

Pesticide residue (Organophosphate and Carbamate) detection kit

# AgriChem<sup>TM</sup>

## Instruction Manual of AgriChem<sup>TM</sup>\* Pesticide Residue Detection Kit

<sup>TM</sup>\*: Under registration in Japan

**Harmful reagents are included. Please read this manual before use**

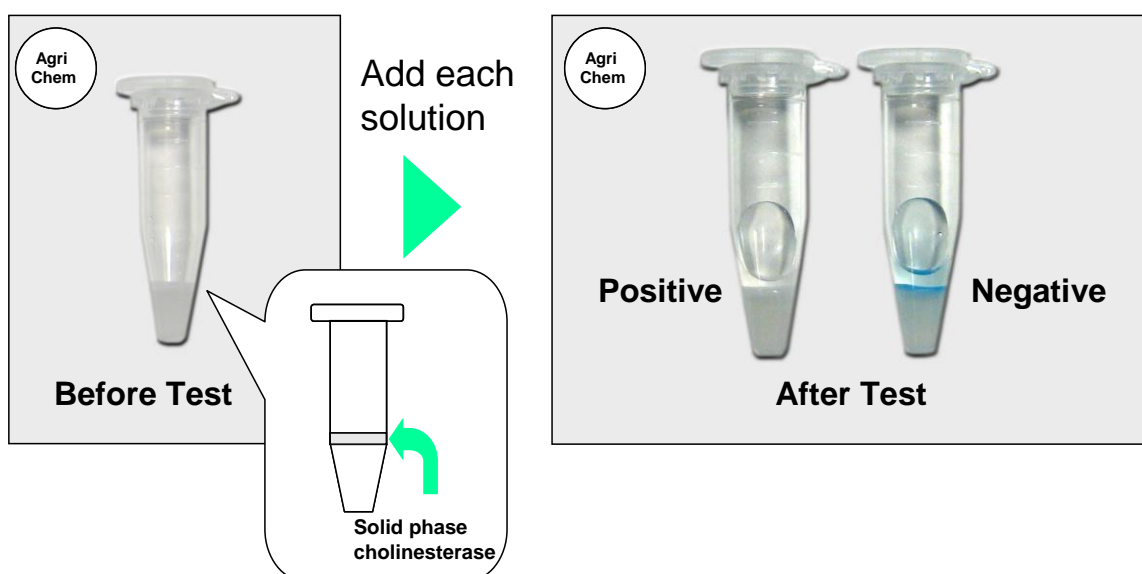
### Intended Use

AgriChem<sup>TM</sup> is designed for rapid test of residual pesticides (organophosphates and carbamates) in food and environment without any measuring instrument. This kit is qualitative testing method and covers most of organophosphates and carbamates and monitoring of pesticide drift.

### Measurement Principle

Cholinesterase(ChE) is an enzyme that catalyzes the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid, is an essential enzyme to allow a cholinergic neuron to return to its resting state after activation, and is inhibited by organophosphates and carbamates.

In this kit, immobilized ChE to the bottom of microtube by hydrophilic resin is colored blue with substrate. When assay sample contains any organophosphates and carbamates, the top layer remains white because of the ChE inhibition.

