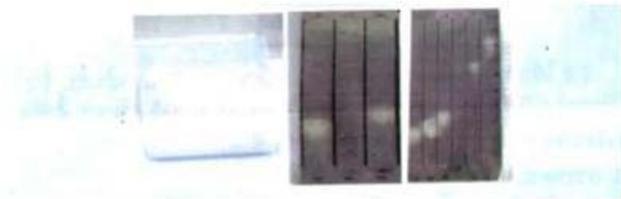


3.4 Use the plastic dropper to draw 6 ml of the mixed enzyme solution in 1 into the tray. Place the tray on a flat surface. Use a forcep to take TLC sheet from 3.3 and place with face down to let the test solution absorbed throughout the sheet. Then bring it to cure on a sieve (dry the sieve before use) which is in a water bath at 37°C for 10 minutes.



4. Colour Test

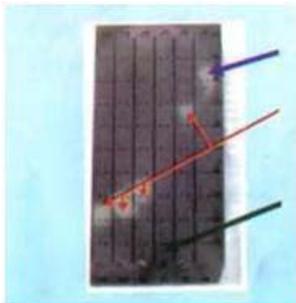
After 10 minutes, face the TLC sheet down in the colour test solution tray (from the mixing of 1 ml of GPO-TM 1 and 4 ml of GPO-TM 2 from the colour test solution preparation in 2). Wait until the colour test solution absorbed throughout the sheet for 3 minutes. Then bring the sheet for result reading.



5. Interpretation

Positive Found white spots on the purple area of the TLC sheet. This shows that there is organophosphate and carbamate pesticides in the sample compare to the standard.

Negative White spot on the purple area of the TLC sheet was not found. This shows that there is no organophosphate and carbamate pesticide in the sample.



Standard pesticide

Found various pesticides

No pesticide was found

Test Result Reading

Standard Name	LOD (mg/kg)	Rf of the Standard
1. Methomyl	1.63	0.76
2. Carbofuran	0.52	0.91
3. Profenofos	0.07	0.92
4. Dicrotophos	1.32	0.28
5. Monocrotophos	1.29	0.35
6. Chlorfenvinphos	0.048	0.94
7. Chlorpyrifos	4.178	0.89
8. Dichlorvos	0.058	0.90

Precaution and Storage

1. Should test under well-ventilated and far from heat or flash sources area.
2. Every chemical are hazardous. Direct inhalation or skin contact should be avoided.
3. Used solution, if contact with hands, wash immediately with water or if contact with eyes, irrigate with water and consult physician immediately.
4. Place the kit out of reach of children and keep in well-ventilated storage.



4 Groups of Pesticide Test Kit in Vegetables, Fruits and Whole Grains



Organophosphate and Carbamate Groups



TEST KIT MANUAL

G9 CO.,LTD

60/3 Inthamara 40 Dindaeng Bangkok, 10400 Thailand

It is a test method for organophosphate and carbamate pesticides in vegetables, fruits and whole grains.

Principle

It uses TLC separation principle and colour test by chemical reactions. If there is organophosphate and carbamate pesticide, white spots on the purple area on the TLC sheet will occur.

Chemical Solutions

1. Extraction	1 bottle
2. Test solution 1	1 bottle (kept frozen)
3. Test solution 1.1	1 bottle (kept cold)
4. GPO-TM 1	1 bottle (kept cold)
5. GPO-TM 1.1	1 bottle
6. GPO-TM 2	1 bottle (kept cold)
7. GPO-TM 2.1	1 bottle
8. GPO-TM 3	1 bottle

Apparatus inside the Box

1. Chromatography set (disk A and TLC sheet)	1 set
2. Standard disk	2 disks
3. Pin	1 pin
4. 3 mm plastic dropper	2 droppers
5. 1 mm plastic dropper	1 dropper
6. Test kit manual	1 set

Additional apparatus

1. Temperature adjustable water bath at 37 ± 2 and 48 ± 2 °C includes sieve.
2. TLC Tank
3. Metal cup
4. Sample bottle and dropper
5. Knife and chopping board for chopping the sample.
6. Forcept
7. Tray for placing TLC sheet
8. Glove
9. Timer

Test Solution Preparation

1. Enzyme Solution

1.1 Bring test solution 1 out from the freezer and leave until the temperature is equal to the room temperature.

1.2 Pour test solution 1.1 into the bottle of test solution1, shake well.

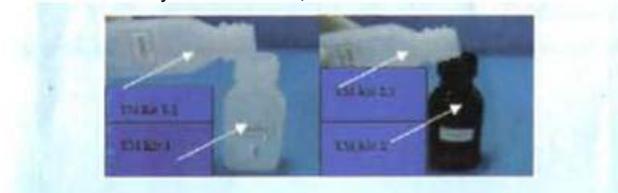
1.3 Place the bottle into the water bath at 37°C of at least 15 minutes before testing and leave it there until finish the test procedure.

