QUALICHEK™ Deoxynivalenol (DON) ELISA

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RUO

Quantitative testing of Deoxynivalenol in samples, such as rice, millet, flour and other crops/feed, etc.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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Introduction:

Vomitoxin, also known as deoxynivalenol (DON), is a type B trichothecene, an epoxy-sesquiterpenoid. This mycotoxin occurs predominantly in grains such as wheat, barley, oats, rye, and corn, and less often in rice, sorghum, and triticale. The occurrence of deoxynivalenol is associated primarily with Fusarium graminearum (Gibberella zeae) and F. culmorum, both of which are important plant pathogens which cause fusarium head blight in wheat and gibberella or fusarium ear blight in corn.

Intended Use:

This kit is for quantitative testing for Deoxynivalenol in sample, such as rice, millet, flour and other crops/feed, etc.

Principle:

This kit uses Indirect-Competitive-ELISA as the method. It can detect DON in samples, such as rice, millet, flour and other crops/feed, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody, standard and other supplementary reagents. The microplate provided in this kit has been pre-coated with coupled antigen. During the reaction, DON in the samples or standard competes with coupled antigen 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.

Materials Provided:

1. Coated Microtiter plate - 1x 96 wells
2. Standards Liquid -1mL each (0 ppb, 3 ppb, 9 ppb, 27 ppb, 81 ppb, 243 ppb)
3. HRP Conjugate - 11 mL
4. Antibody Working Solution - 5.5 mL
5. Substrate A - 6 mL
6. Substrate B - 6 mL
7. Stop Solution - 6 mL
8. Wash Buffer (20X) - 40 mL
9. Plate Sealer - 3 pieces

Material required but not provided:

Instrument: Microtiter plate reader, Printer, Homogenizer, Nitrogen Evaporators/Water bath, Oscillators, centrifuge, Graduated pipette, Balance (sensibility 0.01 g)

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

Reagents: Single channel (20-200 µL, 100-1000 µL), Multichannel (300 µL).

Storage Information:

1. Store main kit components at 2-8°C Avoid freeze / thaw cycles.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

Sample pretreatment:

Bring all reagents and samples to room temperature before use.

Open the microplate 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 Buffer (for sample re-dissolution): Dilute the 2×Reconstitution Buffer with deionized water (1:1). Storage for a month at 4 ℃.
Solution 2: Wash Buffer: Dilute the 20×Concentrated Wash Buffer with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) =1:19).

3. Sample pretreatment procedure:

Pretreatment of grain (rice, corn and millet) and Feed:
1. Weigh 2 g of crushed homogenate into 50 mL EP tube, add 10 mL deionized water, oscillate for 5 min, centrifuge at 4000 r/min for 10 min at room temperature;
2. Take 0.1 mL of supernatant, add 0.9 mL of Reconstitution Buffer, mix fully
3. Take 50 μL for detection and analysis.

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

Pretreatment of corn husk, wheat bran and other strong water absorption feed:
1. Weigh 2 g of crushed homogenate into 50 mL EP tube, add 20 mL deionized water, oscillate for 5 min, centrifuge at 4000 r/min for 10 min at room temperature;
2. Take 0.1 mL of supernatant, add 0.9 mL of Reconstitution Buffer, mix fully;
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 Reconstitution Buffer before determination. For example, take 0.1 mL of the mixed solution in the procedure 3.2 (2), add 0.9 mL of Reconstitution Buffer, mix fully. The final dilution factor of sample is 1000, the minimum detection dose is 3000ppb.

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.**

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells.
2. **Add sample:** add 50 μL of Standard or Sample per well, then add 50 μL of antibody working solution, cover the plate with sealer, oscillate for 5 s gently to mix thoroughly, incubate for 30min at 37℃.
3. **Wash:** uncover the sealer carefully; remove the liquid in each well. Immediately add 300 μL of wash buffer to each well and wash. Repeat wash procedure for 5 times, 30 s 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**: add 100μl of HRP conjugate to each well, incubate shading light for 30 min at 37^0C
5. **Wash**: repeat step 3.
6. **Color Development:** add 50 μL of Substrate Reagent A to each well, and then add 50 μL of Substrate Reagent B. Gently oscillate for 5 s to mix thoroughly. Incubate shading light for 15 min at 37℃ (The reaction time may be shortened or prolonged according to the depth of the color).
7. **Stop reaction:** add 50 μL of stop solution to each well, oscillate gently to mix thoroughly.
8. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm with a microplate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10 min after stop reaction.

Judgment of result:

Absorbance (%) =A/A0×100%

A: Average absorbance of standard or sample

A_0: Average absorbance of 0 ppb Standard

Drawing and calculation of standard curve

Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add average absorbance value of 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. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25℃.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate 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. ELISA Microtiter plate should be covered by plate sealer. Avoid the reagents to strong light.
5. Do not use expired kit or reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard (concentration: 0)＜0.5 unit (A450nm＜0.5), it indicates the reagent may be deteriorated.
7. Stop solution is caustic; avoid contact with skin and eyes.

Technical specification:

Sensitivity: 3 ppb (ng/mL)

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

Detection limit: Grain and Feed ---150 ppb.

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%

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