Version 24-11/25

User's Manual

Zearalenone ELISA Kit

REF **DEIA055V2**

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

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This kit can be used for qualitative and quantitative rapid test of zearalenone in cereals and feed.

General Description

Zearalenone is barriers formed by fungus of sickle fungus. It is a kind of phytoestrogens and has estrogenic characteristics. Resulting in animal fertility disordered or reduced. The clinical symptoms are having more estrogen. This illness not only in pork, but also in cow, horse, sheep and so on. The potential health hazard of body caused by the food of plant or animal organs ingesting mycotoxin is the discussion focus.

This kit is a new generation of drug residue test with enzyme immunoassay technology. It has characteristics of speediness, convenient, accuracy and high sensitivity. The operation time only need 20 minutes and it can furthest reduce the operation error and workload.

Principles of Testing

This ELISA kit is designed to detect zearalenone based on "direct-competitive" enzyme immunoassay. The microtiter wells are coated with BSA-linked zearalenone antigen. Zearalenone in the sample competes with the precoated antigen for binding to the limited number of antibody. After TMB substrate, the signal is measured with an ELISA photometer. The absorption is inversely proportional to the zearalenone concentration in the sample, compared with the standard curve. Then multiply by corresponding dilution ratio. And you can calculate the residue of zearalenone in the sample.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. Standard solutions(5 bottles×1ml/bottle). 0ppb, 20ppb, 80ppb, 240ppb, 1000ppb.
3. Enzyme conjugate solution 12ml. red cap
4. Substrate solution A 7ml. white cap
5. Substrate solution B 7ml.red cap
6. Stop solution 7ml. yellow cap

Materials Required But Not Supplied

1. Equipments

ELISA reader (450nm/630nm)

Homogenizer

Centrifuge

Shaker

Vortex mixer

Analytical balance (inductance: 0.01g)

Graduated pipette: 10ml

Rubber pipette bulb

Triangular flask: 250ml

Graduated cylinder: 100ml

Filter funnel

Whatma#1 filter paper or other same kind product

Volumetric flask: 100ml

Polystyrene centrifuge tube: 50ml, 2ml

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

2. Reagents

Methanol (AR)

Sodium hydroxide (NaOH)

Hydrochloric acid

Deionized water

Storage

Storage condition: 2-8°C.

Storage period: 12months

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 absorbing different reagent.

(b) Make sure that all experimental instruments are clean.

(c) Treated samples can't be stored.

2. Cereals and Feed (raw material, compound feed, concentrated feed)

Obtain a representative sample. Use homogenizer to deal with sample.

a. Method 1:

Weight 20.0±0.05g sample after homogeneity into proper triangular flask (It can be replaced by the clean sealed wild-mouth bottle).

Add 100ml 70% of methanol (see solution 1).Vortex for 5min. Let the sample stand. Filtrate and extract liquid supernatant using Whatman#1 filter paper. Collect the extracted filtrate. (The filter process can be replaced by centrifuge).

Dilute the extract sample with deionzied water in the volume of 1:3, such as take 0.5ml of extract filtrate into 2ml Polystyrene centrifuge tube. Add 1.5ml of deionzied water. Mix them completely.

Take 20µl for assay.

b. Method 2:

Weight 5.0±0.05g sample after homogeneity into 50 ml Polystyrene centrifuge tube (It can be replaced by the clean sealed wild-mouth bottle).

Add 25ml 70% of methanol (see solution 1).Shake for 5min. Centrifuge for separation: 4000r/min / 20-25°C/ 5min. (The centrifuge process can be replaced by Whatman#1 filter)

Dilute the extract sample with deionzied water in the volume of 1:3, such as take 0.5ml of extract filtrate into 2ml Polystyrene centrifuge tube. Add 1.5ml of deionzied water. Mix them completely.

Take 20µl for assay.

Notice: Please keep the PH value of the extract sample id 5-9. Higher or lower PH value can affect the result. The extract sample should be adjusted by NaOH or HCl before detection.

The Whatman#1 filter paper can be replaced by other conventional qualitative filter paper.

Dilution ratio 1

Reagent Preparation

Solution 1: 70% methanol solution

Dilute the methanol (AR) with deionized water in the volume ratio of 7:3(e.g. 7ml of methanol + 3ml of deionized water), which will be used to dilute the sample.

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. Please avoid direct sunlight during the incubation, which means the plate should be covered with the plate cover provided in the kit.

Assay Steps

1. Take all reagents out at room temperature (20-25°C) for more than 30min. Shake gently before use.
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.
3. Number: Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
4. Add standard /sample and Enzyme conjugate solution: Add 20µl of standard solution (kit component) or prepared sample to corresponding wells. Add 100µl of Enzyme conjugate solution (kit component). Mix gently by shaking the plate manually and incubate for 15min at 25°C with cover (or in dark place).
5. Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250µl of

deionized water 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. Coloration: Add 50μl of solution A(kit component), add 50μl of solution B (kit component) to each well. Mix gently by shaking the plate manually and incubate for 5 min at 25°C with cover.
7. Measure: Add 50μl of stop solution (kit component) to each well. Mix gently by shaking the plate manually and measure the absorbance at 450/630nm (It's suggested measure with the dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution). (If in the absence of microplate reader, you can judge it by visual method with no stop solution.).

Calculation

1. Quantitative analysis

(1)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%.

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

B ——the mean absorbance value of each standards or each samples

B_0 ——absorbance value of zero standard

(2)Standard Curve

To draw a standard curve, the absorbance value of standards as y-axis, semilogarithmic of the concentration of the standards (ppb) as x-axis.

The zearalenon 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.

Please notice:

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

If use the professional analysis software, It is more convenient to analyze the sample.

Notice: If the zearalenone concentration of sample is higher than standard's highest concentration (>1000ppb), the first dilute sample solution should be diluted by deionized water to the detection limit of 20-1000ppb. Then get the accurate result.

Please count the dilute ratio to the final result.

Performance Characteristics

Accuracy

Cereals and Feed: 90%±20%

Precision

C.V. of the ELISA kit is less than 10%.

Detection Range

20-1000ppb (Please dilute the sample which is higher than 1000ppb to the detection limit.)

Detection Limit

Detection limit: 20ppb

Sensitivity

Test Sensitivity: 1ppb

Specificity

Zearalenon: 100%

zearalanone: 193%

Zeranol: 117%

αzearalanel: 49%

βzearalanel: 14%

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. Don't use the kits out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.
5. 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.
6. 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).
7. The coloration reaction need 5min after the addition of solution A and solution B, but you can prolong the incubation time ranges to 10min or more if the color is too light to be determined, never exceed 15min, on the contrary, shorten the incubation time properly.
8. The best reaction of temperature will be 25°C. Please make sure the temperature is correct during all steps.

Higher or lower temperature will lead to experiment failure.

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