Pesticide Detection Strip Test

Catalog Number: AU2070

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

1. Background

The Pesticide Detection Strip Test kit is a unique strip-based test to detect organophosphate and carbamate (OPaC) pesticides directly from samples. It is a simple visual test that does not require the use of a reader or other instrument. The unique features of the kit are:

- High sensitivity
- Rapid
- Robust
- High reproducibility
- Flexible format
- No glass ampoules required
- No toxic fumes
- Rapid, convenient one-step activation (oxonation) procedure

2. Kit Contents (24 determinations)

This kit contains sufficient reagents and materials to perform 24 tests.

Component Name	Volumes	Storage **
Test Strips	24	-15 to -25°C
Enzyme Solution	300 µL	-15 to -25°C
Activator Solution	500 µl	-15 to -25°C
Buffer Solution	500 µl	-15 to -25°C
Substrate Solution	500 µl	-15 to -25°C
Negative Control	1400 µL	-15 to -25°C
96-well Microplate	1 each	-15 to -25°C

** The kit can also be stored at 2 – 8 °C for up to 3 months after receipt.

When stored frozen, the kit must be allowed to warm up at room temperature for 75 – 90 minutes before handling or use to ensure integrity of nitrocellulose strips.

3. User Supplied Materials

- Pipet tips and pipettor
- Timer
- Tweezers or forceps

4. Test Method

Organophosphate and carbamate pesticide compounds (OpaCs) have widespread use that have been developed to specifically inhibit the acetylcholinesterase (AChE) enzymes. Once ingested, 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.

The Attogene Pesticide Detection Strip Test kit is designed to specifically detect OPaCs in water samples and liquid samples which have been extracted to recover OPaC.s 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. Therefore, the test includes an oxonation step the pesticides to the active oxon form (using an Activation Solution).

Our rapid Test Strip assay offers a streamlined, visual method for detecting the presence of these pesticides in liquid samples. By coupling a flow-capture design and sensitive visual colorimetric detection, this system delivers semi-quantitative results without the need for readers or other complex laboratory equipment.

The Principle

The assay relies on the specific inhibition of acetylcholinesterase by OPaC compounds. When a sample is introduced, any OPaC compounds present bind and inhibit the enzyme. As this mixture flows up the strip via capillary action, the enzyme is captured at the test line. This concentrates the signal and improves sensitivity.

Signal detection is enabled by a chromogenic Substrate Solution. If the captured enzyme remains active (no inhibitor present), it reacts with the applied substrate to produce a blue color at the test line (Figure1). If inhibitors are present, they block and inactivate the enzyme's active site, preventing the color reaction.

Key Features

- **Visual Interpretation:** Results are easily read by the naked eye based on color intensity.
- **High Sensitivity:** The tight binding affinity between the inhibitor and enzyme ensures detection even at low concentrations.
- **Built-in Verification:** Each assay is designed to be run in parallel with a Negative Control. By comparing the unknown sample to a high-intensity control, users can accurately verify the degree of inhibition.
- **Rapid Workflow:** Moves from sample application to results in minutes.

Understanding Your Results

OpaC compounds in samples are detected by visualizing the intensity of the test line on the strip. The presence of organophosphate or carbamate pesticides in water samples causes a reduction in the observed test line. Samples containing 40 ppb chlorpyrifos cause complete inhibition of the reporter enzyme and no test line on the strip (Figure 1).