***Note** Prepared enzyme solution can be used with all 4 groups. **The temperature is crucial for the reaction of enzyme. Do not take the mixed test solution 1 from the water bath until finish the test procedure. Keep the remaining solution in the freezer (use within 3 days).**

2.1 Test solution GPO-TM 1: Pour GPO-TM 1.1 into the bottle of GPO-TM 1, shake well to get GPO-TM 1 solution. Keep the remaining solution in the refrigerator at $2-8^\circ\text{C}$ (use within 3 days).

2.2 Test solution GPO-TM 2: Pour GPO-TM 2.1 into the bottle of GPO-TM 2, shake well to get GPO-TM 2 solution. Keep the remaining solution in the refrigerator at $2-8^\circ\text{C}$ (use within 3 days).

2.3 When in test procedure 4 (Colour Test), mix GPO-TM 1 solution with GPO-TM 2 solution at the ratio of 1 ml : 4 ml (use immediately after mixed).



TLC Solution

Draw about 10 ml of GPO-TM 3 solution into the TLC Tank of at least 30 minutes before the test.

Test Procedure

1. Sample Preparation and Extraction

1.1 Chop vegetables and fruits thoroughly. Put them in the bottle of approximately 5 grams or 4 lines on the bottle. In case of whole grains, use about 2.5 grams or 2 lines on the bottle. Label every sample bottle.



1.2 Use a dropper to draw 5 ml of the extraction in to the sample bottle. Shake for 1 minute and let it sit for 5 minutes.



2. Evaporation

2.1 Use a pin to punch into the chromatography paper (disk A) for 1 sheet (punch gently to avoid tearing). Then place it into the metal cup which is placed on a sieve. Label the cup (test one sample per one cup). Leave the sieve in the water bath at $48 \pm 2^\circ\text{C}$.



2.2 Use the plastic dropper to draw 1 ml of the clear extraction in 1.2 into the metal cup which is placed on the sieve in 2.1. Let it sits until dry (Do not let disk A stick on the side of the cup).

3. Test

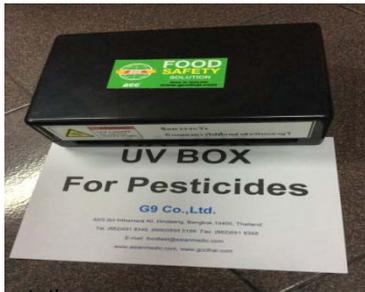
3.1 Use the pin to punch into the standard disk (punch gently to avoid tearing). Adjoin it into the space of TLC sheet. Use the clean paper to overlay and press it gently with the pin to ensure the disk is adjoined.



3.2 Use the pin to punch the disk A in the metal cup of the evaporated sample in 2.2. Put it into the next space of the TLC sheet. Repeat 3.1.

3.3 Use the forcept to take TLC sheet and dip in to the TLC tank. Lean it gently to the side of the bottle. Close the bottle and let it sits still. Wait until the solution moves to the line 10^{th} on the top of the TLC sheet. Grip the TLC and let it dry.





6. Interpretation

Positive Found grey, brown-black spots on the TLC aluminum sheet. This shows that there is organochlorine and pyrethroid pesticides.

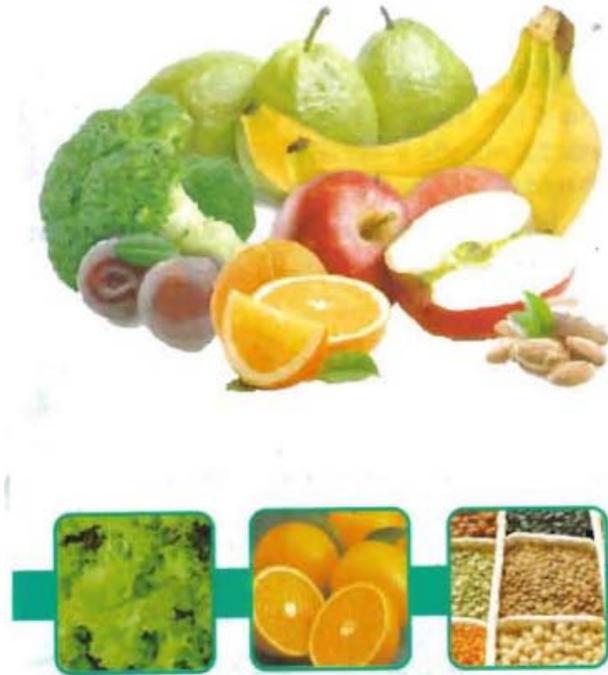
Negative No spot on the TLC aluminum sheet was found. This shows that there is no organochlorine and pyrethroid pesticide.

Test Result Reading

Standard Name	LOD (mg/kg)	Rf of the Standard
1. Cypermethrin	0.3	0.84 , 0.89
2. Permethrin	0.3	0.87
3. Deltamethrin	0.2	0.90
4. Endrin	0.08	0.86
5. Endosulfan	0.04	0.13 , 0.88
6. DDT	0.04	0.90

Precaution and Storage

1. Should test under well-ventilated and far from heat or flash sources area.
2. Every chemical are hazardous. Direct inhalation or skin contact should be avoided.
3. Used solution, if contact with hands, wash immediately with water or if contact with eyes, irrigate with water and consult physician immediately.
4. Place the kit out of reach of children and keep in well-ventilated storage.



