吾酶土生科
WUMAITO BIOTECHNOLOGY

Rapid Bioassay for Pesticide Residues (RBPR) Reagent Kit-1000 tests

綠盾快篩
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Operation Manual

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Product illustration

Acetylcholinesterase, AChE (Enzyme) extracted from special-lined housefly have undergone nearly 20 years of research as well as technologically practical verification, its activity and detected toxicity sensitivity are regarded as the highest in the world; so far, this is unsurpassed by any other product. And applications stemmed from this AChE is further developed into “Rapid detection and test for residues of agricultural chemicals on vegetables and fruits” which can detect and test the residues of agricultural chemicals, and rapidly filter out the accumulated toxicities from nearly hundreds pesticides containing organic phosphorus agent and carbamates.

Principle of test

Pesticides with organic phosphorous agent and carbamates would inhibit AChE activity and its inhibition rate positively correlates to the concentration of agricultural chemical. Under normal condition, AChE can dissolve the substrate (Acetylthiocholine iodide. ATCI) into acetic acid and thiocholine. Then thiocholine interacts with colorimetric agent (5, 5’-dithio-bis-(2-nitrobenzoic acid), DTNB) and this would generate yellowish product. In addition, by using the changed amount (da/min) of absorbance under the wavelength of 412 nm, one can compute the inhibition rate and this can be further used to determine whether are residues from organic phosphorus agent and carbamates within the agricultural chemicals or not.

Product content

Product packaging specification

Item	Content	1000 tests	2000tests
1	Acetylcholinesterase	2 bottles	4 bottles
2	Substrate (ATCI)	2 bottles	4 bottles
3	Colorimetric agent (DTNB)	2 bottles	4 bottles
4	PBS buffer	3 bottles	6 bottles
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※ After receive this set, meticulously inspect to ensure all item contents and quantities consistent to the above specification; if any discrepancy, please report immediately.

※ For rapid testing of the residues of agricultural chemicals on vegetables and fruits, ensure 95% alcohol is readily available.

Prerequisites for storage

1. Besides buffer and bromine, for reagent whether it is in powder form or liquid (diluted), it is recommended to be cryopreserved.
2. In order to prevent buffer powder from being moisturized, it can be stored in a freezing environment—below 0 ℃. After being formulated into solution, it can stay in room temperature for 30 days (if intend to prolong the shelf life, one can separately pack them and store below 0℃).
3. Bromine can be stored under room temperature.

Shelf Life

1. Reagent power can be preserved 2 years if stored below 0oC whereas only 3 months under room temperature.
2. After formulating the reagent power into solution, it can be stored below 0 ℃ for 180 days.
3. Bromine can be stored indefinitely but once discoloration occurs, it loses its effectiveness.

Reagent formulation illustration

Amount of added distilled water

Item	Content	The amount of distilled water used
		1000/2000 Tests
1	Acetylcholinesterase (Enzyme)(AChE)	10ml
2	Substrate (ATCl)	10ml
3	Colorimetric agent (DTNB)	50ml
4	Buffer solution (PBS buffer)	1000ml

Reagent formulating and operation methods

1. For every bottle of AChE, add 10ml distilled water to dissolve. After separately packed, preserve below 0℃.
2. For every bottle of substrate, add 10 ml distilled water to dissolve. After separately packed, preserved below 0 ℃.
3. For every bottle of colorimetric agent, add 50 ml distilled water to dissolve. After separately packed, preserved below 0 ℃.
4. For every buffer solution, add 1000ml distilled water to dissolve and preserve under room temperature.
5. Bromine is preserved under room temperature.
6. Prior the use of reagent, it requires to be warm up back to room temperature before proceeds to test.

