Enzyme Immunoassay Kit for Chlorpyrifos

DEIANJ22

1. Background

Chlorpyrifos (CPS), is an organophosphate pesticide used to kill a number of pests including insects and worms. It is used on crops, animals, and buildings. It was introduced in 1965 by Dow Chemical Company. It acts on the nervous system of insects by inhibiting acetylcholinesterase. Chlorpyrifos ingestion results in more than 10,000 human deaths a year.

Chlorpyrifos is considered moderately hazardous to humans by the World Health Organization. Exposure surpassing recommended levels has been linked to neurological effects, persistent developmental disorders and autoimmune disorders. Exposure during pregnancy may harm the mental development of children, and most home use was banned in 2001 in the U.S. In agriculture, it is “one of the most widely used organophosphate insecticides” in the United States, and before being phased out for residential use was one of the most used residential insecticides.

2. Test Principle

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. Chlorpyrifos residue in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme conjugate, TMB substrate is used to show the color. Absorbance of the sample is negatively related to the chlorpyrifos in it, after comparing with the Standard Curve, multiplied by the dilution factor, chlorpyrifos quantity in the sample can be calculated.

3. Applications

This kit can be used in quantitative and qualitative analysis of chlorpyrifos in fruit (apple, orange) and vegetables (spinach, potato, cabbage).

4. Cross-reactions

Chlorpyrifos……………………………………….100%

5. Materials Required

5.1 Equipments

----Microtiter plate spectrophotometer (450nm/630nm)
----Homogenizer

5.2 Reagents

----Ethyl alcohol (AR)
----Deionized water

6. Kit Components

- Microtiter plate with 96 wells coated with antigen
- standard solution 10× concentrated(1ml×6 bottles)
  0 ppb, 6ppb, 18ppb, 54ppb, 162ppb, 486 ppb
- Spiking standard solution: 1ml, 5ppm
- Enzyme conjugate (12ml)
- Antibody solution (7ml)
- Solution A (7ml)
- Solution B (7ml)
- Stop solution (7ml)
- 20×Concentrated wash solution (40ml)
- 2×Concentrated extraction solution (50ml)

7. Reagents Preparation

Solution 1: Standard Solution

Dilute the concentrated standard solution with the deionized water in the volume ratio of 1:9 based on the requirement(e.g. 0.5ml of concentrated standard solution + 4.5ml of the deionized water).

NOTE: Prepare freshly.

Solution 2: Extraction solution

Dilute the 2×concentrated extraction solution with deionized water in the volume ratio of 1:1(e.g. 10ml of 2×extraction solution + 10ml of deionized water), which will be used for sample extraction, this solution can be stored at 4°C for 1 month.
Solution 3: Wash solution
Dilute the 20×concentrated wash solution with deionized water in the volume ratio of 1:19(e.g. 5ml of 20×wash solution + 95ml of deionized water), which will be used for washing the plates. This solution can be stored at 4°C for 1 month.

8. Sample Preparations
8.1 Notice and precautions before operation
(a) Please use one-off tips in the process of experiment, and change the tips when absorbing different reagent.
(b) Make sure that all experimental instruments are clean.
(c) Keep the untreated sample in refrigerator.
(d) Treated sample can be preserved for 4 hours.

8.2 Fruit (apple, orange) and vegetables (spinach, potato, cabbage)
----Homogenize the sample.
----Take 1.0±0.05g of homogenized sample into a 50ml tube, then add 10ml of ethyl alcohol, vortex for 2min to mix thoroughly.
----Centrifuge for separation: 3000g / ambient temperature(20-25°C/68-77F)/ 5min..
----Transfer 100µl of the supernate into a 2ml tube, add 900µl of extraction solution(Solution 2), vortex for 20s.
----Take 50µl of the prepared solution per well for assay.

9. Assay process
9.1 Notice before assay
9.1.1 Make sure all reagents and microwells are all at room temperature (20-25°C).
9.1.2 Return all the rest reagents to 2-8°C immediately after used.
9.1.3 Washing the microwells correctly is an important step in the process of assay; it is the vital factor to the reproducibility of the ELISA analysis.
9.1.4 Avoid the light and cover the microwells during incubation.

9.2 Assay Steps
9.2.1 Take all reagents out at room temperature (20-25°C) for more than 30min, homogeneize before use(Note: Concentrated wash buffer and extraction buffer should be returned to room temperature as above).
9.2.2 Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.

