Version 20-04/24

User's Manual

Spinosyn ELISA Kit

REF **DEIA6870**

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

The kit can be used for qualitative and quantitative analysis of water samples of spinosyn.

General Description

Spinosyn is a macrolide compound, which has rapid contact and feeding toxicity to pests, has certain permeability to crops, and has rapid insecticidal action. Suitable for controlling cotton, fruits and vegetables, tea, herbal medicine, food, and other pests. Spinosyn is highly toxic to mammals and birds, moderately toxic to aquatic animals, and there is no chronic toxicity.

This kit is a new generation of drug residue detection products developed by ELISA technology. It is fast, simple, accurate, and sensitive compared with instrumental analysis technology. The operation time is only 1.5 hours, which can minimize the operation error and work intensity.

Principles of Testing

The ELISA kit is an indirect competitive ELISA method, and pre-coated the coupled antigen on the microwells of the microplate, and the spinosyn and the pre-coated conjugated antigen on the micro-strips compete for the spinosyn antibody. After the enzyme-labeled secondary antibody added, the TMB substrate is used for coloration, and the absorbance value of the sample is negatively correlated with the content of the spinosyn. Comparing with the standard curve and multiplying it by its corresponding dilution factor, the residual amount of spinosyn in the sample can be obtained. It can be used for qualitative and quantitative analysis of water samples of spinosyn.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with coupling antigen
2. Spinosyn spiking standard solution × 3 tube, 500 ng/ml, 1 ml
3. Empty Brown reagent tube × 7 (Used to dispense different concentrations of the standards)
4. Antibody solution × 1, 7 ml
5. Enzyme labeled Secondary Ab solution, 12 ml/bottle
6 Substrate solution B × 2 bottle, 6 ml/bottle
7. Stop solution, 7 ml/bottle
8. 20× Concentrated wash solution, 50 ml/bottle
9. 2× Sample Diluent, 50 ml/bottle

Materials Required But Not Supplied

1. Microtiter plate spectrophotometer (450 nm/630 nm),
2. Polystyrene centrifuge tube, 2 ml and 5 ml,

3. Micropipettes: 20 µl-200 µl, 100 µl-1000 µl, 250 µl-multipipette

Storage

1. The optimal storage temperature of the kit is 2-8 °C, do not freeze.
2. Unused ELISA strips must be sealed and stored at 2-8 °C.

Specimen Collection And Preparation

Dilute the water sample more than 10 times with Sample diluent.

(e.g.1: water sample 0.1mL+ Sample diluent 0.9mL, e.g.2: water sample 0.1mL+ Sample diluent 1.9mL)

Reagent Preparation

Solution 1: 2× Sample Diluent

Dilute the 2× Sample Diluent with deionized water diluent in the volume ratio of 1:1, called Sample Diluent (10ml 2× Sample Diluent + 10ml deionized).

Solution 2: 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.

Solution 3: Standard solution

Prepare standard solution according to the following table with Brown reagent tube.

Concentration ng/mL	500ppb spiking standard solution µL	Sample Diluent µL	Total volume µL
0	0	1000	1000
1	2	998	1000
3	6	994	1000
9	18	982	1000
27	54	946	1000
81	162	838	1000

Assay Procedure

1. Remove the required reagents from the refrigerated environment and equilibrate at room temperature (20-25 °C / 68-77 °F) for more than 30 min. Note that each liquid reagent should be shaken before use;
2. Take out the required number of microplates, place the unused microplates in aluminum foil bags and vacuum seal them and store them at (2-8 °C / 35.6-46.4 °F).
3. The sample diluent and concentrated wash solution should also be warmed up as described above before

use.