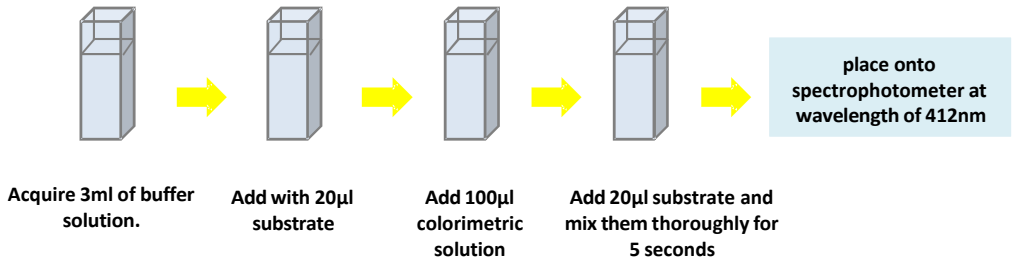
Reagent formulation illustration

1. If no distilled water available, it can be replaced with pure water available on the market. Do not use mineral water, RO reverse osmosis water..etc.
2. Enzyme reagent is recommended to separately pack into 1ml/tube so as to avoid repetitive freezing and thawing.
3. Since bromine is volatile therefore please cut down the time when the bottle cap is open as much as one can. After use, tightly secure the cap. If each time the amount used is minimal, it is then recommended to proceed to separately pack them so as to extend the preservation time.
4. Buffer solution's powder is hard to dissolve; therefore during formulation, please ascertain that it is completely dissolved prior use.

Operating procedures

Enzyme activity test

1. Acquire 3ml of buffer solution and add into colorimetric tube.
2. Add 20µl enzyme.
3. Add 100µl colorimetric solution.
4. Add 20µl substrate.
5. After thoroughly mix the solution, place onto spectrophotometer at wavelength of 412ml in order to measure the absorbance changes (da/min)



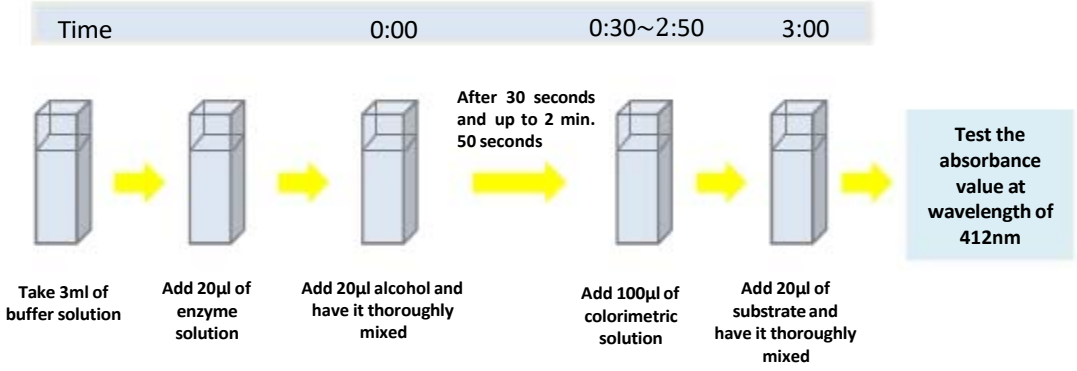
∗ For every bottle of formulated enzyme, it requires the testing for its activity. Enzyme activity must fall between 0.40 ~ 0.50 da/min. When enzyme activity is higher than 0.50 da/min, add distilled water to dilute according to enzyme concentration adjustment formula. If below 0.40 da/min, it requires to add "enzyme powder" to raise the level of activity.

∗ Enzyme concentration adjustment formula:

The amount of added distilled water =
(The actual tested da/min value-0.45)/0.45 X total volume

Corresponding group test

1. Take 3ml of buffer solution and add into the colorimetric tube.
2. Add 20μl enzyme.
3. Add 20μl of 95% alcohol and have the solution mixed thorough then begin the countdown.4. Add 20μl substrate.
4. After 30 seconds and up to 2 min. 50 seconds, add 100μl colorimetric solution.
5. At the mark of 3 minutes, add 20μl substrate.
6. Have the solution thoroughly mixed, place onto spectrophotometer at wavelength of 412ml in order to measure the absorbance changes (da/min).

