Version 15-09/24

User's Manual

T2-toxin ELISA Kit

REF **DEIABL-QB40**

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

The T2-toxin ELISA Kit is developed for quantitative measurement of T2-toxin in feed.

General Description

T-2 is a trichothecene mycotoxin. It is a naturally occurring mold byproduct of Fusarium spp.fungus which is toxic to humans and animals.

Principles of Testing

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. T-2 toxin in sample competes with the antigen coated on the microtiter plate for the antibody added. After the addition of enzyme conjugate, TMB substrate is used to show the color. Absorbance of the sample is negatively related to the T-2 toxin residue in it, after comparing with the Standard Curve, multiplied by the dilution factors, T-2 toxin quantity in the sample can be calculated.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. Standard solutions(6 bottles:1 ml/bottle) 0 ppb, 1 ppb, 3 ppb, 9 ppb, 27 ppb, 81 ppb
3. Concentrated enzyme conjugate 1.2 ml…….red cap
4. Enzyme conjugate diluent 12 ml…….green cap
5. Substrate solution A 7 ml……white cap
6. Substrate Solution B 7 ml……red cap
7. Stop solution 7 ml ……yellow cap

Materials Required But Not Supplied

1. Microtiter plate spectrophotometer (450 nm/630 nm)
2. Vortex mixer
3. Centrifuge
4. Analytical balance (inductance: 0.01 g)
5. Graduated pipette: 10 ml
6. Rubber pipette bulb
7. Volumetric flask: 100 ml;
8. Polystyrene centrifuge tube: 2 ml, 50 ml
9. Micropipettes: 20 µl-200 µl, 100 µl-1000 µl, 250 µl-multipipette
10. Methanol (AR)

11. Sodium chloride (NaCl, AR)
12. Deionized water

Storage

The T2-toxin ELISA Kit is developed for quantitative measurement of T2-toxin in feed.

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. Lean the tube when taking the supernate after centrifuge, which will avoid taking the impurities.
4. Prepared sample should be used for assay within 4h.

Feed (feedstuff, compound and concentrated), etc

1. Homogenize the feed samples with homogenizer.
2. Weigh 5.0±0.05 g of ground sample into a 50 ml polystyrene centrifuge tube, then add 25 ml of 50% methanol (Solution 1), shake fiercely for 5 min and then centrifuge for separation: 5 min / 4000 r/min / ambient temperature;
3. Take 500 µl of the supernatant into a 2 ml polystyrene centrifuge tube, mix with 500 µl of 10% NaCl (Solution 2) completely and shake for 1 min.
4. Take 20 µl of the prepared solution for assay.

Dilution Factor: 10

Reagent Preparation

Solution 1: 50% methanol

Dilute the methanol with deionized water in the volume ratio of 1:1, which will be used for sample preparation.

Solution 2: 10% NaCl

Dissolve 10.0g of sodium chloride with 100ml of deionized water.

Assay Procedure

1. **Notice before assay**

1.1 Make sure all reagents and microwells are all at room temperature (20-25°C).

1.2 Return all the rest reagents to 2-8°C immediately after use.

1.3 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.

1.4 Avoid the light and cover the microwells during incubation.

2. **Assay Steps**

2.1 Take all reagents out at room temperature (20-25°C) for more than 30 min, shake gently before use.

2.2 Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.

2.3 The diluted wash solution should be rewarmed to be at room temperature before use.

2.4 Number: Numbered every microwell positions and all standards and samples should be run in duplicate. Record the standards and samples positions.

2.5 Dilute the concentrated enzyme conjugate: Dilute the concentrated enzyme conjugate (Kit component) with the enzyme conjugate diluent (Kit component) in the volume ratio of 1:10 (e.g. 0.4 ml of concentrated enzyme conjugate + 4 ml of enzyme conjugate diluent).

2.6 Add standard/sample and diluted enzyme conjugate: Add 20 µl of standard solution(Kit component) or prepared sample to corresponding wells. Add 100 µl of diluted enzyme conjugate (2.5). Mix gently by rocking the plate manually and incubate for 10min at 25°C with cover.

2.7 Wash: Remove the cover gently and pure the liquid out of the wells and rinse the microwells with 250 µl 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).

2.8 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 5min at 25°C with cover.

2.9 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 5 min after addition of stop solution.)

Interpretation Of Results

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.

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

B ——absorbance standard (or sample)

B_0 ——absorbance zero standard

Typical Standard Curve

1. To draw a standard curve: Take the absorbance value of standards as y-axis, semi-logarithmic of the concentration of the T-2 toxin standards solution (ppb) as x-axis.
2. The T-2 toxin 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.

Precision

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

Detection Limit

Feed------10 ppb

Sensitivity

1 ppb

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 0.5M H2SO4 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 (450/630 nm) of the zero standard is less than 0.5 (A450nm<0.5).
8. The coloration reaction needs 5min after adding solution A and solution B. And you can prolong the incubation time ranges to 7min if the color is too light to be determined, never exceed 10 min, on the contrary, and shorten the incubation time properly.
9. The optimal reaction temperature is 25°C. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.