Manual of Deoxynivalenol (DON) Elisa Kit

1. Product Code: WSK-E16GS

2. Summary

This product adopts Elisa technique. It takes only about 20minutes to do the test after sample pretreatment.

3. Application

It is suitable for qualitative/quantitative detection of DON in feedstuff and animal feed.

This manual must be read in its entirety before using this product.

Please contact technical service as below if any question about the manual

Phone: 86-27-87582341
Fax: 86-27-87196150
Email: tech@cusabio.com
Web: www.cusabio.com

P.S：Please provide lot number of the kit to us (on the outside of box), so that we respond to your question as soon as possible.

4. Assay Principle

This product adopts competitive inhibition enzyme immunoassay technique. DON antigen is pre-coated on microtiter plate. When adding standard/sample, HRP-conjugate secondary antibody and antibody into microtiter plate in sequence, pre-coated antigen and standard/sample both react with antibody. Meanwhile, HRP-conjugate secondary antibody will combine with antibody. It turns colorful after adding TMB Substrate. Later, add Stop Solution to stop reaction and then read the data. DON concentration is in negative correlation to the OD value. Compare with Standard Curve and multiple dilution times, then you get DON content in sample.

5. Performance Data

1) Detection Range: 200μg/kg-7500μg/kg
2) Limit of Detection: 200μg/kg
3) Recovery Rate: 80%-120%
4) Intra-assay Precision: CV%≤ 10%
Three samples of known concentration were tested twenty times on one plate to assess.
Inter-assay Precision: CV%≤ 10%
Three samples of known concentration were tested in twenty assays to assess.

5) Cross-reaction Rate

Deoxynivalenol (DON)	100%
3-Acetyl-DON	17%
15-Acetyl-DON	<1%

The Limit of Detection (LOD) was defined as the lowest concentration that could be differentiated from zero. It was determined by mean O.D value of 20 replicates of zero standard added by their three standard deviations.

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6. Product Composition

Product Composition	Quantity(96T)
Microtiter Plate	96T*1set
Standard	6 x 1 mL
HRP-conjugate	1 x 7 mL
Antibody	1 x 7 mL
TMB Substrate	1 x 12 mL
Stop Solution	1 x 10 mL
Wash Buffer (10x)	1 x 30 mL
Sample Diluent	1 x 50 mL
Adhesive Strip	2pcs
Manual	1pcs

7. Standard Concentration

Standard	S1	S2	S3	S4	S5	S6
Concentration (μg/kg)	0	200	500	1000	2500	7500

P.S: Sample dilution times is 50, and standard actual concentration is 1/50 of tagged concentration. So, you do not need to multiple sample dilution times when calculating result.

(For strong water-absorbing samples like bran、wheat middling、alfalfa、silage and etc, you need to multiple samples dilution times 2 when calculating result.)

8. Storage

Sealed Kit	Store at 2-8°C for 12 months, avoiding light. Do not use product beyond expiration date.
Opened kit	Put unused microtiter plate back in aluminum foil bag and seal well with provided drying agent. May be stored for up to one month at 2-8° C.

***It is for product within expiration date.**

9. Required Agent and Instrument

- Deionized Water, Absolute Ethyl Alcohol
- Microplate Reader capable of measuring absorbance at 450 nm.
- Incubator which can provide stable incubation conditions up to 25°C±0.5°C.
- Squirt Bottle, Manifold Dispenser, or Automated Microplate Washer.
- Absorbent Paper for blotting the microtiter plate.
- Analytical Balance (2 decimal place), Weighing Scoop
- Centrifuge, Vortex Mixer, Water Bath
- 50 mL Graduated Cylinders.
- Single-channel Micropipette(20-200μL、100μL-1000μL、1000μL-5000μL)
- 300μL Multichannel Micropipette
- Pipettes and Pipette Tips.
- Test Tubes for dilution.

PRECAUTIONS

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The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using it.

