VERSION 1.01

OCHRATOXIN A（OTA）ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE
By Immunomart

Ochratoxin A（OTA）ELISA Test Kit

Catalogue Number. IP100059

Principle

The kit uses indirect competitive ELISA to detect ochratoxin (Ochratoxins A, OTA) in cereals and feed samples such as rice noodles, peanuts, and soybeans. The kit consists of pre-coated antigen plate and enzyme conjugate, antibodies, standards and other supporting reagents. During the test, the standard or sample solution is added, the ochratoxin in the sample and the pre-coated antigen on plate to compete against the ochratoxin antibody, and after adding the enzyme conjugate, via the TMB substrate show color, sample absorbance values of Ochratoxin has a negative correlation with its content, compared with the standard curve and then multiplied by the corresponding the dilution factor, can draw Ochratoxin content in samples.

Technical specifications

Sensitivity: 1ppb
Incubation Temperature: 25℃
Incubation Time: 30min～15min
Detection limit feed, grain :10ppb
Cross-reaction rate: Ochratoxin A,100%
Recovery rate: feed, grain 85±15%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	6× standard solution (1 mL each)	0ppb, 1ppb, 3ppb, 9ppb, 27ppb, 81ppb	
3	Enzyme conjugate	11ml	red cap
4	Antibody working solution	5.5ml	blue cap
5	Substrate A	6ml	white cap
6	Substrate B	6ml	black cap
7	Stop solution	6ml	yellow cap
8	20× concentrated washing buffer	40ml	white 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, $NaHCO_3$, Deionized water

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

1.) 70% Methanol 7 parts of Methanol + 3 parts of deionized water;
2) 0.1M $NaHCO_3$ solution Weigh 4.2g $NaHCO_3$, add 500ml deionized water to dissolve;
3) Washing buffer 1 part of 20X concentrated washing buffer and dissolve with 19 parts of deionized water to obtain the ready to use washing buffer.

a) grain, rice,corn,feed

1 Weigh 2g homogeneous sample into a 50ml centrifuge tube, add 10ml 70% Methanol, shake for 5min, Centrifuge at 4000 r/min at room temperature for 10min;
2) Take 1ml up-layer clear liquid, add 1ml 0.1M $NaHCO_3$ solution, shake to evenly;
3) Take 50ul liquid to test
Dilution factor: 10
Detection limit: 10ppb

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.Take out all the necessary reagents from the kit and place at the room temperature (20 to 25 ℃) for at least 30 minutes. Note that each liquid reagent must be shaken to mix evenly before use.
2. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2-8 ℃, not frozen.
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. Enzyme conjugate preparation: take 1 part 11X Concentrated Enzyme conjugate, add 10 parts Enzyme conjugate dilution, dilute at 1:10, get the ready to use Enzyme conjugate.
5. Add 50µL of the sample or standard solution to separate duplicate wells, then add enzyme conjugate, 50 µL each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 25 ℃ at dark for 30 minutes.

6. Pour liquid out of micro well, add 300 µL/well of washing buffer for 15-30 seconds, repeat four to five times, and then flap to dry (if there are the bubbles after flapping, cut them with the clean tips).
7. Coloration: add 50 µL of the substrate A, then add 50uL of the substrate B into each well. Mix gently by shaking the plate manually, and incubate at 25 ℃ for 15 minutes at dark for coloration.
8. Determination: add 50 µL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value (Recommend to read the OD value at the dual-wavelength 450/630 nm 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 Ochratoxin A in the sample.

Qualitative determination

The concentration range (ng/mL) of AMOZ can be obtained from comparing the average OD value of the sample with that of the standard solution. Assuming that the OD value of the sample Ⅰ is 0.3, and that of the sample Ⅱ is 1.0, the OD value of standard solutions is: 2.243 for 0ppb, 1.816 for 0.03ppb, 1.415 for 0.09ppb, 0.74 for 0.27ppb, 0.313 for 0.81ppb, 0.155 for 2.43ppb, accordingly the concentration range of the sample Ⅰ is 0.81 to 2.43ppb, and that of the sample Ⅱ is 0.09 to 0.27ppb.

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 (B_0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

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
Draw the standard curve with the absorption percentages of the standard solution and the semilogarithm values of the the Ochratoxin A (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, finally obtaining the AMOZ concentration in the sample.

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.