Version 15-04/24

User's Manual

Coumaphos ELISA Kit

REF **DEIANS002**

Σ **96T**

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 coumaphos in honey.

General Description

Coumaphos is non-systemic organophosphorus insecticide which is particularly effective against diptera pests. And also it is used to control ectoparasites and drosophila, however it is with high toxicity to human and animal. It will reduce the activity of whole blood cholinesterase, causing headache and dizziness, nausea, pupil reduction, jerking, andcyanosis, pulmonary edema, brain edema, respiratory failure.

This kit is a new product based on ELISA technology, which is fast, easy, accurate and sensitive compared with common instrumental analysis and only needs 45 min in one operation, it can considerably minimize operation error and work intensity.

Principles of Testing

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

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. Standard solutions(6 bottles,1 mL/bottle)
   0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb
3. Spiking standard control: (1 mL/bottle) 1 ppm
4. Concentrated enzyme conjugate 1 mL, red cap
5. Antibody solution 7 mL, green cap
6. Solution A 7 mL, white cap
7. Solution B 7 mL, red cap
8. Stop solution 7 mL, yellow cap
9. 20×concentrated wash solution 40 mL, transparent cap
10. Sample diluent, blue cap

Materials Required But Not Supplied

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

2. Rotary evaporator or nitrogen drying instruments
3. Shaker
4. Vortex mixer
5. Centrifuge
6. Analytical balance (inductance: 0.01 g)
7. Graduated pipette: 10 mL
8. Rubber pipette bulb
9. Glass test tube: 10 mL
10. Polystyrene centrifuge tubes: 50 mL
11. Micropipettes: 20-200 µL, 100-1000 µL, 250 µL-multipipette
12. Deionized water
13. Trichloroacetic acid

Storage

Storage condition: 2-8°C.

Storage period: 12 months.

Specimen Collection And Preparation

1. Notice and precautions before operation:

a. Please use one-off tips in the process of experiment, and change the tips when absorb different reagent.

b. Make sure that all instruments are clean.

2. Honey

a. Take 1.0±0.05 g honey sample into a 10 mL polystyrene centrifuge tube, add 2 mL 1% trichloroacetic acid (see solution 1), vortex for 30 s;

b. Centrifuge at room temperature (20-25°C) for 5min ,

at least 3000 g.

c. Take 100 µL sample solution, add 375 µL sample dilution, 25 µl 0.2M NaOH solution (see solution 2), vortex for 30 s to mix it completely;

d. Take 50 µL of the prepared solution for assay.

Dilution factor of samples

Honey: 10

Reagent Preparation

1. Solution 1: 1% trichloroacetic acid

Weigh 5.0 g trichloroacetic acid and add deionized water to 500 mL and mix it completely.

2. Solution 2: 0.2M NaOH solution

Weigh 4.0 g NaOH and add deionized water to 500 mL and mix it completely.

3. Solution 3: wash solution

Dilute 20×Concentrated wash solution with deionized water in the volume ratio of 1:19, which will be used to wash the plates. This diluted solution can be stored for 1 month at 4°C.

Assay Procedure

1. Notice before assay:

a. Make sure all reagents and microwells are all at room temperature (20-25°C).

b. Return all the rest reagents to 2-8°C immediately after used.

c. 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.

d. Avoid the light and cover the microwells during incubation.

2. Assay Steps:

a. Take all reagents out at room temperature (20-25°C) for more than 30 min, shake gently before use.

b. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.

c. The diluted wash solution should be rewarmed to be at room temperature before use.

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

e. **Add standard solution/sample:** Add 50 µL of standard solution or prepared sample to corresponding wells.

f. **Dilution of concentrated enzyme conjugate with antibody solution:** dilute the concentrated enzyme conjugate with antibody solution in the volume ratio of 1:10 according to your use (1 fold concentrated enzyme conjugate +10 folds antibody solution). **Notice: this mixture can not be stored, please use it immediately.**

g. **Add antibody and enzyme conjugate mixture:** add 50 µL antibody and enzyme conjugate mixture to each well immediately. Mix gently and incubate for 30 min at 25°C with cover.

h. **Wash:** Remove the cover gently and pure the liquid out of the wells and rinse the microwells with 250 µL 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).

i. **Coloration:** Add 50 µL solution A and 50 µL solution B to each well. Mix gently and incubate for 15 min at 25°C with cover.

j. **Measure:** Add 50 µL the stop solution to each well. Mix gently and measure the absorbance at 450 nm against an air blank (It' s suggested measure with the dual-wavelength of 450/630 nm. Read the result within 5 min after adding stop solution. We can also measure by sight without stop solution if there is no ELISA reader).

Calculation

1. Percentage absorbance

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%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

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

B ——absorbance standard (or sample)

B_0 ——absorbance zero standard

2. Standard Curve

To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the coumaphos standards solution (ppb) as x-axis.

The coumaphos concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding Dilution factor of each sample followed, and the actual concentration of sample is obtained.

3. Please notice:

For data reduction of the ELISA kits, special software has been developed, which can be ordered on request.

Performance Characteristics

Accuracy: 95±25%

Precision

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

Detection Limit

3.0 ppb

Sensitivity

0.1 ppb

Specificity

Cross-reactions

Coumaphos: 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 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 (450/630 nm) of the zero standard is less than 0.5(A450 nm < 0.5).
8. The coloration reaction need 10-15 min after the addition of solution A and solution B; But you can prolong the incubation time ranges from 20 min to more if the color is too light to be determined. Never exceed 25 min, on the contrary, shorten the incubation time properly.
9. The optimal reaction temperature is 25°C. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.