Note:

- Kindly use graduated containers to prepare reagent.
- Put all reagents at room temperature (20-25°C) for an hour before use.
- Only disposable tips can be used for experiment and tips must be changed when using different reagents.
- Deionized Water is recommended to use to make preparation of reagents or samples. Contaminated water or container for reagent preparation will influence detection result.

10. Reagent Preparation

- **Wash Buffer:** If crystals appeared in concentrate, warm up to room temperature and mix gently until crystals have been completely dissolved. Dilute **Wash Buffer (10x)** with **Deionized Water** at the ratio of 1 piece **Wash Buffer (10x)**: 9 pieces **Deionized Water.** It is valid at 4℃ for one month.

- **40% (v/v) Ethanol Water:** Put 400mL Absolute Ethyl Alcohol in 600mL Deionized Water. Mix well.

Note:

1. CUSABIO is only responsible for the kit itself, but not for samples consumed during assay. The user should calculate possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

2. If samples are not indicated in the manual, it is necessary to do preliminary experiment to determine validity of the kit.

3. Please predict sample concentration before assaying. If values are not within Range of standard curve, users must determine optimal sample dilutions for their particular experiments.

11. Key Note

- The kit should not be used beyond expiration date.
- Do not mix or substitute reagents with those coming from other lots or sources.
- Prepare all necessary reagents before experiment. Mix well when diluting reagent or sample, avoiding foam. If crystals appeared in reagent, put it in Water Bath at 37℃ until crystals have been completely dissolved.
- Must use disposable pipette tips. Change tips when taking different reagents.
- When using Single-channel Micropipette, you can add two samples one time at most. Total Time for adding standard/sample and antibody shall not exceed five minutes. And time for adding antibody shall be within 1minute (Highly recommended to use Eight-channel Micropipette for adding HRP-conjugate and antibody).
- Please soak used bottles(loading standard)、 centrifuge tube、 pipette head、 gloves etc which touched positive sample with 10% Sodium Hypochlorite/Household Bleaching Powder for two hours. Then adjust PH value of solution to seven and dispose. As of liquid waste containing mycotoxin, adjust PH value of solution to seven and then add Sodium Hypochlorite/Household Bleaching Powder at 10%(m/v) according to liquid waste volume. Wait for two hours and then dispose.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference that caused by soluble receptors, binding proteins, and other factors present in biological samples. Unless all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

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12. Sample Pretreatment

Note: Sample needs to be pulverized or ground and passes 20mesh screen (particle size smaller than 1mm). Then mix well for storage (Ground sample shall avoid moisture absorption. It is better measure moisture content before storage and regularly measure moisture content in storage).

A. Feedstuff and Animal Feed---Extracting by Ethanol Water

1) Extraction:

Weigh 5.00±0.05g well mixed sample, then put into 50mL centrifuge tube. Later, add 25mL 40% Ethanol Water (Alternative using 300mL Conical Flask if you do not have 50mL centrifuge tube. Then, you shall increase sample amount to 20±0.2g). P.S: Sample Extracting Ratio is 1:5(m/v), namely, one piece sample weight to five pieces extracting solution volume.

Vibrating by Vertical Multi-tube Vortex Mixer or equivalent device for three minutes; or vibrating by Horizontal Shaking Table at 250rpm for ten minutes.

2) Sample Cleansing:

Centrifuge at 4000rpm for five minutes or wait for 5-10minutes.

3) Sample Dilution:

Take 40μL supernatant and then add 360μL Sample Diluent. Mix well.

Sample Dilution Times: 1.

B. Feedstuff and Animal Feed---Extracting by Deionized Water

1) Extraction:

Weigh 5.00±0.05g well mixed sample, then put into 50mL centrifuge tube. Later, add 25mL Deionized Water (Alternative using 300mL Conical Flask if you do not have 50mL centrifuge tube. Then, you shall increase sample amount to 20±0.2g). P.S: Sample Extracting Ratio is 1:5(m/v), namely, one piece sample weight to five pieces extracting solution volume.

