to -25°C
96-well Microplate	1 each	15-35°C

3. User Supplied Materials

- Micro-pipettes with disposable plastic tips to pipet 5-20 µL.
- Micro-pipettes with disposable plastic tips to pipet 20-200 µL.
- Methanol (optional)
- Timer
- Microtiter plate reader (wavelength 412 nm)
- Nix QC Pro

4. Organophospthate Test Method

Organophosphate and Carbamates (OPaC) are a target in the agricultural industry because of their role as a pesticide. The AttoTector OPaC detection kit is designed specifically to detect OPaC in liquid or solid samples which have been extracted to recover OPaC. The format to detect OPaC is simple and sensitive. The reaction uses an enzymatic reaction that can be detected using absorbance at 412 nm. In the presence of OPaC, the rate of chromophore production is reduced in a concentration dependent fashion. The higher the concentration of OPaC, the less color change is produced.

The Attogene test uses the property of OPaC to inactivate the acetylcholinesterase enzyme (AChE) to block the formation of the colored product. The OPaC is detected by analyzing the absorbance of each sample well at 412 nm using a plate reader. The OPaC in each sample is then directly detected from the reduced change in absorbance at 412 nm during a 10-minute reaction time.

5. Instructions

Method control: It is best to run a set of Negative and Positive controls with each sample set run to ensure comparable readings from the day, time and user. Depending on the Pesticide residue being detected, a spike solution that can be used to generate the positive control can be made and added into control tubes.

- Thaw out Oxonation Reagent 1 and Oxonation Reagent 2 at room temperature prior to performing the test.
- Allow the other reagents to warm to room temperature for 60 minutes prior to performing the test.

6. Protocol Summary

1. Add 50 µL of sample solution (and Negative and Positive controls) into microplate wells (duplicates or triplicates recommended).
2. Add 5 µL of Oxonation Reagent 1 and 10 µL of Reaction Buffer to each well containing sample or control. Mix and incubate 10 minutes at room temperature.
3. Add 5 µL of Oxonation Reagent 2 to each well containing sample or control. Mix and incubate 5 minutes at room temperature.
4. Carefully add 20ul of AchE solution into each well. Mix and incubate 15 minutes at room temperature.
5. Add 100 µL of Reaction Buffer and 20 µL Chromagen solution to each well.
6. Begin the reaction by adding 100 µL Substrate Solution to each well.
7. Measure the increase in absorbance at 412 nm over a 10-minute interval for each well.

7. Example Microplate Layout:

	A	B	C	D	E
1	Neg				
2	Neg				
3	Pos				
4	Pos				
5	Sample 1				
6	Sample 1				
7	Sample 2				
8	Sample 2				

If quantitative results are required, it is possible to set up a set of standards at known concentrations of specific pesticides which can be used to extrapolate the concentration in the sample being analyzed, loading into a 96 well plate and reading the samples at 412nm.

8. 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. 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.

9. Determination of Pesticide in Samples

If the enzyme activity in the sample is 20% lower than the negative control it is indicative that the sample may contain an Organophosphate or Carbamate residue at the concentration above the limit of detection.

It is important to run the control reaction of known negative sample to ensure that matrix from the sample is not non-specifically inhibiting the reaction. Specificity of the presence of a pesticide can be further confirmed using an analytical method such as HPLC and Mass Spec as needed.

Notes on the measurement: The color of the reaction may continue to change after the specified reaction time has elapsed. The rate of the reaction is impacted by the room temperature thus, incubating the plate in a set temperature incubator at 25°C, it can help ensure consistency.

Note: If the test shows the concentration may actually be higher than a diluted standard. In this case, we recommend carrying out a stepwise dilution of the sample with distilled water, to bring the OPaC content into the measuring range of the known concentration positive control. The dilution factor must be taken into account when calculating the OPaC content.

Sensitivity for many common organophosphate or carbamate represented in ppb: Malathion 4.1; Chlorpyrifos 1.4; Diazinon 73; Phorate 42.

Because detection limits of the various organophosphate or Carbamate pesticides differ depending on their ability to inhibit the enzyme. If it has been established that only a single organophosphate or carbamate is present, the test can be used in conjunction with appropriate standards for quantitative testing.

NOTE: FOR INFORMATION ON SAMPLE PREPARATION METHODS, CONTACT ATTOGENE AT SUPPORT@ATTOGENE.COM FOR DETAILED INSTRUCTIONS.

Who we are:

Attogene is a biotechnology company located in Austin, Texas. Our focus is to enhance health and wellness by offering and developing customer focused Life Science Products domestically and internationally.

Our mission is to:

- Enhance detection technologies
- Enable rapid responses
- Enable impactful research discoveries

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