4 Groups of Pesticide Test Kit in Vegetables, Fruits and Whole Grains



Organochlorine and Pyrethroid Groups



TEST KIT MANUAL

G9 CO., LTD

60/3 Inthamara 40 Dindaeng Bangkok 10400, Thailand

It is a test method for organochlorine and pyrethroid pesticides in vegetables, fruits and whole grains.

Principle

It uses TLC separation principle and test by chemical reactions and expose to UV with wavelength of 254 nm to create colour. If there is organochlorine and pyrethroid pesticide, grey, brown-black spots on the TLC sheet will occur.

Chemical Solutions

- | | |
|---------------|----------|
| 1. Extraction | 1 bottle |
| 2. GPO-TM 4 | 1 bottle |
| 3. GPO-TM 5 | 1 bottle |
| 4. GPO-TM 5.1 | 1 bottle |

Apparatus inside the Box

- | | |
|--------------------------|------------|
| 1. TLC Aluminum Sheet | 1 set |
| 2. Capillary Tube | 1 set |
| 3. 3 mm plastic dropper | 3 droppers |
| 4. 1 mm plastic dropper | 1 dropper |
| 5. Test kit manual | 1 set |
| 6. Powder carbon tablets | 1 pack |

Additional apparatus

- Temperature adjustable water bath at 48 ± 2 °C includes sieve.
- UV Box (UV 254 nm)
- TLC Tank
- Metal cup
- Sample bottle and dropper
- Knife and chopping board for chopping the sample.
- Forcept
- Tray for placing TLC sheet
- Glove
- Timer

Test Solution Preparation

Draw 10 ml of GPO-TM 5 solution and 2 ml of GPO-TM 5.1 into the TLC Tank. Shake well and let it sits for at least 30 minutes before the test.

Test Procedure

1. Sample Preparation and Extraction



1.1 Chop vegetables and fruits thoroughly. Put them in the bottle of approximately 5 grams or 4 lines on the bottle. In case of whole grains, use about 2.5 grams or 2 lines on the bottle. Label every sample bottle.



1.2 Use a dropper to draw 5 ml of the extraction in to the sample bottle. Shake sturdily for 1 minute and let it sit for 5 minutes.

2. Evaporation

Use the plastic dropper to draw 1 ml of the clear extraction in 1.2 into the metal cup which is placed on the sieve. Leave the sieve in the water bath at 48 ± 2 °C. Wait until the extraction in the metal is almost all evaporated or is about 2 drops left.



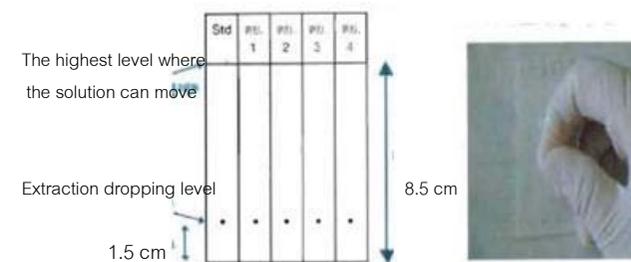
3. TLC Aluminum Sheet Preparation

3.1 Write the sample ID on the TLC aluminum sheet. Do not touch the TLC sheet directly with your hands. This will create grease on the sheet and interfere the test.

3.2 Use a pencil to mark a spot in the middle of each column. This is the spot to drop the extraction. It is 1.5 cm measured from the bottom of the sheet.

3.3 The highest level where the solution can move is 8.5 cm measured from the bottom of the sheet. Mark the level lightly with a pencil.

3.4 The remaining area from the marked level where the solution can move, write the sample and standard ID.



4. Test

4.1 Use the capillary tube to draw the sample extraction in 2 (if the solution in the metal cup are dried, drop 2 drops of the extraction into the cup. Tilt the metal cup gently to dissolve all the dried extraction inside the cup.). Drop all the extraction by touching the tip of the capillary tube to the marked extraction dropping spot on the TLC aluminum sheet. Raise the capillary tube up and wait until the dropped extraction dried. Repeat about 4-6 times until the extraction runs out.

4.2 Take TLC aluminum sheet and dip in to the TLC tank. Lean it to the side of the bottle and close the lid (Do not shake or move the bottle).

4.3 When the solution moves to the specified level, open the lid and use the forcept to grip the TLC aluminum sheet. Place it on the side of the bottle and let it dry.

5. Colour Test

5.1 Spray the TLC aluminum sheet with GF-TM 4 with the distance of 4.5 inches until it soaks. Let it dry (1 minute).



5.2 Expose the TLC aluminum sheet with UV at the wavelength of 254 nm for 3-5 minutes (if expose too long, the sheet will burn). If there is organochlorine and pyrethroid pesticides, it will turn grey, brown-black. Read the colour comparing result and Rf of the standard pesticide.