4. Number: Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
5. Adding: Add 50 µl of standard working solution/sample solution to the corresponding microwells, add 50 µl of antibody solution to each well, Mix gently by shaking, and cover with a cover film cover at 25 °C (98.6 °F) for 30 min in the dark.
6. Wash: After carefully remove the cover film, pour out the liquid in the well, add 300 µl of washing solution (Solution 2) to each well, soak for 10 s, then pour off the washing solution, then add the washing solution repeatedly. After washing 4-5 times, the micro plate is placed on the absorbent paper and patted dry (Bubbles that have not been removed after patted can be punctured with unused tips).
7. Add Enzyme labeled secondary Ab: Add 100 µl/well of the enzyme-labeled secondary antibody, mix gently by shaking, cover with a cover film and cover at 25 °C (98.6 °F) for 30 min in the dark environment, and take out step 6 of repeated washing.
8. Coloration: Add 100 µl/well of substrate solution, and cover with a cover film and cover at 25 °C (98.6 °F) for 15 min in the dark.
9. Calculation: Add stop solution 50 µl/well, gently shake and mix, set the microplate reader at 450 nm (recommended to use dual-wavelength 450/630 nm detection, please read the data within 5 min), determine the OD value of each well.

NOTES

1. The temperature of all reagents and strips required was raised to room temperature (20-25 °C / 68-77 °F) before use.
2. All reagents were returned (2-8 °C / 35.6 - 46.4 °F) immediately after use.
3. The reproducibility in the ELISA assay is highly dependent on the consistency of the wash plate, and the correct plate wash operation is the point in the ELISA assay procedure.
4. During all incubations, avoid light exposure and seal the microplate with a cover film.

Calculation

As a result, there are two methods of judging, and the first method can be used for the rough judgment, and the second method can be used for the quantitative judgment. Note that the absorbance value of the sample is inversely related to the amount of its spinosyn. The semi-logarithmic system was used to substitute the percentage of absorbance corresponding to the standard substance and fit the standard curve with the standard substance concentration.

1. The concentration range (ppb) is obtained by comparing the average absorbance value of the sample with the standard absorbance value.The absorbance values of the standard are: 1.824 for 0ppb; 1.458 for 1ppb; 0.936 for 3ppb; 0.469 for 9ppb; 0.217 for 27ppb; and 0.140 for 81ppb. The concentration range of spinosyn residues in the sample can be derived.
2. Quantitative analysis

   Percentage absorbance: The mean values of the absorbance values obtained from 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 of standards or samples

B0 - absorbance of zero standard (0ng/ml)

Note: The standard curve is drawn by taking the percent absorbance of the standard as the ordinate and the logarithm of the spinosyn standard concentration (ppb) as the abscissa. The percent absorbance of the sample is substituted into the standard curve, and the concentration corresponding to the sample is read from the standard curve, and the corresponding dilution factor is multiplied by the actual concentration of spinosyn in the sample. If the kit professional analysis software is used for calculation, it is more convenient for accurate and rapid analysis of a large number of samples.

Sensitivity

Kit sensitivity: 1 ppb

Reproducibility

Intra-plate variation coefficient: <10%

Inter-plate variation coefficient: <10%

Precautions

1. Room temperature below 20 °C or reagents and samples not returned to room temperature (20-25 °C / 68-77 °F) will result in low standard OD values.
2. If the plate well is dry during the washing process, the standard curve is not linear and the repeatability is not good. Therefore, the next step should be taken immediately after the plate is patted dry.
3. Shake the reagents before use and avoid bubbles when mixing.
4. The reaction stop solution is a high concentration of sulfuric acid to avoid contact with the skin.
5. Do not use kits that exceed the expiration date; do not use any reagents that exceed the expiration date. Dosing a kit that exceeds the expiration date will cause a decrease in sensitivity; do not exchange reagents from different batches.
6. Store the kit at 2-8 °C (36-46 °F), do not freeze, and put the unused microplate back into the foil pouch to reseal. Standard materials and colorless substrate fluids are sensitive to light, so avoid direct exposure to light.
7. Any color of the substrate liquid indicates deterioration of the substrate fluid and should be discarded. A 0 standard absorbance (450/630 nm) value of less than 0.5 (A450 nm < 0.5) indicates that the reagent may deteriorate.
8. After adding the substrate A and the substrate B, the general color development time is 15 minutes. If the color is lighter, the reaction time can be extended to 20 minutes, but not more than 25 minutes. Otherwise, the reaction time is shortened.
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 will be changed, which will directly affect the detection result.