Elabscience®

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# DON(Deoxynivalenol) ELISA Kit

Catalog No: E-TO-E003

96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

## Test principle

This kit uses Indirect-Competitive-ELISA as the method. It can detect Deoxynivalenol(DON) in samples, such as rice, millet, flour and other crops/feed, etc. This kit is composed of Micro ELISA Plate, HRP conjugate, antibody, standard and other supplementary reagents. The microplate provided in this kit has been pre-coated with DON. During the reaction, DON in the samples or standard competes with DON on the solid phase supporter for sites of DON antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is for color development. There is a negative correlation between the OD value of samples and the concentration of DON. The concentration of DON in the samples can be calculated by comparing the OD of the samples to the standard curve.

## Technical indicator

**Sensitivity:**3ppb (ng/mL)

**Reaction mode:**37℃, 30min~30 min~15min

**Detection limit:** Grain andfeed---150ppb

**Cross-reactivity:**Deoxynivalenol($C_{15}H_{20}O_6$)---100%,3-Acetyldeoxynivalenol($C_{17}H_{22}O_6$)---＜1%,

**Sample recovery rate:** Grain and formula feed---85%±15%

## Kits components

| Item | Specifications |
|---|---|
| Micro ELISA Plate | 96 wells |
| Standard Liquid(black cap) | 1 mL each: (0ppb,3ppb,9ppb,27ppb,81ppb,243ppb) |
| HRPConjugate(red cap) | 11 mL |
| Antibody Working Solution(blue cap) | 5.5 mL |
| Substrate Reagent A(white cap) | 6 mL |
| Substrate Reagent B(black cap) | 6 mL |
| Stop Solution(yellow cap) | 6 mL |
| 20×Concentrated Wash Buffer(white cap) | 40mL |
| 2×Redissolved Buffer(yellow cap) | 50 mL |
| Product Description | 1 copy |

## Other supplies required

**Instrument:**Micro-platereader, Printer, Homogenizer, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01g).

**High-precision transferpettor:**Single channel (20-200 μL, 100-1000 μL), Multichannel (300 μL).

## Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the micro-plate reader in advance, preheat the instrument, and set the testing parameters.

1. **Sample pretreatment Notice:** experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. **Solution preparation**

   Solution 1(Reconstitution solution): for sample re-dissolution. Dilute the2×Reconstitution solutionwith deionized water (1:1). Store at 4℃, valid for 1 month.

   Solution 2(1×wash working buffer): 20×Concentrated Wash Buffer(volume): deionized water (volume) = 1:19.

3. **Sample pretreatment procedure**

**3.1 Pretreatment ofgrain (rice, corn and millet) and feed:**

(1) Weigh2g of crushed homogenate into 50mL EP tube, add 10mLdeionized water, oscillate for 5 min, centrifuge at 4000r/min for 10min at room temperature;

(2) Take 0.1mLof supernatant, add 0.9mLof Reconstitution fluid, mix;

(3) Take 50μL for detection and analysis.

**Note: Sample dilution factor: 50, minimum detection dose: 150 ppb**

**3.2 Pretreatment of corn husk, wheat bran and other strongwater absorption samples:**

(1) Weigh 2 g of crushed homogenate into 50mL EP tube, add 20mLdeionized water, oscillate for 5 min, centrifuge at 4000r/min for 10min at room temperature;

(2) Take 0.1mLof supernatant, add 0.9mLof Reconstitution fluid, mix;

(3) Take 50 μL for detection and analysis.

**Note:Sample dilution factor: 100, minimum detection dose: 300 ppb**

For the sample containing high level of toxins, it can be diluted by 35% methanol before determination. For example, take 0.1mL of the mixed solution in the procedure 3.2 (2),add 0.9mL of 35% methanol, mix up. The final dilution factor of sample is 1000, the minimum detection dose is 3000ppb.

As the toxins distribute unevenly in samples, we need make multidraw and mix fully first, then take 2g of the solution to detect.

## Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature for 30 min before use. **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.**Dilute 40 mL of concentrated wash buffer into 800 mLwash working buffer with deionized or distilled water before experiment.

1. **Number:** number the sample and standard in order (multiplewell), and keep a record of standard wells and sample wells.
2. **Add sample:** add 50μL of Standard, Blank, or Sample per well, then add 50μL antibody working solution, cover the plate with sealer we provided, oscillate for 5s gently to mix thoroughly, shading light incubation for 30min at37℃.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 250μLof wash working buffer to each well and wash.Repeat wash procedure for 5 times, 30s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **HRP conjugate:** add100μL HRP conjugate to each well, incubate for 30min at 37℃ in dark.
5. **Wash:** repeat step 3.
6. **Color Development:** add 50μLof substrate solution A to each well, and then add 50μLof substrate solution B.Gently oscillate for 5s to mix thoroughly. Incubate for 15min at 37℃ in dark.
7. **Stopreaction:**add 50μLof stop solution to each well, gently oscillate for 5s.
8. **OD Measurement:** determine the optical density (OD value) of each wellat 450 nm with a micro-plate reader (the 450/630 nm double wavelength is recommended).This step should be finished in 10min after stop reaction.

## Result analysis

1 **Absorbance(%)=A/$A_0$×100%**

A: Average absorbance of standard or sample

$A_0$: Average absorbance of 0ppb Standard

2 **Drawing and calculation of standard curve:**

Create a standard curve by plotting the absorbancepercentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add average absorbance valueof sample to standard curve to get corresponding concentration. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

For this kit, it is more convenient to use professional analysis software for accurate and fast analysis on a large number of samples.

## Notes

1. Overall OD value will be lower when reagents is not brought to room temperature before use or room temperature is below25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curve andpoor repeatability, move on to the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. Micro ELISA plate should be covered by plate sealer. Avoid the reagents to strong light.
5. Do not use expired kit and reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0)＜0.5 unit($A_{450nm}$＜0.5), it indicates reagent is deteriorated.
7. Stop solution is caustic, avoidcontact withskinandeyes.

## Storage and valid period

**Storage**: Store at 2-8℃. Avoid freeze / thaw cycles.

**Valid Period**: 1 year, production date is on the packing box.