

Zearalenone ELISA Kit

ZEN ELISA

Product #: F1002-96T

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1. Description

Zearalenone, also known as RAL and F-2 mycotoxin, is a potent estrogenic metabolite produced by some *Fusarium* and *Gibberella* species. Zearalenone is the primary toxin, causing infertility, abortion or other breeding problems, especially in swine.

This ELISA Kit is based on indirect competitive ELISA to detect zearalenone in sample within 45 minutes.

2. Principle

This ELISA Kit is based on indirect competitive ELISA, the coupled antigen was pre-coated in the microwells, the zearalenone will compete the antibody with the pre-coated antigen, then add enzyme conjugate and TMB substrate successively, the OD value of the sample is negatively correlated with the content of zearalenone contained in the sample. The standard curve is fitted by regression and multiplied by the corresponding dilution ratio to obtain the content of zearalenone in the sample.

3. Application

This kit is applicable for quantitative determination of zearalenone in cereals (wheat, corn, sorghum, red bean, green bean, mung bean, etc) and feed.

4. Instrument and material required

- 1) ELISA reader, with 450/630nm
- 2) Incubator (25°C)
- 3) Centrifuge
- 4) Balance, 0.0001g
- 5) Centrifuge tube, 2mL, 15mL, 50mL
- 6) Vortex mixer
- 7) Micropipette, 20-200 μ L, 100-1000 μ L
- 8) Multi-channel pipette, 250 μ L

5. Reagent required

Ultrapure water, Methanol (AR), acetonitrile (AR).

6. Kit components

- 1) Microtiter plate, 96wells, 1 plate
- 2) Zearalenone standards, 1mL/vial, 6 vials, 0, 0.1, 0.3, 0.9, 2.7, 8.1 ng/mL
- 3) Enzyme conjugate, 12mL, with brown cap
- 4) Antibody Solution, 7mL, with green cap
- 5) TMB Substrate, 12mL, with brown cap
- 6) 20X Wash buffer, 50mL, with transparent cap
- 7) 2X feed buffer, 50mL, with transparent cap
- 8) 2X cereal buffer, 50mL, with transparent cap
- 9) manual, 1 set

7. Buffer preparation

Wash buffer: Dilute 20X wash buffer with ultrapure water, in the volume ratio of 1:19, for example, 10mL 20X wash buffer + 190mL ultrapure water, mix thoroughly. This diluted wash buffer can be stored at 2-8°C for 1 month.

Feed buffer: Dilute 2X feed buffer with ultrapure water, in the volume ratio of 1:1, for example, 10mL 2X feed buffer + 10mL ultrapure water, mix thoroughly, which can be used in the feed dilution. This diluted feed buffer can be stored at 2-8°C for 1 month.

Cereal buffer: Dilute 2X cereal buffer with ultrapure water, in the volume ratio of 1:1, for example, 10mL 2X cereal buffer + 10mL ultrapure water, mix thoroughly, which can be used in the cereal sample dilution. This diluted cereal buffer can be stored at 2-8°C for 1 month.

50% methanol: Dilute methanol with ultrapure water, in the volume ratio of 1:1, for example, 10mL methanol + 10mL ultrapure water, mix thoroughly, which can be used in the extraction of cereal sample.

84% acetonitrile: Dilute acetonitrile with ultrapure water, in the volume ratio of 84:16, for example, 84mL acetonitrile + 16mL ultrapure water, mix thoroughly, which can be used in the extraction of feed sample.

Stop solution: 2M sulfuric acid, which should be prepared by customer in the lab. Protocol can be provided upon request.

8. Notice and precautions before assay

- 1) Make sure the ELISA kit and all reagents are returned to room temperature (20-25 °C). For example, keep these reagent and kits at room temperature for at least 30min.
- 2) Return unused kit components to 2-8 °C.
- 3) Washing step is important for the reproducibility of the kit, please follow this instruction carefully.
- 4) Cover the ELISA plate during all incubation. Avoid direct sunlight.

9. Sample preparation

Precautions before prepare samples:

- 1) Use disposable tips during the test. Change new tip for different sample / reagent.
- 2) Make sure all lab wares are clean and ready to use.
- 3) Prepared sample shall be analyzed immediately after dilution.

