Zearalenone (ZEN) ELISA Kit

NE070200301 – 96 wells

Standard Range: 0 – 729 ppb

Sample Types: Grains, Feed etc.

Cross-Reactivity: Zearalenone: 100%; Zearalanone: 13%; Zearalanol: <1%

General Description

Zearalenone is one of the metabolites of Fusarium sp. fungi. Zearalenone has a strong estrogen-like effect, can cause animal estrogen hyperactivity, resulting in infertility or abortion animals. As a result, countries have strict legal requirements or surveillance systems for the toxin. Zearalenone ELISA Kit is a highly sensitive testing product, capable of qualitative and quantitative analysis of the toxin residues in grains, feed and cereals.

Test Principle

This kit uses the indirect competitive enzyme-linked immunoassay (ELISA) method to detect Zearalenone (ZEN) in samples such as grains and feed. The kit consists of an antigen-coated plate, HRP conjugate, antibodies, standards, and other supporting reagents. During detection, when standards or samples are added, ZEN in the samples will compete with the paired antigens to bind to anti-ZEN antibodies. After the addition of the HRP conjugate, staining is performed with TMB substrates. The absorbance value of the samples has a negative correlation with the ZEN content. Finally, by comparing the obtained absorbance values with the standard curve, the ZEN toxin content in the sample can be calculated.

Kit Contents and Storage

The shelf life of the kit is 12 months. Store the kit at 2-8 ℃ and avoid freezing.

Components	Quantity
ELISA Plate	8 wells x 12 strips
Standards (0ppb, 0.3ppb, 0.9ppb, 2.7ppb, 8.1ppb, 24.3ppb) (black cap) Corresponded to: **0ppb, 9ppb, 27ppb, 81ppb, 243ppb, 729ppb**	1 mL / each
Antibody Solution (blue cap)	5.5 mL
HRP Conjugate (red cap)	5.5 mL
Substrate Reagent A (white cap)	6 mL
Substrate Reagent B (black cap)	6 mL
Stop Solution (yellow cap)	6 mL
20X Concentrated Wash Buffer (white cap)	40 mL
Adhesive Membrane	1 pcs
Manual	1 pcs

Materials Required but Not Supplied

- Microplate reader,
- Grinder (for homogenizing solid samples),
- Vortex mixer (for shake and mix),
- Centrifuge or filter paper,
- Balance with a division value of 0.01 g,
- Micropipettes (single-channel and multi-channel),
- Methanol.

Precautions

- Prior to beginning the assay procedure, bring all reagents to room temperature (20 – 25°C).
- Mix all reagents gently by inverting them before use. Do not foam.
- Complete all steps in the study uninterrupted and within the recommended time limits.
- Please mix the contents in the wells evenly and wash the plate thoroughly. Reproducibility largely depends on the consistency of the washing step.
- After washing, ensure the wells are sufficiently dry before proceeding to the next step quickly.
- Cover the microplates with an adhesive membrane during incubation.
- Close all reagent caps immediately after use and do not replace bottle caps.
- Use a separate disposable tip for each sample and solution to prevent cross-contamination.
- Since the OD values of the standard curve may vary depending on real-time test performance conditions (e.g., operator, pipetting technique, washing technique, or temperature effects), the user should create a standard curve for each test.
- Do not use reagents that have expired.
- Do not mix reagents from different lots.
- If the absorbance value of 0 ppb is below 0.5 (A450nm < 0.5), it means the reagent may be metamorphic.
- The substrate solution should be colorless; otherwise, discard it.
- Check both the sensitivity and accuracy of the laboratory equipment used during the procedure (micropipets, ELISA reader, etc.).
- Do not smoke, eat, drink, or perform pipetting by mouth in the laboratory.
- Always wear disposable gloves during the procedure.
- Avoid contact of substrate and stop solution with skin and mucous membranes (possible risk of irritation, burns, or toxicity). In case of contact, wash the affected area with plenty of water.
- The usage and disposal of chemical products should be carried out in accordance with good laboratory practices (GLP).

Preparation

Please remember that all laboratory equipments must be clean. Use disposable pipette tips to prevent contamination of results.

Reagent Preparation:

Reagent 1: 90% Methanol Solution

Dilute the methanol with distilled water at a volume ratio 9 : 1 (9 unit Methanol : 1 unit Distilled Water).

Reagent 2: 1X Wash Buffer

Dilute the 20X wash buffer with distilled water at a volume ratio 1 : 19 (1 unit 20X Wash Buffer : 19 unit Distilled Water).

