VERSION 1.01

ZEARALENONE ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Zearalenone ELISA Test Kit

Catalogue Number. IP100056

Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Zearalenone. The coupling antigen is pre-coated on the micro-well stripes. The Zearalenone in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti- Zearalenone antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with the Zearalenone in the sample. This value is compared to the standard curve and the Zearalenone residues is subsequently obtained.

Technical specifications

Sensitivity: 0.1ppb
Incubation Temperature: 25℃
Incubation Time: 30min～15min
Detection limit Feed, 10ppb,rice, corn, peanut, ,5ppb
Cross-reaction rate: Zearalenone,100%
Recovery rate: Feed, rice, corn, peanut, 100±30%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	6× standard solution (1 mL each)	0ppb, 0.1ppb, 0.3ppb, 0.9ppb, 2.7ppb, 8.1ppb	
3	Enzyme conjugate	7ml	red cap
4	Antibody working solution	7ml	blue cap
5	Substrate A	7ml	white cap
6	Substrate B	7ml	black cap
7	Stop solution	7ml	yellow cap
8	20× concentrated washing buffer	40ml	white cap
9	20× concentrated redissolving solution	50ml	transparent cap

Materials required but not provided

Equipments: microplate reader, printer, homogenizer, nitrogen-drying device, vortex, centrifuge, measuring pipets, balance (a reciprocal sensibility of 0.01 g), incubator, water bath;

Micropipettors: single-channel 20-200μL, 100-1000μL, and multi-channel 30～300μl;

Reagents: Methanol, n-hexane.

Sample pre-treatment Instructions

The following points must be dealt with before the pre-treatment of any kind of sample:

1） Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents;

2） Before the experiment, each experimental equipment must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.
Solution preparation before sample pre-treatment:

Samples preparation

Use 7 parts of Methanol and dissolve with 3 parts of deionized water to obtain the ready to use sample extract solution.

A) Preparation of feed sample

1) Take 1.0±0.05g grinded sample into 50ml centrifuge tube, add 5ml Sample extract solution, shake for 3min, centrifuge at above 4000r/min at 20℃ for 10 min;
2) Take 50ul supernatant, add 95ul Sample redissolving solution, shake to evenly;
3) Take 50μl to test
Dilution factor: 100

b) Preparation of corn, rice sample

1) Take 1.0±0.05g grinded sample into 50ml centrifuge tube; add 5ml sample extract solution;
2) Shake completely for 3min(or shake by hand for above 5min), centrifuge at above 4000r/min at 20℃ for 10 min;
3) Take 100ul supernatant(up-layer), add 900ul sample redissolving, shake to evenly;
4) Take 50μl to test
Dilution factor: 50

c) Preparation of peanut sample

1) Take 1.0±0.05g grinded peanut sample into 50ml centrifuge tube; add 5ml sample extract solution, then add 4ml n-hexane, shake for 3min, centrifuge at above 4000r/min at 20℃ for 10 min;
2) Discard the supernatant, take 100ul of the middle-layer liquid, add 400ul Sample redissolving solution, shake to evenly;
3) Take 50μl to test
Dilution factor: 50

ELISA procedures

Instructions

1) Bring all reagents and micro-well strips to the room temperature (20-25 ℃) before use;
2) Return all reagents to 2-8 ℃ immediately after use;
3) The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in ELISA the procedures;
4) For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

Operation procedures

1. Bring test kit to the room temperature (20-25 ℃) for at least 30 min, note that each reagent must be shaken to mix evenly before use, put the required micro-well strips into plate frames. Re-sealed the unused microplate, store at 2-8 ℃, not frozen.

2. Solution preparation: dilute 40mL of the 20 × concentrated washing buffer with deionized water at 1:19 (1 part 20 × concentrated washing buffer + 19 parts deionized water). Or prepare as needed.
3. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate, record their positions.
4. Add 50μL of the sample or standard solution into separate duplicate wells; add 50ul enzyme conjugate then 50μL of the antibody working solution into each well, mix gently by shaking the plate manually. Seal the microplate with the cover membrane, and incubate at 25 ℃ for 30min.
5. Pour liquid out of microwell, flap to dry on absorbent paper, add 250 μL/well of washing buffer to wash microplate for 15-30s, then take out and flap to dry with absorbent paper, repeat 4-5 times. (If there are the bubbles after flapping, cut them with the clean tips).
6. Coloration: add 50 μL of the substrate A and then 50 μL of the substrate B into each well. Mix gently by shaking the plate manually, then incubate at 25 ℃ for 15 min at dark for coloration.
7. Determination: add 50μL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450nm to determine the OD value of every well. (Recommend to read the OD value at the dual-wavelength 450/630nm within 5 minutes).

Result judgment

There are two methods to judge the results; the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the Zearalenone in the sample

Qualitative determination

The Concentration range (ppb) can be obtained by compared the average absorbance value with standards. Suppose absorbance value of Sample One is 0.3, Sample Two is 1.0, and the standards are: 0ppb of 2.243; 0.03ppb of 1.816; 0.09ppb of 1.415; 0.27ppb of 0.74; 0.81ppb of 0.313; 2.43ppb of 0.155. Then the concentration of the sample one is in the range of 0.81ppb ~ 2.43ppb; Sample Two is 0.09ppb ~ 0.27ppb. The concentration range of aflatoxin in the samples can be obtained by multiplied by the corresponding dilution of the sample.

Quantitative determination

The mean values of the absorbance values is obtained for the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

Percentage of absorbance value = $(B / B_0) \times 100\%$

B—the average OD value of the sample or the standard solution

B_0—the average OD value of the 0 ng/mL standard solution

The zero standard is thus made equal to 100 % and the absorbance values are quoted in percentages. The values calculated for the standards are entered in a system of coordinates on semilogarithmic graph paper against the the Zearalenone concentration [ng/L]. The aflatoxin concentration in ng/L (ppb) corresponding to the absorbance of each sample can be read from the calibration curve.

Precautions

1. The room temperature below 25 ℃ or the temperature of the reagents and the samples being not returned to the room temperature (20-25 ℃) will lead to a lower standard OD value.

2. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility; So continue to next step immediately after washing.
3. Mix evenly, otherwise there will be the undesirable reproducibility.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
5. Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lots to use.
6. Put the unused microplate into an auto-sealing bag to re-seal it. The standard solution and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light.
7. Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of the standard solution 1(0 ppb) of less than 0.5 indicates its degeneration.
8. The optimum reaction temperature is 25 ℃, and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.

Storage: store at 2-8 ℃, not frozen.
Expiry date: 12 months; date of production is on box.