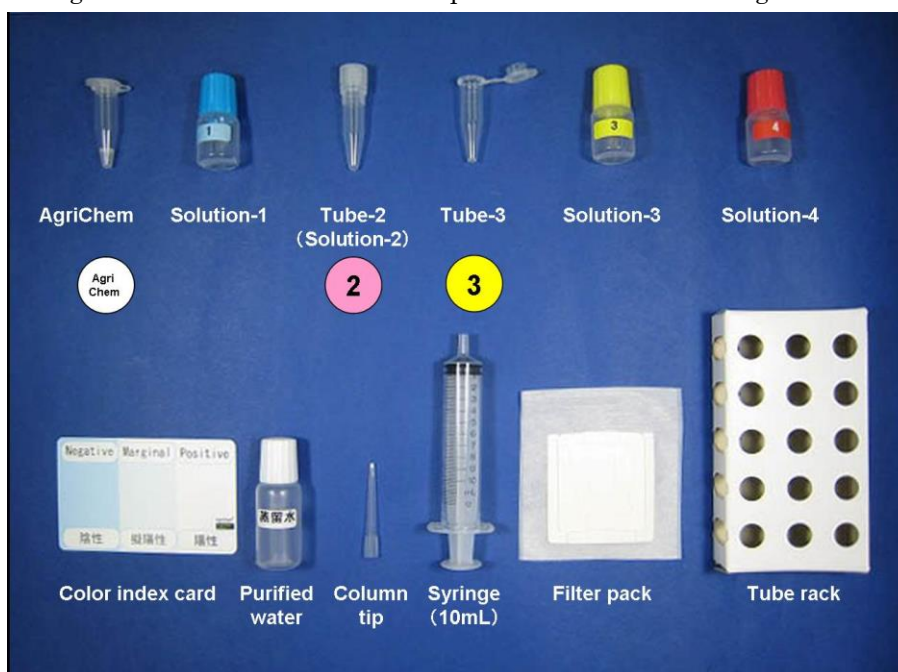


## Features

1. Rapid: Compared with instrumental analysis and ELISA method, AgriChem™ is rapid testing.
2. Ease of use: AgriChem™ does not need any specific skills nor instruments and is testable even on site.
3. Low cost : Not necessary to use any detection equipment.
4. High sensitivity: More sensitive than conventional enzyme kits by column concentration.
5. Safety: AgriChem™ is designed for minimal exposure to reagents by performing reactions in tubes.

## Components (contents)

Appearance (Below figure is the one for five tests. A part of bottles sizes change for other tests.



List

Name	Type	IAG-20	IAG-60
		20test	60test
1. AgriChem		22	66
2. Solution-1 (Blue cap)	Solution	1	3
3. Tube-2 (Pink label)	Powder	20	60
4. Tube-3 (Yellow label)		20	60
5. Solution-3 (Yellow cap)	Solution	1	3
6. Solution-4 (Red cap)	Solution	1	3
7. Color Index Card		1	1
8. Purified water (White cap)	Solution	1	2
9. Column tip		20	60
10. Syringe (10mL)		1	1
11. Filter pack		20	60
12. Tube rack		1	1

For control reaction, **AgriChem** and **Tube 3** are contained 1 more in 5-tests kits, 2 more in 20-tests kit, and 6 more in 60-tests kit than number of tests.

## Assay Protocol

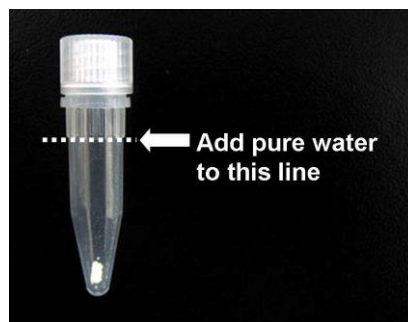
### Materials required but not provided in kit box

1. Kitchen knife and chopping board
2. Plastic bags for samples
3. Scales in grams for samples (1g~500g)
4. 100mL beaker (or equivalent)
5. 200mL beaker (or equivalent)
6. Timer (in second)
7. City water (or purified water)
8. Waste tank

### Preparation of the Reagents (Prepare below reagents before test)

**Solution 2** (Activator Solution) : Add 1ml (up to long line of picture (below))

of **Purified water** into **Tube 2**. Shake and solve the white powder well (**Solution 2**).



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※Prepared **Solution-2** is not possible to preserve nor re-use. Adjust only the inspection numbers of this solution and use once and daily. Do not re-use.

## Testing Procedures & Instructions

Testing of negative control needs to be run always at same time of each sample testing to make sure that the kit performs properly. There may be a case that the degree of color development changes by room temperature for enzyme reaction. **AgriChem** for control test is not operated up to ⑰ after ①

**Preparation and Extraction of Residual Pesticides in Crops** : Extraction of Adherent Pesticides on the Surface of Crops.

