



GPO-TM/1 KIT



(For detection of 2 pesticide compound residues in vegetables, fruits, and whole grain)

Pesticide compounds: **ORGANOPHOSPHATE AND CARBAMATE**

Manufacturer: The Government Pharmaceutical Organization

Objective: To detect pesticide compound residues (organophosphate and carbamate) in vegetables, fruits, and whole grain.

Detection principle: Separation of pesticide compounds by using TLC (Thin Layer Chromatography) and chemical reaction to form color. If sample contains Organophosphate and Carbamate substances, a white spot will be detected on purple background on the TLC sheet.

Chemical substances:

1. Extraction solution	1	bottle
2. Testing solution 1	1	bottle (keep refrigerated)
3. Testing solution 1.1	1	bottle (keep refrigerated)
4. GPO TM 1 reagent	1	bottle (keep refrigerated)
5. GPO TM 1.1 solution	1	bottle
6. GPO TM 2 reagent	1	bottle (keep refrigerated)
7. GPO TM 2.1 solution	1	bottle
8. GPO TM 3 solution	1	bottle
9. Water	1	bottle
10. Charcoal powder	1	bag

Testing equipment (inside the box)

1. TLC Sheet (GPO-TM/1)	2	sheets
2. Plastic bottle with sprayer	2	bottles
3. Plastic dropper (3 ml)	2	tubes
4. Plastic dropper (1 ml)	1	tube
5. Glass dropper	10	tubes
6. Instruction manual	1	set

Additional equipment needed

1. Warm water bath (with controlled temperature between 37 ± 2 and 48 ± 2 °C with wire mesh rack.
2. TLC Tank
3. Metal cup
4. Bottle with dropper (for collecting sample)
5. Knife and cutting board (for cutting sample)

6. Forceps
7. TLC cover tray
8. Gloves
9. Stopwatch

Preparation of Testing solutions

1. Enzyme Solution

- 1.1 Take Testing solution 1 out of refrigerator and leave to obtain room temperature.
- 1.2 Pour Testing solution 1.1 into Testing solution 1 bottle, shake well and pour the mixture into spray bottle.
- 1.3 Put spray bottle in warm water bath at 37°C for at least 15 minutes before starting the test and keep the bottle there until the test is finished.

Note: Constant temperature control is essential for enzyme chemical reaction. Thus, mixture in spray bottle must be kept in warm water bath throughout the testing procedure. After testing is completed, the rest of the mixture can be kept frozen and must be used within 3 days.

2. Color Testing Solutions

- 2.1 GPO TM 1 Testing solution : Pour GPO TM 1.1 solution into GPO TM 1 reagent bottle and shake well to obtain GPO TM1 solution.
- 2.2 GPO TM 2 Testing solution: Pour GPO TM 2.1 solution into GPO TM 2 reagent bottle and shake well to obtain GPO TM 2 solution.
- 2.3 In Testing procedure step 4 (Color test), mix GPO TM 1 Testing solution with GPO TM2 Testing solution at ratio 1 ml : 4 ml and use immediately.

Note: GPO TM 1 and GPO TM 2 Testing solutions obtained from 2.1 and 2.2 must be kept in refrigerator at 2-8°C and can be used within 3 days.

3. **TLC Solution:** Pipet 10 ml of GPO TM 3 solution into TLC Tank and leave at least 30 minutes before starting the test.

Testing Procedure

1. Preparation and Extraction of sample

- 1.1 Prepare sample in a bottle by following instruction directed in the below table (do not use blender) and label code on each bottle.

Sample	Preparation method	Quantity	Volume of Extraction Solution used (ml)
1 General vegetables and fruits such as Chinese kale, Chinese cabbage, cabbage, and yard long bean	Finely chopped	5 gram (approximate quantity at 4 th mark label of the bottle).	5
2 Juicy vegetables and fruits such as cucumber, tomato, orange	Coarsely chopped	2.5 gram (approximate quantity at 3 rd mark level of the bottle).	5
3 Whole grains such as dried beans, local vegetables such as coriander, parsley, neem, acacia, fingerroot, chili	Finely grinded	0.50 gm	5

1.2 Add approximately 0.25 gm (~ 1 capsule) of charcoal powder into sample bottle from 1.1, shake for 1 minute and leave for another 5 minutes to obtain complete extraction.



2. Evaporation

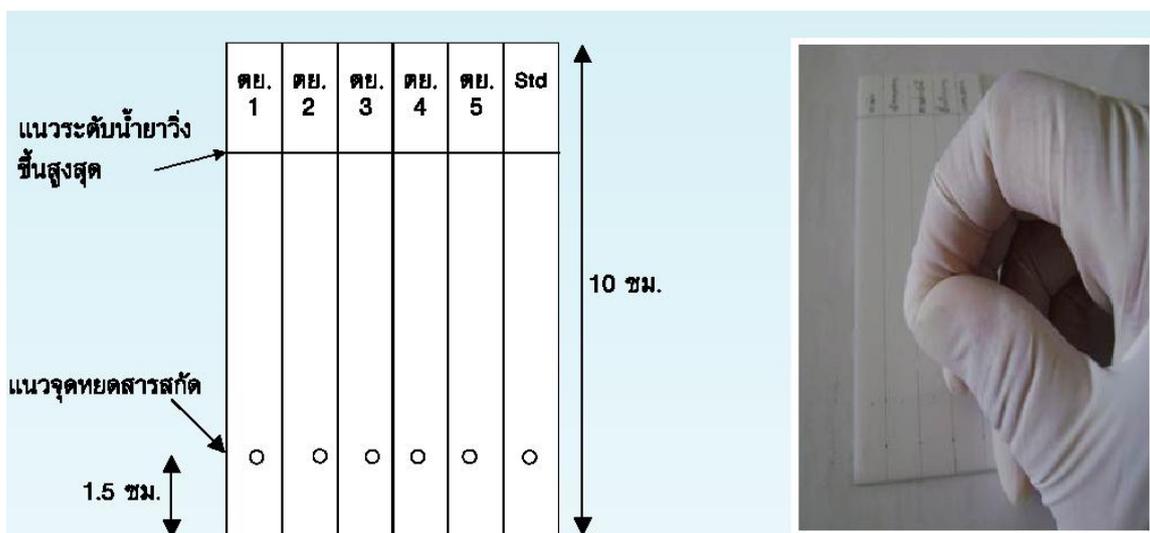
Use plastic dropper to draw 1 ml of extract from clear layer in 1.2 into a metal cup on the rack of warm waterbath and leave at 48°C until the solution is almost evaporated (2-3 drops left).