Vibrating by Vertical Multi-tube Vortex Mixer or equivalent device for three minutes; or vibrating by Horizontal Shaking Table at 250rpm for ten minutes.

2) Sample Cleansing:

Centrifuge at 4000rpm for five minutes or wait for 5-10minutes.

3) Sample Dilution:

Take 40μL supernatant and then add 360μL Sample Diluent. Mix well.

Sample Dilution Times: 1.

For strong water-absorbing sample like bran、wheat middling、alfalfa、silage and etc.

1. Extracting by Ethanol Water

Weigh 10.0±0.05g well mixed sample into 300mL Conical Flask, then add 100mL 40% Ethanol Water. Vibrating by Horizontal Shaking Table at 250rpm for ten minutes. Centrifuge at 4000rpm for five minutes or wait for 5-10minutes. Take 100μL supernatant and then add 900μL Deionized Water. Mix well.

Sample Dilution Times: 2.

2. Extracting by Deionized Water

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Weigh 10.0±0.05g well mixed sample into 300mL Conical Flask, then add 100mL Deionized Water. Vibrating by Horizontal Shaking Table at 250rpm for ten minutes. Centrifuge at 4000rpm for five minutes or wait for 5-10minutes. Take 100μL supernatant and then add 900μL Deionized Water. Mix well.
Sample Dilution Times: 2.

13. Assay Procedure

Put all reagents at 20~25℃ for an hour at least before use. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and samples as directed in previous sections.

2. Take out required wells and rack and put back the rest into aluminum foil bag and seal it. Store at 2-8°C. Avoid wetting.

3. Add 50μL **Standard** or **Sample** into wells in sequence. It is recommended that all samples and standards be assayed in duplicate.

4. Add 50μL **HRP-conjugate** into each well in sequence. Then add 50μL **Antibody** into each well in sequence. Seal plate with adhesive strip and shake plate for 30s. Later, incubate at 25°C for 30 minutes.

5. Take out microtiter plate and remove adhesive strip and then spin-dry liquid in wells on absorbent paper. Then wash the plate four times with diluted wash buffer. Every time, soak each well with 250μL wash buffer for 30s, and then spin-dry liquid in wells on absorbent paper. If you use squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, let it stand for 15~30 seconds, because complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. Add 100μL **TMB Substrate** into each well in sequence, then shake it softly for a while. Later, seal plate with adhesive strip again and then incubate at 25°C for 5 minutes.

7. Add 50μL **Stop Solution** into each well in sequence so as to stop reaction (Blue turns Yellow). Gently tap the plate to ensure thorough mixing. The sequence of adding **Stop Solution** shall be the same sequence of adding **TMB Substrate**. In order to guarantee result accuracy, you shall add **Stop Solution** as soon as **TMB Substrate** reaction time reaches to 5minutes.

8. Read OD value with Microplate Reader at 450nm/630nm. Please read data in 5minutes after adding **Stop Solution**.

Key Note

1. Final experimental results will be closely related to validity of products, operation skills of end users and experimental environment.

2. Samples or reagents addition: Please carefully add samples into wells and mix gently to avoid foaming. Do not touch the well wall. Duplication of all standards and specimens is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent. Do experiment fluently. Put aside microplate for a long time without moving to next step, which may cause dry wells, degeneration of antibody or antigen, and finally bad result.

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3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added into the well strips, DO NOT let the strips DRY at any time during assay. Incubation time and temperature must be observed.

4. Washing: Wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding 30second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

5. Control of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10minutes). TMB Substrate shall change color from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.

6. TMB Substrate is easily contaminated. It shall remain colorless or light blue until it is added into the plate. Store it avoiding light. Discard it with any color that indicates the degeneration of this solution; when the absorbance value of standard solution S1 is less than 0.5, which indicates its degeneration.

7. Wells that turn green indicate that Stop Solution has not mixed thoroughly with TMB Substrate.

8. Best reaction temperature of this product is 25±3°C. Too high or too low reaction temperature will influence result.

14. Data Analysis

Please check details at https://www.cusabio.com/m-225.html.

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