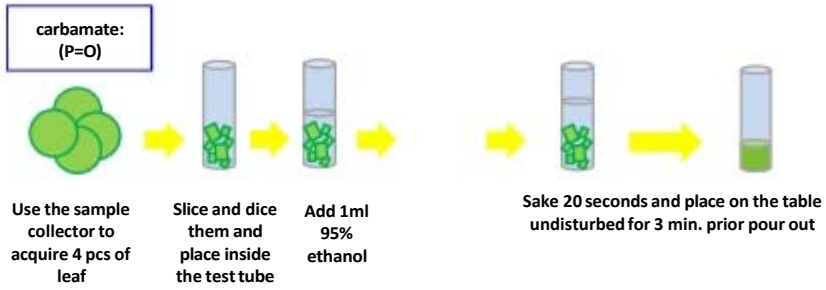


＊ Error for two consecutive corresponding group should be less than 0.014 so that sample group test can proceed.

Sample extraction procedure

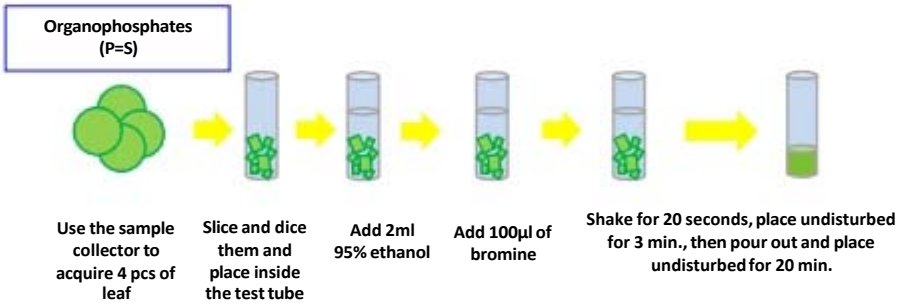
Test for carbamate:

1. Use a stainless steel pipe with diameter about 1 inch to cut and retrieve 4 pcs of vegetable samples or 1 gram of vegetable and fruit sample, slice and dice them and place inside glass test tube.
2. Add 1ml of 95% alcohol into the test tube, shake for 20 seconds and place on the table undisturbed for 3 min.
3. Pour the extracted solution into a clean test tube.



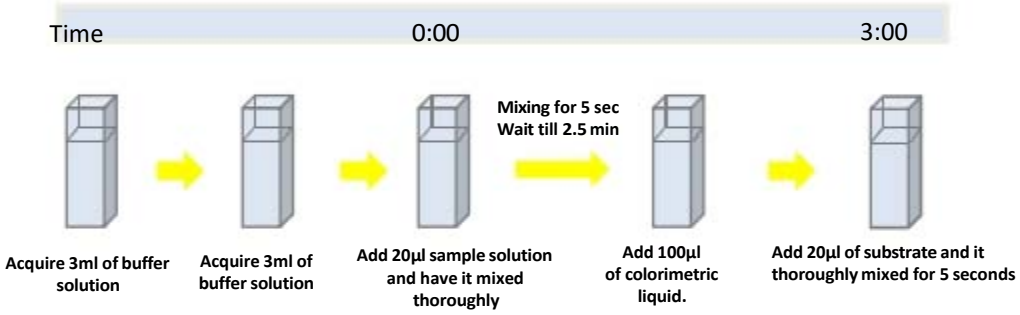
Detect and test for organic phosphorus compounds

1. Use a stainless steel pipe with diameter about 1 inch to cut and retrieve 4 pcs of vegetable samples, slice and dice them and place inside glass test tube.
2. Add 2ml of 95%alcohol and 100 µl of bromine into test tube. Shake it for 20 seconds and place on the table undisturbed for 3 min.
3. Pour the extracted solution into clean test tube, place on the table undisturbed for 20 min. till the bromine evaporated prior proceed to test.



Sample Test

1. Acquire 3ml buffer solution and add into colorimetric tube.
2. Add 20μl of enzyme.
3. Add 20μl of sample solution, have it thoroughly mixed and start countdown.
4. Between 30 seconds and two min. 50 seconds time mark, add 100μl of colorimetric liquid.
5. On 3 min. mark, add 20μl of substrate.
6. Have the solution mixed thoroughly, place onto spectrophotometer set at 412 nm to test the absorbance (da/min) change.
7. According to the change speed of absorbance and the changes from corresponding group to calculate its inhibition rate.



