Version 18-04/24

User's Manual

Deoxynivalenol (DON) ELISA Kit

REF **DEIA056**

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 Deoxynivalenol in cereals and feed.

General Description

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

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. Standard solutions(5 bottles × 1ml/bottle)
   0ppb, 150ppb, 500ppb, 1500 ppb, 5000ppb
3. Enzyme conjugate: 7ml……..red cap
4. Substrate solution A 7ml ……white cap
5. Substrate solution B 7ml……..……red cap
6. Stop solution 7ml ……..……yellow cap
7. 20× concentrated wash solution 40ml……. Transparent cap

Materials Required But Not Supplied

Equipment

1. ELISA reader (450nm/630nm)
2. Homogenizer
3. Centrifuge
4. Shaker
5. Analytical balance (inductance: 0.01g)

6. Graduated pipette: 10ml
7. Rubber pipette bulb
8. Triangular flask: 250ml
9. Graduated flask: 100ml
10. Filtering funnel
11. Whatman#1 filter paper or the similar alternative products
12. Polystyrene centrifuge tube: 50ml, 2ml
13. Micropipettes: 20μl-200μl, 100μl-1000μl, 250μl-multipipette

Reagent

1. Deionized water

Storage

2-8 °C for 12 months.

Specimen Collection And Preparation

Notice and precautions before operation:

1. Please use one-off tips in the process of experiment, and change the tips when absorbing different reagent.
2. Make sure that all experimental instruments are clean.
3. When take the liquid supernatant after centrifuge, the centrifuge tube need be slightly inclined to avoid taking the impurity.

Bran, wheat middling

Method 1:

1. Homogenize the sample.
2. Weight 20.0±0.05g of sample after homogenizer into a Triangular flask. Add 400ml deionized water, shake for 5min. Keep it still and extract the supernatant with the Whatman#1 filter paper. Gather the extraction (the filtration can be replaced by the centrifuge).
3. Take 50μl for assay.

Method 2:

1. Homogenize the sample.
2. Weight 5.0±0.05g of sample after homogenizer into a Triangular flask. Add 100ml deionized water, shake for 5min. Centrifuge: 5min/3000g/room temperature. (the centrifuge can be replaced by the filter paper).
3. Take 50μl for assay.

Cereals and feed (raw feed, compound feed, concentrated feed)

Method 1:

1. Homogenize the sample.
2. Weight 20.0±0.05g of sample after homogenizer into a Triangular flask. Add 100ml deionized water, shake for 5min. Keep it still and extract the supernatant with the Whatman#1 filter paper .Gather the extraction (the

filtration can be replaced by the centrifuge).

3. Dilute the extraction with the deionized water in the volume of 1:3.
4. Take 50µl for assay.

Method 2:

1. Homogenize the sample.
2. Weight 5.0±0.05g of sample after homogenizer into a 50ml polystyrene centrifuge tube . Add 25ml deionized water, shake for 5min. Centrifuge: 5min/at least 3000g/room temperature.
3. Dilute the extraction with the deionized water in the volume of 1:3.
4. Take 50µl for assay.

Notice: The PH of the sample extraction solution should be 5-9. Use the NaOH or the HCl to adjust the PH if the PH value is higher or lower.

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

Reagent Preparation

Solution 1: Wash solution

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

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

Advice: Because of the short test time, if using the eight channel pipette, the number of sample and standard should not be more than 48 in one test. If using the single channel pipette, the number of sample and standard should not be more than 24 in one test.

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: Add 50µl of standard solution or prepared sample to corresponding wells. Add 50ul of enzyme conjugate to each well. 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 wash

solution (solution 1) 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) and 50µl of solution B (kit component) to each well. Mix gently by shaking the plate manually and incubate for 5min 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 (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

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%.

Absorbance (100%)=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 Deoxynivalenol 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.

Sample dilution ratio: 1

Notice: If the concentration of the Deoxynivalenol in the sample is higher than the 5000ppb, the concentration of the sample solution should be diluted into 150-5000ppb and test again.

Please calculate the dilution ration when calculate the result.

Precision

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

Accuracy

Cereals,Feed……..……90%±20%

Detection Range

150-5000ppb (please dilute the concentration of sample solution into 150-5000ppb when the concentration of

the Deoxynivalenol is higher than 5000ppb.)

Detection Limit

Cereals, Feed…….....……150ppb

Sensitivity

Test Sensitivity: 7.5ppb

Specificity

Deoxynivalenol: 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 the high concentration of 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 (450/630nm) of the zero standard is less than 0.5(A450nm<0.5).
8. The coloration reaction need 5min after the addition of solution A and solution B, but you can prolong the incubation time ranges to 7min or more if the color is too light to be determined, never exceed 10min, on the contrary, shorten the incubation time properly.
9. 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.