

TRICHOTHECENES (T-2) ELISA TEST KIT
MANUAL

Trichothecenes (T-2) ELISA Test Kit

Catalogue Number. IP100060

Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Trichothecenes (T-2). The coupling antigen is pre-coated on the micro-well stripes. The Trichothecenes (T-2) in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti-Trichothecenes (T-2) 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 Trichothecenes (T-2) in the sample. This value is compared to the standard curve and the Trichothecenes (T-2) residues is subsequently obtained.

Technical specifications

Sensitivity: 0.05ppb

Incubation Temperature: 25°C

Incubation Time: 30min~15min

Detection limit feed, peanut, rice :3ppb

Cross-reaction rate: Trichothecenes(T2),100%

Recovery rate: feed, rice 90±25%,peanut 85±25%,

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	6× standard solution (1 mL each)	0ppb, 0.05ppb, 0.15ppb, 0.45ppb, 1.35ppb, 4.05ppb	
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	15ml	white cap
9	5× concentrated redissolving solution	50ml*2	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,

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) Use 1 part of (5X)concentrated redissolving solution and dissolve with 4 part of deionized water to obtain the ready to use sample redissolving solution
- 2) Use 1 part of Methanol and dissolve with 1 parts of sample redissolving solution to obtain the ready to use sample extract solution.

peanut, rice,,feed

- 1) Take 1.0 ± 0.05 g grinded sample into 50ml centrifuge tube, add 5ml sample extract solution, shake for 3min, centrifuge at above 4000r/min at 20°C for 10 min;
 - 2) Take 100ul supernatant(up-layer), add 400ul sample redissolving solution, shake to evenly;
 - 3) Take 50 μ l to test
- Dilution factor: 25

ELISA procedures

Instructions

- 1) Bring all reagents and micro-well strips to the room temperature (20-25 °C) before use;
- 2) Return all reagents to 2-8 °C 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 °C) 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 °C, 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 °C at dark for 30 minutes.
6. Pour liquid out of microwell, add 300 µL/well of washing buffer for 15-30 seconds, repeat four to five times, 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 °C 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 Trichothecenes (T-2) 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; 0.05ppb of 1.816; 0.15ppb of 1.415; 0.45ppb of 0.74; 1.35ppb of 0.313; 4.05ppb of 0.155. Then the concentration of the sample one is in the range of 1.35ppb ~ 4.05ppb; Sample Two is 0.15ppb ~ 0.45ppb. The concentration range of Trichothecenes (T-2) 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,

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

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

B₀—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 Trichothecenes (T-2) (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 °C or the temperature of the reagents and the samples being not returned to the room temperature (20-25 °C) 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 °C, and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.

Storage: store at 2-8 °C, not frozen.

Expiry date: 12 months; date of production is on box.