10. Sample handling

10.1 Cereal

Take 5±0.01g sample into a 50mL centrifuge tube, then add 25mL 50% methanol, vortex for 2min to dissolve. Centrifuge for 5min at 5000g, and then take 100µL supernate into a 2mL centrifuge tube, add 900µL cereal buffer, mix thoroughly. **Then take 200µL and mix with 400µL cereal buffer** again, mix thoroughly by vortex. Take 50µL sample for assay.

10.2 Feed

Take 5±0.01g sample into a 50mL centrifuge tube, then add 25mL 84% acetonitrile, vortex for 2min to dissolve. Centrifuge for 5min at 5000g, and then take 100µL supernate into a 2mL centrifuge tube, add 900µL feed buffer, mix thoroughly. **Then take 100µL and mix with 500µL feed buffer** again, mix thoroughly by vortex. Take 50µL sample for assay.

11. Assay procedures

- 1) Return the ELISA kit and all reagents to room temperature (20-25 °C). For example, keep these reagent and kits at room temperature for at least 30min.
- 2) Take needed microwells and zip rest in the zip-bag and return to 2-8 °C.
- 3) Layout the plate and record sample and standard well positions. It is recommended to run all tests in duplicates.
- 4) **Add sample/standard, antibody and enzyme conjugate:** add **sample/standard** into the wells, **50µL per each**, then add **antibody solution, 50µL per well**, add **enzyme conjugate, 50µL per well**, shake gently and then cover the plate and incubate at **25 °C for 30min**.

- 5) **Wash:** take out the plate and pour the liquid out. Use the diluted wash buffer to wash the plate, 250µL per well. Wash for 4-5 times with interval of 10s. The pour the liquid out and tap the plate against absorbent paper. Eliminate the air bubble in the wells with micropipette tip if the bubble exists.
- 6) **Coloration:** add TMB substrate, 100µL per well, and then cover the plate and incubate **25 °C for 15min.**
- 7) **Stop the reaction:** add stop solution, 100µL per well, shake gently and read the plate with ELISA reader at 450nm. Read the plate within 5min after adding stop solution.

12. Result Calculation

This kit is based on competitive ELISA, thus the OD values is inversely proportional to the zearalenone content contained in sample.

12.1 Quantitative analysis

Calculation of absorbance: the percentage absorbance of standard product or sample is equal to the average absorbance value of standard product or sample (double hole) divided by the average absorbance value of blank standard product, and then multiplied by 100%, i.e

$$\text{percentage absorbance (\%)} = \frac{B}{B_0} \times 100\%$$

B - the average absorbance value of standard product or sample

B₀- the average absorbance value of blank standard product

12.2 Standard curve

The standard curve was drawn by taking the percentage absorption of standard substance as the vertical coordinate and the logarithm of concentration of standard substance zearalenone as the horizontal coordinate. Put the percentage absorption rate of samples into the standard curve, read the corresponding concentration of samples from the standard curve, and multiply the corresponding dilution times to obtain the actual concentration of zearalenone in samples.

13. Sample dilution factor:

Feed: 360, Cereal: 180

14. LOD of sample

Feed: 36 ng/g Cereal: 18 ng/g

15. Specifications of the kit

- 1) Sensitivity: 0.1ng/mL
- 2) Specificity: 100% to zearalenone, 13% to zearalanone, and <1% to zeranol/zeralanol
- 3) Recovery: 80% - 120%
- 4) Precision: CV<10%.

16. Cautions and tips for the test

- 1) Lower room temperature, e.g., lower than 20 °C may cause lower OD values. Please make sure all reagent and kit components are returned to room temperature.
- 2) Wash step is vital for the reproducibility of the kit. Please wash according to the kit instruction. Do not let the plate dry during wash. Continue the next operations immediately after wash step.
- 3) Shake each reagent gently before use.
- 4) Stop solution is acidic, please handle with care.
- 5) Do not use expired kits and reagents. Do not mix the reagent and kits from different LOT.
- 6) The kit is stored at 2-8°C(36-46°F), do not freeze.

- 7) TMB substrate is sensitive to sunlight. Avoid direct sunlight.
- 8) If Standard 1 (0ng/L) OD is lower than 0.5, please do not use. The kit may be expired or deteriorated.
- 9) The coloration step takes 15min. You can prolong it to 20min-25min if the color of the well is too light. On the contrary, please reduce the incubation time.
- 10) The incubation is 25°C /98.6 °F, lower or higher temperature will cause changes of OD and sensitivity of the kits, which may affect the result of the assay.

17. Storage and expiration

The kit is valid for 12months when stored at 2-8 °C. LOT and Expiry information are printed on the package.