9.2.3 Number: Numbered every microwell positions and all standards and samples should be run in duplicate. Record the standards and samples positions.

9.2.4 Add standard solution/sample and antibody solution: Add 50μl of standard solution(Solution 1) or prepared sample to corresponding wells. Add 50μl of antibody solution(Kit provided). Mix gently by rocking the plate manually and incubate for 30min at dark place at 25°C with cover.

9.2.5 Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250μl diluted wash solution (solution 3) at interval of 10s for 4-5 times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tip).

9.2.6 Add Enzyme conjugate: Add 100μl of enzyme conjugate(Kit provided) to each well, Mix gently by rocking the plate manually and incubate for 30min at dark place at 25°C with cover. Repeat the wash step 5 again.

9.2.7 Coloration: Add 50μl solution A(Kit provided) and 50μl solution B(Kit provided) to each well. Mix gently by rocking the plate manually and incubate for 15min at 25°C with cover.

9.2.8 Measure: Add 50μl of stop solution(Kit provided) to each well. Mix gently by rocking the plate manually and measure the absorbance at 450nm (It’s suggested measure with the dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution; If no microplate reader, the result can be determined by nare eye without adding stop solution).

10. Results Interpretation
Introduction to the judgment method: Method 1 can be used for qualitative and method 2 for quantitative judgment.
(Note: The sample absorbance value is inversely realted to the amount of chlorpyrifos contained in it.)

10.1 Method 1 -- qualitative analysis
Compare the sample average absorbance value with the standard value, to get the concentration range (pb) of Chlorpyrifos in samples.
Assuming sample 1 absorbance value is 0.680, sample 2 absorbance value is 1.320, and the standard absorbance values are: 0pb 2.070; 0.6pb 1.598; 1.8pb 1.170; 5.4pb 0.703; 16.2ppb 0.32; 48.6pb 0.151. Then the concentration range of sample 1 is between 5.4pb and 16.2pb and multiplied by its corresponding dilution factor to obtain the concentration range of chlorpyrifos residues in the sample;
the concentration range of sample 2 is 0.6ppb-1.8pb and multiplied by its corresponding dilution factor to obtain the concentration range of chlorpyrifos residues in the sample.

10.2 Method 2—quantitative analysis
--Calculation of Absorbance percentage: The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

\[ \text{Absorbance (\%)} = \frac{B}{B_0} \times 100\% \]

B ——absorbance standard (or sample)
B0 ——absorbance zero standard

--To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the chlorpyrifos standards solution (ppb) as the x-axis, drawing a standard curve. Substitute the absorbance percentage of the sample into the standard curve, and read the corresponding concentration from the standard curve, then the actual residual amount of chlorpyrifos in the sample can be obtained after multiply the dilution factor.

If the professional analysis software is used for calculation, it is more convenient for accurate and rapid analysis of a large number of samples.

11 Sample dilution factor
Fruit and vegetables dilution factor: 100.

12. Sensitivity, accuracy and precision
Test Sensitivity: 0.6ppb
Detection limit
Fruit, vegetables........................................100ppb
Accuracy
Tea, fruit, vegetables.................................100±30%
Precision
Variation coefficient of the ELISA kit is less than 10%.

13. Notice
13.1 The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25°C).
13.2 Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tap the microwells holder.
13.3. Shake each reagent gently before use.
13.4. Keep your skin away from the stop solution for it is the 0.5M H2SO4 solution.
13.5 Don’t use the kits out of date. Don’t exchange the reagents of different batches, or else it will drop the sensitivity.
13.6 Keep the ELISA kits at 2-8°C, do not freeze. Seal rest microwell plates. Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.
13.7 Substrate solution should be abandoned if it turns colors. The reagents may be turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5(A450nm<0.5).
13.8 The coloration reaction needs 15min after the addition of solution A and solution B. And you can prolong the incubation time from 20min to more if the color is too light to be determined. Never exceed 25min, on the contrary, shorten the incubation time properly.
13.9 The optimal reaction temperature of the kit is 25°C (7 TF). If the temperature is too high or too low, the absorbance value and sensitivity may change, which directly affects the detection result.

14. Storage
Storage condition: 2-8°C.
Storage period: 12 months