- ① Prepare the tests number + 1 (for negative control) of **AgriChem**, open the caps, and apply 2 drops of **Solution 1** into **AgriChem**. After applying, close the caps.



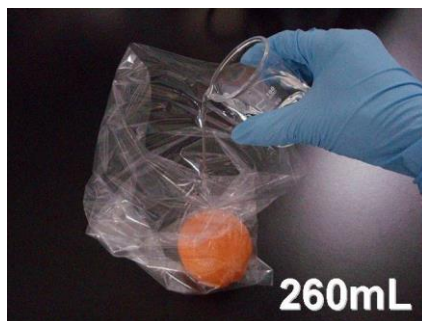
1



- ② Weigh the crop sample and put into a plastic bag. About 100g~300g of crop is proper to handle for elution (to be varied according to the type of crop). When the size and weight become problems, cut it in a suitable size with the cutting board and the kitchen knife.



- ③ Add an equal weight of water <sup>※1</sup>(1g=1mL) into the bag by beaker (of 100mL)



※<sub>1</sub> If available, purified (distilled or deionized) water is recommended.

- ④ Seal and shake the bag vigorously for 30 seconds for preparation of sample extraction.



- ⑤ Pour 100ml of the extraction water in **the bag** into **beaker (of 100mL)**.

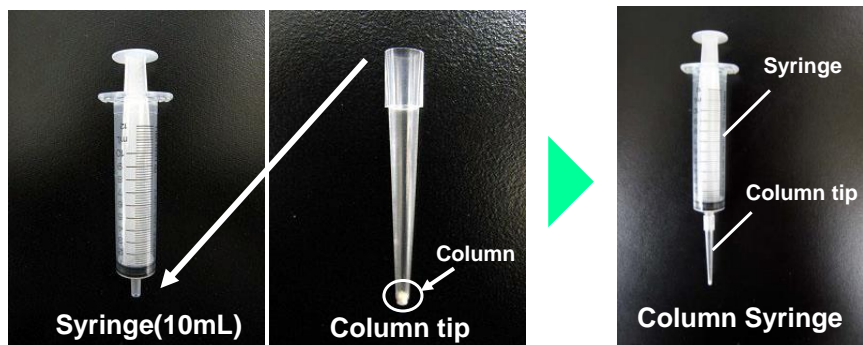


- ⑥ For removing solid contaminants in extraction water, set the filtration pack onto beaker (of 200mL) and add whole extraction water to the filtration pack. Collect more than 10ml of filtrated extraction water and discard the filtration pack and the remaining extraction water on filter.

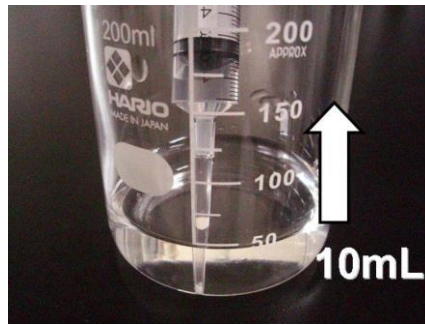


**Adsorption and Activation of Pesticides** : Concentration and Activation of Pesticides in Extract on Column.

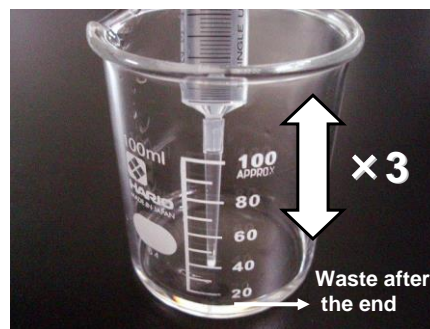
- ⑦ Put the column tip firmly onto the top of 10mL syringe (**Column Syringe**).



- ⑧ Aspirate **slowly**<sup>※2</sup> 10mL of the filtrated extraction water from the **beaker (of 200mL)** into the **Column Syringe**.