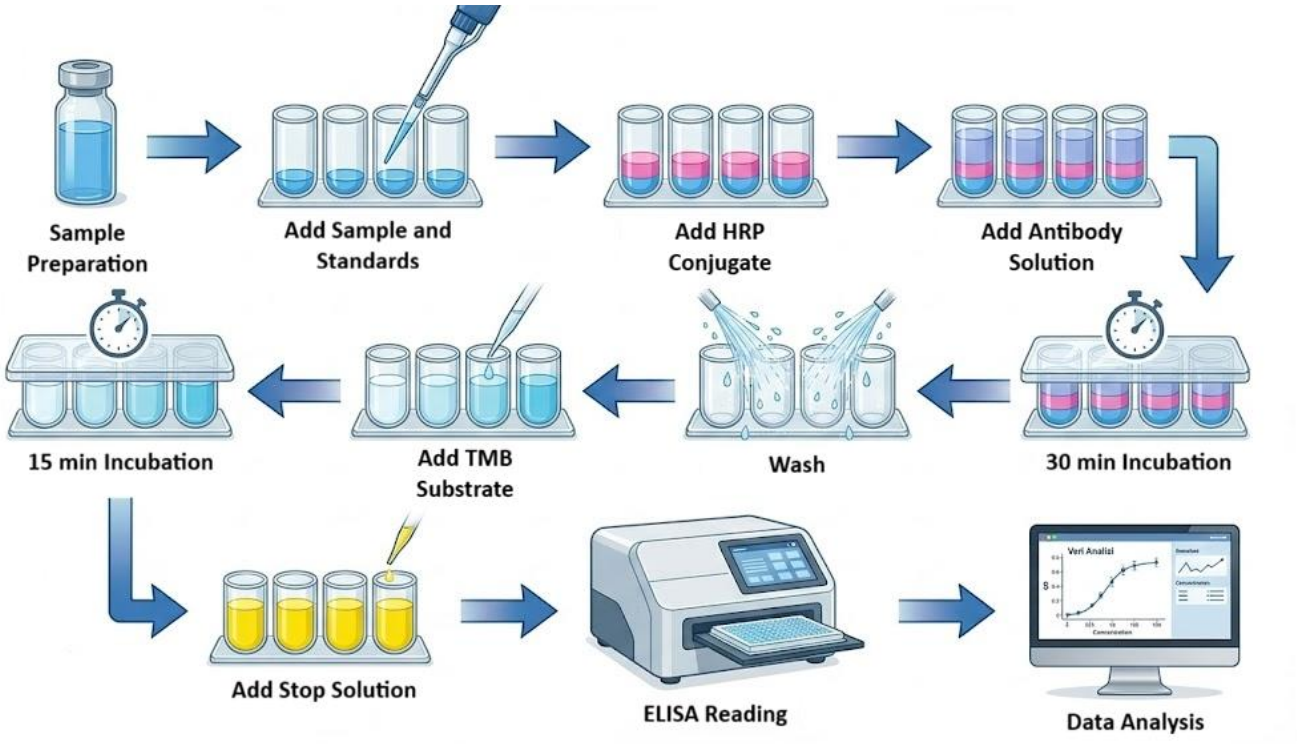
Sample Preparation:

Grain and Feed treatment:

1. Mix the well ground sample (passing a 20 mesh sieve) thoroughly and sub-sampling.
2. Suspend 5.0 g of ground sample in 50 mL of 90% Methanol Solution (Reagent 1), vortex or shake extraction for 5 min.
3. Filter the extraction with filter paper or centrifuge at 4000 r/min for 5 min.
4. Transfer the filtrate (or supernatant) 250 µL to a new centrifuge tube, add 500 µL of distilled water and mix well.
5. Take out 50 µL for test.

Dilution times of the sample:30 – Detection limits: 9ppb

ELISA Protocol Template



ELISA Protocol

Place all reagents and samples to room temperature (adjust to around 25℃) for 30min. Gently shake the reagent bottles before use.

Take out the frame of the microplate along with the required number of wells. Then place the unused microplate wells into the sealed bag with the desiccant provided. Store the remaining kit in the refrigerator at 2-8℃.

1. **Number:** Number the standards and samples sequentially into the corresponding microwells; repeating with 2 wells for each sample and standard is recommended.
2. **Incubation:** Add 50 µL of standard to each numbered standard well and 50 µL of sample to each sample well, then add 50 µL of HRP conjugate to all wells. Finally, add 50 µL of antibody solution to each well. Cover the microplate with an adhesive membrane and incubate at room temperature for 30 minutes.
3. **Wash:** Carefully open the adhesive membrane and invert the wells to pour out the liquid. Pipette 300 µL of 1X Wash Buffer into each well and invert to pour out, repeat this process for 5 times. Invert the plate and tap it onto a paper towel to drain any remaining liquid. (Ensure no bubbles remain).
4. **Substrat:** Add 50 µL of Substrate Reagent A to each well, followed by 50 µL of Substrate Reagent B. Allow to react in the dark at room temperature for 15 minutes. (If the blue color is very faint, the reaction can be extended in a controlled manner.)
5. **Stop:** Add 50 µL of Stop Solution to each well.
6. **Reading:** Determine the Optical Density (OD value; absorbance value) by reading at 450 nm (Reference wavelength 595 or 630 nm) with a microplate reader. (The reading should be completed within 10 minutes of adding the stop solution.)

Interpretation of the Result

- **Calculate the Percentage of Absorbance Value:**

Percentage of absorbance value (%) = A / A0 × %100

A—the average OD value of the sample or standard;

A0—the average OD value of the 0ppb standard.

It is used to calculate the percentage absorbance of a standard or sample.

- **Draw the Standard Curve and Calculate:**

Take the absorbance percentage of the standards (A/A0) as the Y-axis and the corresponding logarithmic concentration of the standards (ppb) as the X-axis.

Plot the standard semi-logarithmic curves with the X-axis and Y-axis.

Plot the absorbance percentage of the samples on the standard curve, then obtain the corresponding concentration from the standard curve. Finally, multiplying the obtained concentration values by the corresponding dilution times gives the actual ZEN concentration of the samples.

Using the kit's professional analysis software for calculations makes it easier to analyze a large number of samples accurately and quickly.