3. Testing procedure

3.1 Label names of samples to be tested on the upper end of GPO-TM/1 TLC sheet. Avoid direct hand touch to the sheet which may cause contamination of fat stain that can interfere the result interpretation.

3.2 Use capillary tube to draw extract from No.2. If extract in the cup is dried out, add another 2 drops of Extraction solution and lean the cup from side to side to dissolve the dried substance. Spot extract from capillary tip on the spot point (at the bottom end) on GPO-TM/1 TLC sheet once, then raise the capillary up and wait to air dry, then repeat spotting for another 4-6 times until the extract in the capillary is finished.



3.3 Use forceps to pinch GPO-TM/1 TLC sheet into TLC Tank and gradually lean it against the inner wall, close the tank cover and leave stable to let the solution diffuse upward until reaches solvent front line. Then, open the cover and use forceps to take the sheet out and leave to air dry. Spray Testing Solution 1 over GPO-TM/1 sheet until surface is all wet and use forceps to lay the sheet facing up on warm water bath at 37°C and cover with tray for 10 minutes to obtain constant temperature.



4. Color Test

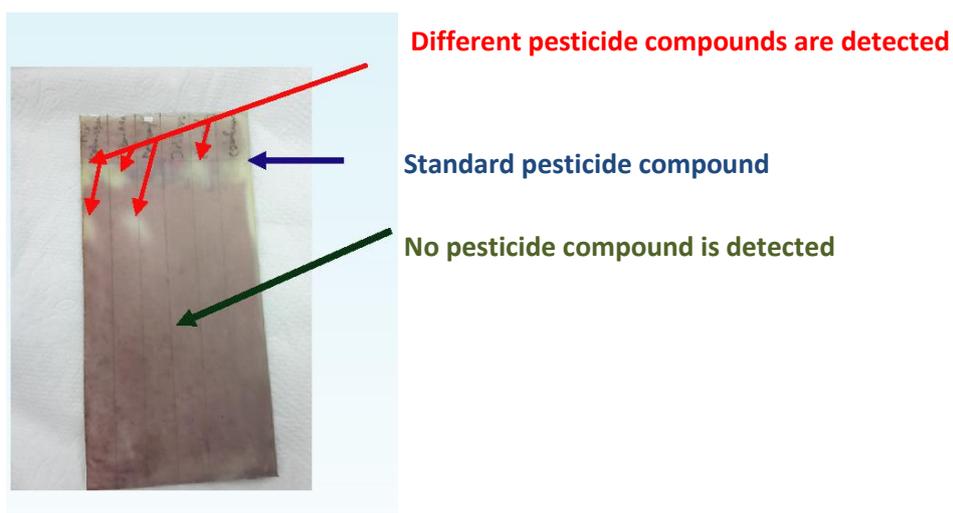
After 10 minutes, spray GPO-TM/1 TLC sheet from 3.3 with Color Testing Solution (prepared by mixing 1 ml of GPO-TM/1 Solution and 4 ml of GPO-TM/2 Solution from above) by leaving spray distance at 15 cm to allow complete absorption all over the sheet, then leave for 3 minutes and interpret the result.



5. Result Interpretation

Positive : If white rounded spot is detected on the purple background on GPO-TM/1 TLC sheet, it means there are organophosphate and carbamate residues in the sample.

Negative: If no white rounded spot is detected on the purple background on TLC sheet, it means the sample contains no organophosphate and carbamate residues.



Result interpretation

Name of Standard reagent	LOD (mg/kg)	RF value (Standard reagent)
1. Dicrotophos	0.126	0.09
2. Monocrotophos	0.118	0.17
3. Methomyl	0.663	0.67
4. Carbofuran	0.594	0.89
5. Diazenon	3.68	0.66
6. Dichlorvos	0.026	0.81
7. Carbaryl	0.597	0.91
8. Chlorfenvinphos	0.097	0.90
9. Profenofos	0.35	0.90
10. Chlorpyrifos	1.79	0.90
11. Bendiocarb	0.597	0.90

Cautions and Storage

1. The test should be conducted in airy room and avoid exposure to source of direct heat and flame.
2. All chemical substances are dangerous; avoid direct inhalation or direct contact.
3. In case of skin contact with chemical solutions, immediately wash with of water. In case of eye contact, immediately wash with plenty of water and seek immediate medical attention.
4. Keep the test kit out of reach of children and store in a place with good ventilation.

Storage : Keep in temperatures as directed