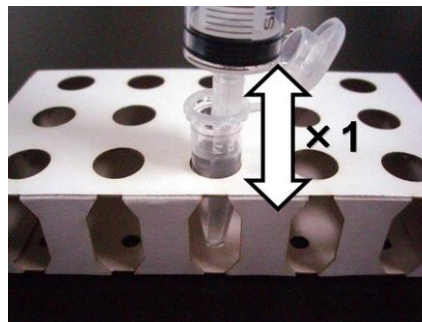


- ⑨ With the **Column Syringe**, pipette (dispense and aspirate) **slowly**<sup>※2</sup> the extraction water into and from 100mL beaker. Repeat three times. At this step, pesticides in the extraction water are trapped on the resin on the top of column tip. **Discard the extraction water in Column Syringe**.



※2 Strong aspiration may cause detachment of the resin from the top of tip. Aspirate slowly. Aspirate less than 1ml per 2 sec. and idle at every 1ml aspiration

- ⑩ Set the tube of **Solution 2** on tube rack, with the **Column Syringe**, pipette whole volume of the prepared **Solution 2** from and into the Tube-2 once. (Organophosphorus pesticides adsorbed on the column are converted to activated (oxon) form.)



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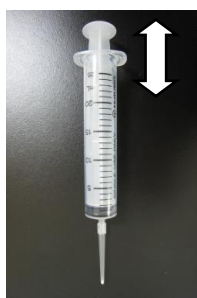
- ⑪ The remained activator solution in the **Column Syringe** needs to be completely drained by moving the piston of the syringe up and down



- ⑫ Pour about 20 ml of fresh (city) water into beaker (of 100mL). Pipette 5mL of this water for washing inside of the **Column Syringe**. If **Solution 2** remained in **Column Syringe**, enzymatic reaction is affected. Repeat 3 times with changing the fresh (city) water every time.



- ⑬ The remained water in the **Column Syringe** needs to be completely drained by moving the piston of the syringe up and down.



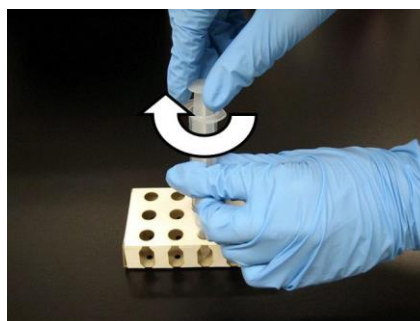
**Elution of Pesticides and Inhibition of Cholinesterase:** Transfer Pesticides from Column Syringe to AgriChem™.

- ⑭ Pour 3 drops of **Solution 3** into **Tube 3** and set **Tube 3** on tube rack.



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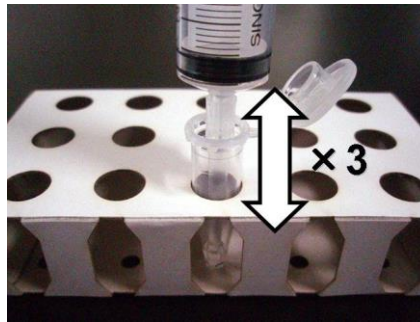
- ⑮ Pipette the whole volume of **Solution 3** from and into the **Tube 3** with the **Column Syringe**. It must be cautioned that the liquid doesn't go up to the syringe<sup>※3</sup>.



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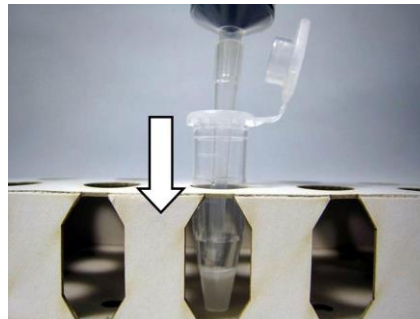
※<sub>3</sub> For small amount aspiration, aspirate slowly while turning the piston of the syringe. Dispense normally.

