Organophosphate/Carbamate Detection Assay

(Electric Eel Acetylcholinesterase)

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:

- Rapid
- Robust
- High reproducibility
- Flexible format

2. Kit Contents (96 determinations)

Component Name	Volumes	Storage
Chromogen Solution (DTMB)	2 x 1 mL	2-8°C
AChE Solution (Acetylthiocholine esterase derived from Eel)	200 µL	2-8°C
Reaction Buffer	10.56 mL	2-8°C
Substrate Solution (Acetylthiocholine -ATC)	5 x 1 mL	-15 to -25°C
Oxonation Reagent I	4 x 120 µL	-15 to -25°C
Oxonation Reagent II	4 x 120 µL	-15 to -25°C
OP Pesticide Mix A (0ppm, 0.4ppb, 0.8ppm, 1.6ppm, 3.2ppm, 4.8ppm)	0.8mL x 6	-15 to -25°C
Negative Control	100 µL	-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

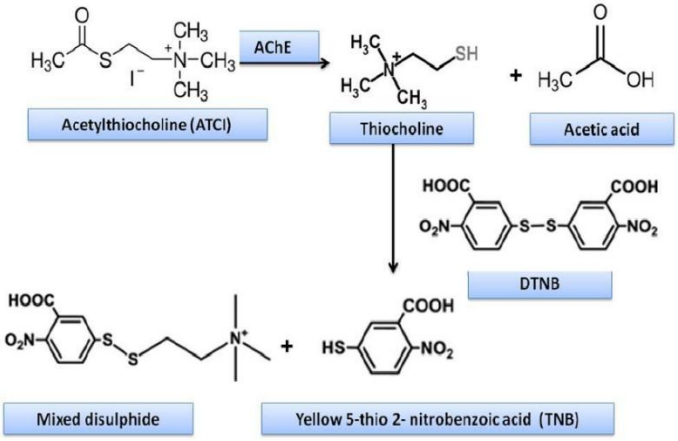
- Microtiter plate reader (wavelength 412 nm)

4. Test Method

OPaC compounds are a class of pesticides with tremendous worldwide use that have been developed to specifically inhibit the acetylcholinesterase (AChE) enzymes. Once inside an organism, OPaCs result in the buildup of acetylcholine (Ach) in the nerve synapse, causing excessive excitation of the nerves. OPaCs are known to be harmful to humans where they can have serious health consequences with varying extremes of severity. In turn, regulatory agencies both domestically and internationally have instituted regulations and guidelines to monitor OPaC accumulation to mitigate their impact on human health.

This Attogene OPaC detection kit is designed specifically to detect OPaC in liquid or solid samples which have been extracted to recover OPaC. Because the majority of OP designed pesticides are thiophosphates (P=S) that do not readily inhibit acetylcholinesterase they need to be chemically converted to the cognate oxon organophosphate (oxo-) via an oxonization reaction where P=S is converted to P=O. Conversion of organothiophosphates to activated pesticides in the laboratory requires specific chemical treatment. Therefore, we designed a system in which the sample can react with oxination reagents supplied in this kit (Oxonation Reagent I and Oxonation Reagent II).

This test uses the property of OPaC to inactivate the electric eel acetylcholinesterase enzyme (AChE) to block the formation of thiocholine and acetic acid (as shown in the reaction schematic below). 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 and can be quantified using a set of OPaC standards to create a standard curve and quantify the amount of OPaC in the sample.



5. Instructions

Method control: It is best to run a set of standards with each sample set 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 fortified sample which are often used as an extraction control.

- Thaw out Oxonation Reagent I, Oxonation Reagent II and ATC at room temperature prior to performing the test.
- Return Oxonation Reagent I, Oxonation Reagent II and ATC to the freezer directly after 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 or standard into microplate wells (duplicates or triplicates recommended).
2. Add 5 µL of Oxonation Reagent I and mix well.
3. Incubate 10 minutes at room temperature.
4. Add 5 µL of Oxonation Reagent 2 to each well and mix well.
5. Incubate 5 minutes at room temperature.
6. Carefully add 2ul of AchE solution into each well and mix well.

7. Add 100 µL of Reaction Buffer and mix well.
8. Add 20 µL Chromagen (DTMB) Solution to each well and mix well.
9. Add 100 µL Substrate Solution (ATC) to each well and mix well.
10. 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	0ppb				
2	0ppb				
3	400ppb				
4	400ppb				
5	800ppb				
6	1,600ppb				
7	3,200ppb				
8	4,800ppb				

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 10%-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 standard. In this case, we recommend carrying out a stepwise dilution of the sample with distilled 10% methanol, to bring the OPaC content into the measuring range of the standards. The dilution factor must be taken into account when calculating the OPaC content.

Sensitivity for many common organophosphate or carbamate represented in ppm: Malathion 4; Chlorpyrifos 1; Diazinon 7; Phorate 4. Detection limits of the various organophosphate or Carbamate pesticides differ depending on their ability to inhibit the Eel 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.

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