The intensity of the Test Line is inversely proportional to the concentration of the inhibitor in your sample:

- **No Inhibitor (Negative):** A strong, distinct blue line matching the Negative Control.
- **Low Concentration:** A visible but noticeably fainter line compared to the Negative Control.
- **High Concentration (Positive):** Little to no color development at the Test Line

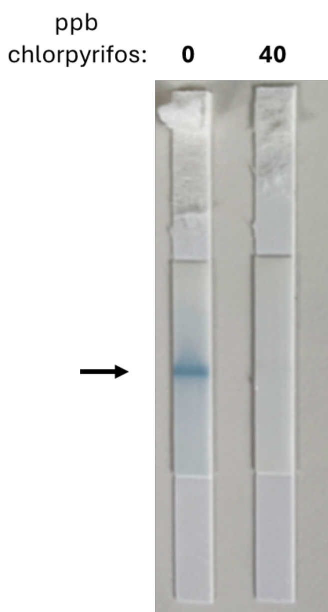

Figure 1. Visual detection of chlorpyrifos inhibition of acetylcholinesterase using the Attogene Acetyl-cholinesterase Inhibition Strip Test. Water samples containing 0 ppb and 40 ppb chlorpyrifos were tested in the assay. Both samples were treated with Activator Solution to attain maximum test sensitivity. A blue colored line appears at the test line (indicated by the arrow) for the 0 ppb sample, while 40 ppb oxonated chlorpyrifos abolishes the color intensity of the test line.

The assay can be used with water and other liquid samples to detect low levels ($\leq$10 ppb) of common pesticides including malathion, diazinon, chlorpyrifos, parathion, coumaphos.

5. Preparation and Notes

Method control: It is best to run a Negative Control test with each sample set to ensure comparable readings from the day, time and user.

- Thaw out the kit at room temperature for at least 90 minutes. If the kit has been stored at 4 °C, allow the kit to warm up to room temperature for at least one hour before performing the test.
- Each test strip contains a thin glass fiber pad at the bottom and a thicker wick at the top. A nitrocellulose membrane is between the glass fiber pad and the wick. To prevent cross-contamination, always handle the strips by the upper wick and avoid touching the lower pad.

6. Protocol

1. Add 200 µL of sample or Negative Control into microplate wells.
2. Add 10 µL of Activation Solution and gently mix by pipetting up and down.
3. Incubate 10 minutes at room temperature.
4. Add 10 µL of Buffer Solution to each well and mix well.
5. Incubate 5 minutes at room temperature.
6. Carefully add 10 ul of Enzyme Solution into each well and mix well.
7. Incubate 10 minutes at room temperature.
8. For each sample (and Negative Control), immerse the lower end of a strip into the well.
9. Incubate 10 minutes at room temperature. The liquid will flow up the glass fiber pad into the nitrocellulose membrane.
10. Remove strips from the wells. Lay on clean surface with pads facing up and glossy plastic backing facing down.
11. Remove the wick and sample pad from each strip using tweezers or forceps.
12. Add 20 µL Substrate Solution onto the center of the nitrocellulose membrane.
13. Incubate 5 minutes at room temperature.
14. Compare the test line color intensity of the sample strip to that of the Negative Control strip

Note: The Activation steps (Steps 2 and 3) are specifically designed to convert phosphorothioate and phosphorothionate pesticides to a more active and readily detectable form. If phosphorothioate and

phosphorothionate pesticides are known not to be in the test samples, it is acceptable to skip Steps 2 and 3.

7. Limitations of the OPaC , Possible Test Interference

This test is recommended for use with water samples or other aqueous samples containing low conentrations of miscible organic solvents (<20%). 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. If possible, it is useful to test a known negative sample (in parallel) to ensure that matrix from the sample is not non-specifically inhibiting the reaction.

8. Determination of Pesticide in Samples

If the color intensity of a sample test is significantly 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.

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. Be sure to observe and record the color change 5 minutes after adding the Substrate Solution.

Sensitivity : Detection limits of the various organophosphate or Carbamate pesticides differ depending on their ability to inhibit the enzyme. The assay can be used with water and other liquid samples to detect low levels (<10 ppb) of common organophosphate and carbamate pesticides including malathion, diazinon, chlorpyrifos, parathion, and coumaphos. Assay sensitivity can be further increased by increasing the incubation time at Step 7 (> 10 minutes). 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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