- ⑩ Repeat step ⑨ three times. At this step, pesticides on resin are eluted into **Solution 3**.



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- ⑪ Set the tube of **AgriChem** on tube rack, aspirate the whole volume of **Solution 3** from the **Tube 3**, and dispense the whole solution into **AgriChem** (for testing). Apply 3 drops of **Solution 3** from **Bottle 3** in to **AgriChem** (for negative control). After closing the cap of **AgriChem**, shake lightly and wait for 15 minutes for inhibiting enzymatic reaction.



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### **Color Development** : Confirmation of the Presence of Pesticides by the Enzyme Reaction.

- ⑫ Apply one drop of the **Solution 4** into **AgriChem** (for testing and negative control). After closing the cap of **AgriChem**, shake lightly and wait for 15 minutes. **Handle Solution 4 carefully, because Solution 4 is easily spilled from the bottle.**



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- ⑬ Compare the color of top layer of **AgriChem** with the **Color Index Card**



Agri Chem



## Read Result



Compare with the top layer of resin (area of dotted circle-line in the left picture) and **Color Index Card**. If necessary, overlap **AgriChem** onto the **Color Index Card**.



Negative: The pesticide is not detected.

Positive, Marginal: The pesticide or enzyme inhibitor remains in the sample. If necessary, re-examine and confirm with instrumental test method.

## Examples of Detection Limit of Pesticides by AgriChem™ with Standard Solutions

Pesticides	Detection Limit(ppb)	Pesticides	Detection Limit(ppb)
EPN	60	TERBUFOS	60
AZINPHOS-ETHYL	2	PARATHION	1
AZINPHOS-METHYL	10	PYRIDAFENTHION	2
ISOXATHION	0.6	PIRIMIPHOS-METHYL	20
ETHION	20	FENAMIPHOS	6
ETRIMFOS	10	FENITROTHION	80
QUINALPHOS	0.4	PROPAPHOS	6
COUMAPHOS	6	BROMOPHOS-ETHYL	20
CHLORPYRIFOS	0.4	PHOSALONE	8
CHLORPYRIFOS-METHYL	2	PHORATE	100
CHLORFENVINPHOS	40	MONOCROTOPHOS	100
SULPROFOS	60	PIRIMICARB	10
DIAZINON	0.8		

The expression “1 ppb” means that given property exists at relative proportion of one part per billion parts examined, as would occur if water-borne pollutant was present at concentration of one-nano gram per gram of sample solution (0.000001%).

Confirm the latest maximum residue limits (MRLs) of agricultural compounds in foods in each country.



## Storage and Stability

Store between 4°C and 8°C. Protect from light. Do not freeze. The expiration date is indicated on box.

## Transportation

Keep cool between 4°C and 8°C. Protect from light. Do not freeze.

## Cautions

### ① For Safety

- Do not drink and do not touch the reagents. This kit contains the harmful reagents. For use this kit, treat carefully for safety by wearing lab coat, protection glove, and protection glass. If you feel abnormality in your body, see medical doctor immediately.
- After testing, AgriChem™ is possible for containing high concentration of pesticides. Do not open the cap of AgriChem™ after testing.

### ② For General

- This kit is designed for self-inspection related to food hygiene. Operate and judge the result at your responsibility.
- Waste the surplus reagents and inspection samples according to the regional regulations, under considering health and environment well. If AgriChem™ judged the positivity, there is a possibility that pesticides exist in the AgriChem™, and treat the AgriChem™ as polluted waste.
- Store this kit at 2-8°C under the dark condition.
- Keep and refer to this kit manual carefully at testing and when necessary.
- In farm products, influence of crops origin materials and impurities might be received, and the false positive can be caused. Contact below address when such a result is doubted.

## Contact Information

Further information on this product is available directly from Institute of Microchemical Technology.  
Contact via website <http://www.i-mt.co.jp/>.

Any change of the specifications of this product may be applied without any advance notice.

## Manufactured by:

### Institute of Microchemical Technology

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