

ZEARALENONE ELISA TEST

Enzyme immunoassay for in vitro diagnostic use

1. INTENDED USE

Zearalenone ELISA Kit is a direct method competitive enzyme-linked immunoassay for the quantitative detect the presence of Zearalenone in samples of grains and feed.

Incubation Time: 10 min + 15 min

Assay Time Requirement: For grains and feed, approximately 1 hour

Detection Limit: Zearalenone, 2 ppb (2 ng/mL)

Detection Range: 50-2500 ppb

Cross-reactivity: Zearalenone 100%

Recovery Rate: >90%

Precision: Intra-assay CV<8% Inter-assay CV<15%

2. PRINCIPLE OF THE ASSAY

Zearalenone ELISA kit is a direct method competitive enzyme-linked immunoassay. The assay is performed in ELISA microwells, which has been precoated with one anti-Zearalenone antibodies. When standards or samples are added into the microwells, the free Zearalenone antigens and the enzyme conjugate will compete to combine the antibody binding site on the microwells during incubation. After that, the unbound materials will be removed in a washing step. Then, two substrates are added into the microwells. The bound enzyme conjugate will convert the colorless substrate into blue. The color will change from blue to yellow after adding stop solution. Absorbance is then measured spectrophotometrically (450 nm) and colour intensity results are inversely proportional to the original Zearalenone amount in the sample. Sample concentrations are then calculated on the basis of a calibration curve derived from the standards.

3. KIT COMPONENT

- 1 X Microtiter plate (8 microwells X 12 removable strips) precoated with anti-Zearalenone antibodies
- 6 X Zearalenone standard solutions (1mL each): 0 ng/mL, 2 ng/mL, 4 ng/mL, 8 ng/mL, 20 ng/mL, 100 ng/mL
- 1 X Enzyme-linked conjugate (6 mL)
- 1 X Washing buffer concentrate (10 X, 50 mL)
- 1 X Substrate A: Carbamide Peroxide, (6 mL)
- 1 X Substrate B: TMB solution (6 mL)
- 1 X Stopping solution, 2 M Sulphuric acid (6 mL)
- Product Manual

4. MATERIALS REQUIRED BUT NOT PROVIDED

- ELISA Microtiter plate reader equipped with 450nm and 620nm filters
- 50 - 200 µl multichannel micropipette
- 50, 100 and 200 µl precision micropipette
- Microplate washer or squeeze bottle
- Pulverizer
- Agitator
- Graduated cylinder
- Centrifuge
- Centrifugal tubes (50 mL)
- Methanol (AR Grade)
- Distilled water

5. PREPARATION OF WORKING SOLUTIONS

Washing buffer: dilute 10X concentrate with distilled water (i.e. 1 mL + 9 mL).

70% Methanol/water solution: dilute methanol with distilled water (i.e. 7 mL + 3 mL).

35% Methanol/water solution: dilute 75% methanol solution with distilled water (i.e. 1 mL + 1 mL).

Standard solution, Enzyme-linked conjugate, substrate A, substrate B and stopping solution are ready-to-use.

6. SAMPLE PREPARATION

- 6.1 Grains and Feed (dilution factor 25)
- Finely grind and pulverize the sample into a fine powder.
 - Weigh 2 g of the sample powder into a 50 mL tube.
 - Add 25 mL of 70% methanol solution and shake thoroughly for 3 minutes.
 - Add 25 mL of distilled water and mix well.
 - Do centrifugation at 5000 g for 10 minutes.
 - Collect 50 µL of extract liquid for use in the assay.

* If the concentration is out of the detection range, please use 35% methanol for further extraction.

7. ELISA TESTING PROTOCOL

7.1 Assay Preparation

- Bring all reagents to room temperature (1-2 hours at 20-25°C) before use.
- Predispose a duplicate for each standard point and a duplicate for each sample.

7.2 Testing procedures

- Place 50 µL of each standard into the standard wells.
- Place 50 µL of each sample into the sample wells.
- Place 50 µL of enzyme conjugate solution into each well.
- Mix gently by rocking the plate manually.
- Incubate 10 min at room temperature ($25 \pm 2^{\circ}\text{C}$). Tapping the microwells holder in incubation can decrease the inner errors between the duplicate wells.
- Pour the liquid out of the wells and tap the microwells holder upside down against absorbent laboratory paper to ensure complete liquid removal.
- Fill completely all the wells with washing buffer (approx 50 µL/well). Repeat the washing step 4 times. After the last washing step, tap the microwells holder upside down against absorbent paper to ensure complete liquid removal.

- Add 50 µL of substrate A solution into each well.
- Add 50 µL of substrate B solution into each well.
- Tap the microwells holder to make them mix thoroughly.
- Incubate 15 min at room temperature (25 ± 2°C).
- Add 50 µL of stopping solution into each well. Mix gently by rocking the plate manually.
- Read the absorbance at 450 nm filter with a Microplate reader in 5 min.

8. CALCULATION

The unknown values for Zearalenone concentration in samples are determined from a calibration curve.

- Calculate the mean absorbance value for zero standard and subtract it from the absorbance value of all other wells. (This step is commonly skipped in practical operation.)
- Calculate the mean absorbance value for the Maximum Binding, the standards and the samples.
- Calculate the relative absorbance.

$$\text{Relative absorbance} = \frac{\text{Absorbance standard (or sample)}}{\text{Absorbance zero standard}} \times 100 = \frac{B}{B_0} (\%)$$

A special program with Excel functionality, Quicking ELISA Analysis Program in Excel, is available for giving the ELISA test results.

- Enter the Relative absorbance values calculated for each standard in a semi-logarithmic system of coordinates against the standard concentration; draw the calibration curve
- Take the relative absorbance value for each sample and interpolate the corresponding log concentration from the calibration curve. Calculate the actual concentration of the sample by multiplying the dilution factor to the extraction

concentration.

9. STORAGE

- The kit should be stored at 2~8°C, NO FREEZING.
- Unused test wells should be sealed and dryly stored.

10. PRECAUTIONS

- Please carefully read the instruction before use.
- Reagents should be brought to room temperature, 20-25°C prior to use.
- Do not use reagents after expiration date; Do not misuse reagents from other kits with different Lot numbers; Do not substitute reagents from any other manufacturer into the test kit.
- Avoid contact of skin and mucous membranes with reagents and sample extraction; If exposure should occur, please immediately flush with water.
- Please wear protective gloves when using the kit. Consider all materials that are exposed to standards or samples to be contaminated with Zearalenone.
- The stop solution contains sulphuric acid. Keep it away from skin and eyes.

11. LIMITATION

Zearalenone ELISA kit is used as a screening system. If a more accurate result is required, other method such as HPLC or GC/MS is suggested to give a confirmation.