Result interpretation

Computational method for inhibition rate

Inhibition rate (%) =

$$\frac{\text{absorbance of corresponding group} - \text{absorbance of sample}}{\text{absorbance of corresponding group}} \times 100\%$$

Inhibition criteria:

Inhibition rate	Recommended processing
≥ 45%	Probable excessive agricultural chemicals with organic phosphorous agent and carbamate
35% - 44%	Suspect residues of agricultural chemicals with organic phosphorous agent and carbamate, it is recommended to clean thoroughly
≤ 34%	Smaller residues of agricultural chemicals with organic phosphorous agent and carbamate, it can be safely consumed after washing

Criteria for agricultural chemical type

Agricultural chemical type	Free of bromine	Added with bromine	Result
Carbamates (P=O)	Positive reaction (Inhibition rate>35%)	Positive reaction (Inhibition rate>35%)	Inhibition rates are similar to each other for free of bromine and added with bromine
Organophosphates (P=S)	Negative reaction (Inhibition rate>35%)	Positive reaction (Inhibition rate>35%)	Huge difference in inhibition rate between free of bromine and added with bromine

Notes

1. Prior use reagent, have temperature brought back to room temperature, mix thoroughly. After use, store below 0℃.
2. Bromine handling should take place at location that is well ventilated so as to ensure the health safety of personnel.
3. Unused bromine can be stored inside glass bottle with screw-on cap so as to prevent bromine from being evaporated. Do not use discolored bromine.
4. Enzyme reagent formulated with added distilled water can be frozen preserved 180 days.
5. Enzyme activity would change along the temperature. Temperature goes up and enzyme activity would be higher; therefore it is recommended to proceed test under constant temperature environment.
6. Enzyme activity would change along the temperature whereas for the scope of activity falls in the range of 0.20~0.60 da/min., it can be used normally. Nonetheless, for the first formulated batch, the enzyme activity should be adjusted between 0.40~0.50 da/min.
7. While using colorimetric tube, please hold the tube at both sides of the matte surface so as to prevent from interfering test result.

Criteria for agricultural chemical type

Instrument	1. Spectrophotometer: it can read the absorbance changes at wavelength of 412ml.(SL-1569) 2. 20μl automate straw 3. 100μl automate straw 4. 5000μl automate straw 5. Timer
Consumable	1. 4ml colorimetric tube 2. 200μl automate straw 3. 5000μl automate straw 4. Glass test tube 5. Plastic centrifuge tube 6. Parafilm
Reagent	95% alcohol (Medicinal alcohol made by Taiwan Tobacco and Liquor Corp.)

Troubleshooting

Issue	Probable cause and recommendation
Negative inhibition rate	**Experimental error:** • It could be experiment operational error. It is recommended that pay attention to the amount extracted is accurate while extracting samples and reagents. **Excessive environment temperature change:** • If there are 3 consecutive negative values occurred, it is probable because of excessive environment temperature change and cause this. It is recommended that redo the corresponding group in addition to avoiding from testing under the excessive temperature change environment. **Excessive sample water content:** • Suppose the water content in vegetable and fruit sample is excessive, this is prone to produce suspended solids which can cause negative inhibition rate value. **Special sample** • Partial of the itemed sample, because of higher starch content, it is prone to have negative value.

Issue	Probable cause and recommendation
Excessive or extremely low enzyme activity	**Excessive temperature change:** • Enzyme activity changes along temperature change. Temperature rises so the enzyme activity would be higher therefore it is recommended that test be proceeded in a constant temperature environment. **Enzyme deterioration:** • During the enzyme formulation, if one is unable to properly use distilled water or the enzyme itself has not been properly preserved, this could cause enzyme deterioration so as to impact the enzyme activity.
Unable to exhibit colorimetric reaction	**Not added with enzyme, colorimetric reagent or substrate:** • During the test session, the enzyme, colorimetric reagent or substrate are not added, it is recommended to redo the test.
Substrate turning yellowSubstrate turning yellow	**Substrate contaminated:** • During experimentation operation, pay attention that while drawing different reagents, the automate straw is required to be replaced so as to prevent reagent from cross-contamination.