Version 19-01/24

User's Manual

Chlorpyrifos ELISA Kit

REF **DEIANJ22**

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

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

General Description

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.

Principles of Testing

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.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. 10ppm standard solution (1ml)
3. Enzyme conjugate (12ml)
4. Antibody solution (7ml)
5. Substrate solution (2×6ml)
6. Stop solution (7ml)
7. 20× Concentrated wash buffer (50ml)
8. 2× Concentrated sample solution (50ml)

Materials Required But Not Supplied

Equipments

1. Microtiter plate spectrophotometer (450nm/630nm)

2. Homogenizer
3. Vortex mixer
4. Centrifuge
5. Analytical balance (inductance: 0.01g)
6. Graduated pipette: 10ml
7. Rubber pipette bulb
8. Polystyrene centrifuge tubes: 2ml, 50ml
9. Volumetric flask: 100ml, 500ml
10. Micropipettes: 2-20ul, 20ul-200ul, 100ul-1000ul
11. Multipipette: 50-300ul

Reagents

1. Methanol
2. Deionized water

Storage

Store all reagents at 2-8°C.

Valid Period: six months.

Specimen Collection And Preparation

Notice and precautions before operation

1. Please use one-off tips in the process of experiment and change the tips when absorbing different reagent.
2. Make sure that all experimental instruments are clean.
3. Keep the untreated sample in refrigerator.
4. Treated sample can be preserved for 4 hours.

Fruit (apple, orange) and vegetables (spinach, potato, cabbage)

1. Homogenize the sample.
2. Take 1.0±0.05g of homogenized sample into a 50ml tube, then add 10ml of methanol, vortex for 20s to mix thoroughly.
3. Centrifuge for separation: 3000g/ambient temperature(20-25°C/68-77℉)/ 5 min.
4. Transfer 100ul of the supernatant into a 2ml tube, add 900ul of sample solution (Solution 1, see "Reagent Preparation"), vortex for 20s.
5. Take 50ul of the prepared solution per well for assay.

Cereals (barley, sesame seeds, quinoa)

1. Take 1.0±0.05g of sample into a 50ml tube, then add 10ml of methanol, vortex for 20s to mix thoroughly.
2. Shaking for extraction: ambient temperature(20-25°C/68-77℉)/ 20 min.
3. Transfer 100ul of the supernatant into a 2ml tube, add 900ul of sample solution (Solution 1, see "Reagent Preparation"), vortex for 20s.

4. Take 50ul of the prepared solution per well for assay.

Reagent Preparation

Solution 1: Sample solution

Dilute the 2× concentrated sample solution with deionized water in the volume ratio of 1:1(e.g. 10ml of 2× sample solution + 10ml of deionized water), which will be used for sample extraction, this solution can be stored at 4°C for 1 month.

Solution 2: Standard solution

Prepare 10 x concentrated standard solution

1. First dilute the 10ppm standard solution to 1ppm with Methanol in the volume (e.g. 0.1ml of 10ppm concentrated standard solution + 0.9ml of Methanol).
2. Then prepare 10 x concentrated standard solution as below.

10 x concentrated standard solution	Methanol	1ppm standard solution
1ppb	999uL	1uL
3ppb	997uL	3uL
9ppb	991uL	9uL
27ppb	973uL	27uL
81ppb	919ppb	81uL

Prepare standard solution

Dilute the 10 x concentrated standard solution above with the deionized water in the volume ratio of 1:9 based on the requirement separately (e.g. 0.1ml of concentrated standard solution + 0.9ml of the sample solution).

NOTE: Prepare freshly.

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.

Assay Procedure

Notice before assay

1. Make sure all reagents and microwells are all at room temperature (20-25°C).
2. Return all the rest reagents to 2-8°C immediately after used.
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.
4. Avoid the light and cover the microwells during incubation.

5. The standard solution must be prepared for immediate use and cannot be stored.

Assay Steps

1. Take all reagents out at room temperature (20-25°C) for more than 30min, homogenize before use (**Note:** Concentrated wash buffer and Concentrated sample solution should be returned to room temperature as above).
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.
3. **Number:** Numbered every microwell positions and all standards and samples should be run in duplicate. Record the standards and samples positions.
4. **Add standard solution/sample and antibody solution:** Add 50ul of standard solution (Solution 2) or prepared sample to corresponding wells. Add 50ul of antibody solution (Kit provided). Mix gently by rocking the plate manually and incubate for 30min at dark place at 25°C with cover.
5. **Wash:** Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250ul 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).
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.
7. **Wash:** Repeat the wash step 5 again.
8. **Coloration:** Add 100ul substrate solution to each well. Incubate for 15min at 25°C with cover.
9. **Measure:** Add 50ul 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).

Calculation

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%.

Absorbance (%) = B/ B0 ×100%

B ——absorbance standard (or sample)

B_0 ——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.

sample solution factor

Fruit and vegetables dilution factor: 100.

Precision

Variation coefficient of the ELISA kit is less than 10%.

Detection Limit

Fruit, vegetables, cereals……10ppb

Accuracy

Tea, fruit, vegetables, cereals.……100±30%

Sensitivity

Test Sensitivity: 0.1ppb

Specificity

Chlorpyrifos…….....…….....100%

Precautions

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).
2. Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tap the microwells holder.
3. Shake each reagent gently before use.
4. Keep your skin away from the stop solution for it is the 2M H_2SO_4 solution.
5. Don't use the kits out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.
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.
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).
8. The coloration reaction needs 15min after the addition of substrate solution. 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.
9. The optimal reaction temperature of the kit is 25°C (77°F). If the temperature is too high or too low, the absorbance value and sensitivity may change, which directly affects the detection result.