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User's Manual

Glyphosate ELISA Kit

REF **DEIANS007**

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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This kit can be used in quantitative and qualitative analysis of Glyphosate residue in honey.

General Description

Glyphosate is a a new internal absorption, high efficiency and low toxicity organophosphate herbicide which is widely used all over the world. In recently decades, it has been widely used due to its good conductivity, excellent weeding effect and the glyphosate-resistant plants have been developed and used widely. The herbicide mechanism is to block the activity of 5-enolpyruvylshikimate-3-phosphonic synthase(EPSP). This synthase is present in the biosynthesis of aromatic amino acids in the green plants, therefore, glyphosate is toxic and harmful to green plants. Its main metabolite in plants, soil and water is aminomethy Iphosphonic acid(AMPA). Although it is a low-toxicity organophosphorus herbicide, its unreasonable use will also lead to some agricultural products (such as sugar cane) damage; Secondly, it will lead to excessive residues of glyphosate in plant products, which will affect the food safety of consumers, and even cause disease, poisoning and death. Meanwhile, it will also affect the international trade of related products and death. Meanwhile, it will also affect the international trade. This kit is a new product for herbicide residue detection based on ELISA technology which only costs 45 min in each operation and can considerably minimize operation errors and work intensity.

Principles of Testing

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

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. Standard solutions(6×2ml/bottle)
   0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb
   Spiking standard solution: (1 ml/bottle) 1 ppm
3. Enzyme conjugate-Antibody solution 7 ml, red cap
4. Solution A 7 ml, white cap
5. Solution B 7 ml, red cap
6. Stop solution 7 ml, yellow cap
7. 20×concentrated Wash solution 40 ml, transparent cap
8. 2×concentrated sample diluent 50 ml, transparent cap

9. 2.5xconcentrated derivatization reagent 2 ml, black cap
10. Derivatization diluent 3 ml, black cap

Materials Required But Not Supplied

1. Equipments

Microtiter plate spectrophotometer (450 nm/630 nm)

Rotary evaporator or nitrogen drying instruments

Shaker

Vortex mixer

Centrifuge

Analytical balance (inductance: 0.01 g)

Graduated pipette: 10 ml

Rubber pipette bulb

Volumetric flask: 100 ml, 500 ml

Glass test tube: 10 ml

Polystyrene Centrifuge tubes: 2 ml, 5 ml, 50 ml

Glass centrifuge tube: 10 ml

Micropipettes: 20 µl-200 µl, 100 µl-10000 µl, 250 µl-multipipette

2. Reagents

Deionized water

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. Derivatization reagent can be kept at 2-8°C for one week;

c. Diluent working reagent can be kept for three months at 2-8°C.

2. Sample preparations

Honey

Weigh 1.0±0.05 g honey sample into a 10 ml polystyrene centrifuge tube, then add 4ml deionized water and shake it with shaker to mix it completely;

Take 100 µl honey sample into 2 ml polystyrene centrifuge tube , add 900 µl sample diluent working solution(solution 1), then add 25 µl derivatization reagent working solution (solution 3), mix it complete and then incubate for 60 min at room temperature(20-25°C);

Take 100 µl of the prepared solution for assay.

3. Dilution factor: 50

Reagent Preparation

Solution 1: Sample diluent working solution

Dilute the 2×concentrated sample diluent solution with deionized water in the volume ratio of 1:1, which can be conserved for 6 months at 4°C.

Solution 2: Wash solution

Dilute the 20×concentrated wash solution with deionized water in the volume ratio of 1:19, which will be used to rinse the plate. The diluted wash solution can be conserved for a month at 4°C.

Solution 3: Derivatization reagent working solution

Dilute the 2.5×concentrated derivatization reagent with derivatization reagent in the ratio of 1:1.5 (1 fold 2.5×concentrated derivatization reagent+1.5 fold derivatization reagent) which is used for sample derivatizated and it can be conserved for one week 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 use.

c. Washing the microwells correctly is an important step in the process of assay; it is the vital factor to the repetitiveness of the ELISA analysis.

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

2. Assay process:

a. Take all reagents out at room temperature (20-25°C) for more than 30min, 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 and antibody: Add 100 µl of the standard solution or prepared sample to corresponding wells. Add 50 µl of Enzyme conjugate-antibody solution(Kit component). Mix gently by rocking the plate manually and incubate for 30 min at 25°C with cover.

f. Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250 µl of diluted wash solution (solution 2) 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).

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

h. Measure: Add 50 µl of the stop solution(Kit component) to each well. Mix gently by rocking the plate manually and measure the absorbance at 450 nm (It' s suggested measure with the dual-wavelength of 450/630 nm. Read the result within 5min after addition of stop solution.)

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 Glyphosate standards solution (ppb) as x-axis.

The Glyphosate 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:

Special software has been developed for data reduction, which can be provided upon request.

Performance Characteristics

Honey: 90±20%

Precision

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

Detection Limit

Honey: 5 ppb

Sensitivity

0.1 ppb

Specificity

Glyphosate: 100%

Aminomethylphosphonic acid: <1%

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 repetitiveness and operate the next step immediately after tap the microwells holder.
3. Mix the homogenate and elute the plate adequately.
4. Keep your skin away from the stop solution for it is the 0.5M 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 without frozen. 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 needs 15min after the addition of solution A and solution B. And you can prolong the incubation time ranges from 20 min or more if the color is too light to be determined. Never exceed 25 min. On the contrary, shorten the incubation time properly.
9. The optimum reaction temperature is 25°C. Too high or too low temperature will cause the changes of sensitivity and absorbance values.