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User's Manual

Cyhalothrin ELISA Kit

REF **DEIA-LL005**

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

Cyhalothrin ELISA kit can be used for qualitative and quantitative determination of cyhalothrin residues in tobacco samples.

General Description

Cyhalothrin is a representative variety of pyrethroid insecticides. Cyhalothrin is a pair of isomers with the highest insecticidal activity among 16 stereoisomers. It has the characteristics of insecticidal spectrum, high efficacy, safety, long duration of efficacy, and resistance to rain scouring.

Principles of Testing

This kit is based on indirect-competitive ELISA technology.The microtiter wells are coated with coupling antigen.Cyhalothrin 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 cyhalothrin in it, after comparing with the Standard Curve, multiplied by the dilution factor, cyhalothrin quantity in the sample can be calculated.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. Standard solution (0.5ml×5 bottles):0 ppb, 450ppb, 1350ppb, 4050ppb, 12150ppb
3. Enzyme conjugate (7ml)
4. Antibody solution (7ml)
5. Solution A (7ml)
6. Solution B (7ml)
7. Stop solution (7ml)
8. Concentrated wash solution 20× (40ml)
9. Concentrated reconstitute solution 4× (15ml)
10. Concentrated sample diluent 4x (50ml)

Materials Required But Not Supplied

1. Microtiter plate spectrophotometer (450nm/630nm)
2. Vortex mixer
3. Analytical balance (inductance: 0.01g)
4. Polystyrene centrifuge tubes: 2ml, 50ml
5. Micropipettes: 20ul-200ul,100ul-1000ul,0.5-5mL

6. Shaker
7. Acetonitrile (analytical purity)
8. Deionized water

Storage

The kit may be stored at 2-8°C for 12 months.

Specimen Collection And Preparation

Notes before sample treatment

1. Please use one-off tips in the process of experiment, and change the tips when absorb different reagent.
2. Make sure that all experimental instruments are clean, otherwise it will affect the assay result.
3. The processed samples cannot be stored and should be used immediately for detection.

Pretreatment method of tobacco leaf samples

Tobacco samples should be crushed before testing

1. Take 1.0 ± 0.05 g sample to 50 mL polystyrene centrifuge tube, add 5 mL acetonitrile, vortex mixer for 3 minutes, mixing.
2. Room temperature (20-25 °C), 3000 g, centrifuged for 5 min, marry 50 uL supernatant to 2 mL polystyrene centrifuge tube, add 950 uL sample diluent (see mixture 3), vortex for 20 seconds
3. Take 50uL for analysis

Reagent Preparation

Mixture 1: Redissolving reconstitute solution

Dilute the 4× concentrated reconstitute solution with deionized water according to the volume ratio of 1: 3 (3part of deionized water +1 parts of 4× concentrated reconstitute solution) for diluting the concentrated solution of standard substance.

Mixture 2: Standard working solution

Dilute the standard solution with reconstitute solution (see Mixture 1) in the volume ratio of 1: 49. The prepared curve shall not be stored for more than 12 hours, and the standard working solution shall be prepared and used now.

Mixture 3: Sample dilution
Dilute the 4× sample diluent with deionized water in the volume ratio of 1:3, which will be used for sample dilution. This solution can be stored at 4°C for 1 month.

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

Assay Procedure

Notices before test:

1. Make sure all reagents and microwells are all at room temperature (20-25°C).
2. 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.
3. All reagents were returned to 2-8°C immediately after use.
4. Avoid the light and cover the microwells during incubation.

Operating steps:

1. Take all reagents out at room temperature (20-25°C) for more than 30min, homogenize before use.
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.
3. Concentrated washing solution, concentrated sample diluent and concentrated reconstitute solution should also at room temperature
4. Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions
5. Add standard/sample, enzyme conjugate and antibody solution: add 50μL of standard working solution (see Mixture 2)/ sample into the corresponding microwells, and then add 50μL / well of enzyme conjugate, and then add 50μL / well of antibody solution. Gently shake and mix well, and then react in a dark environment at 25°C for 30 min with a cover film.
6. Washing plate: carefully uncover the cover film, spin off the liquid in the hole, add 250μL of washing working liquid (see mixture 4) to the hole, and wash it thoroughly for 4-5 times, with an interval of 10s each time, pour off the washing liquid in the hole of the plate, and pat it dry with absorbent paper (the bubbles that have not been removed after pat dry can be punctured with a clean gun head).
7. Add 50μL of solution A and 50μL of solution B to each well. Mix gently by shaking the plate manually and incubate for 15 min at 25°C with cover.
8. Add 50μL of stopping solution per well, shake gently and mix evenly, set the microplate reader at 450nm (it is recommended to use dual wavelength 450/630nm for detection, and read the data within 5min), and determine the OD value of each well. (If there is no microplate reader, it can be judged by visual inspection without stopping solution)

Quality Control

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Calculation

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 cyhalothrin contained in it.)

1. Compare the sample average absorbance value with the standard value, to get the concentration range (ppb) of Cyhalothrin in samples.

Assuming sample 1 absorbance value is 0.680, sample 2 absorbance value is 1.320, and the standard absorbance values are: 0ppb 2.070; 9ppb 1.598; 27ppb 0.903;81ppb 0.372; 243ppb 0.211. Then the

concentration range of sample 1 is between 27-81ppb and multiplied by its corresponding dilution factor to obtain the concentration range of butralin residues in the sample; the concentration range of sample 2 is 9-27ppb and multiplied by its corresponding dilution factor to obtain the concentration range of cyhalothrin residues in the sample.

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%

Absorbance (%) =B/B_0

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 cyhalothrin 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 cyhalothrin 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.

Precision

Variation coefficient of the ELISA kit is less than 10%.
Accuracy:
tobacco leaves:100±30%

Detection Limit

900 ppb

Sensitivity

9 ppb

Specificity

Cyhalothrin: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 be dry between steps to avoid unsuccessful repetitiveness and operate the next

step immediately after tap the microwells holder.

3. Mix the homogenate and elute the plate adequately.
4. Avoid the stop solution touching skin for the 2M H_2SO_4.
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. Storage constitution: Keep the ELISA kits at 2-8°C without frozen. Avoid direct sunlight during all incubations. Covering the microtiter plates is recommended.
7. The reagents go bad: Substrate solution should be abandoned if its color has changed. 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 15 min after the addition of solution A and solution B. If the color is lighter, the reaction time can be prolonged to 20min (or longer). But not more than 25min. Otherwise, the reaction time is shortened.
9. The best reaction temperature of the kit is 25°C, too high or too low temperature will lead to changes in absorbance value and sensitivity, which will directly affect the detection results.