Trichothecenes (T-2) ELISA test kit

INSTRUCTION MANUAL

FOR PRODUCT No: LT53001AYSL

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MANUAL VERSION 1.01

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 are subsequently obtained.

MATERIALS SUPPLIED WITH THIS KIT

1	Micro-well strips	12 strips with 8 removable wells each	6	Substrate B	7 ml, black cap
2	6× standard solution (1 ml each)	0ppb, 0.05ppb, 0.15ppb, 0.45ppb, 1.35ppb, 4.05ppb	7	Stop solution	7 ml, yellow cap
3	Enzyme conjugate	7 ml, red cap	8	20× concentrated wash	15 ml, white cap
4	Antibody working solution	7 ml, blue cap	9	5× concentrated redissolving solution	100 ml, transparent cap
5	Substrate A	7 ml, white cap			

MATERIALS REQUIRED BUT NOT PROVIDED

1) Micropipettors and disposable tips: 0.5µl-10µl, 10µl-100µl, 100µl-1000µl.
2) 37 ℃ Incubator.
3) Measuring cylinder: 500 ml.
4) 96 wells microplate reader.
5) Distilled/De-ionized water.
6) Microplate Washer.

TECHNICAL SPECIFICATIONS

Sensitivity: 0.05 ppb

Incubator temperature: 25℃

Incubator time: 15min-30min

Detection limit:

Food, rice, peanut - 3ppb

Cross-reaction rate:

Trichothecenes (T-2) - 100%

SAMPLE PRE-TREATMENT

Instructions (The following points must be dealt with before the pre-treatment)

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 utensil 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:

1) Sample redissolving solution

Use 1 part of 5X concentrated re-dissolving solution and dissolve with 4 parts of deionized water to obtain the ready to use sample re-dissolving solution.

2) Sample extract solution

Use 1 part of Methanol and dissolve with 1 parts of deionized water to obtain the ready to use sample extract solution.

SAMPLES PREPARATION

Preparation of Feed, peanut, rice 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 100μl supernatant (up-layer), add 400μl sample redissolving solution, shake to evenly;

3) Take 50μl to test

Dilution factor: 25

ELISA PROCEDURES

Instructions

1. Bring ELISA reagents to room temperature (20 - 25 °C) before use.
2. Put ELISA reagents back to 2-8 °C immediately after use
3. The ELISA reproducibility in the analysis process is largely depends on the consistency of the washing plate, the correct washing plate operation is the point of determination ELISA program
4. In all process of constant temperature incubation, avoid light exposure, seal the microplate with the cover membrane

PROTOCOL

1. Bring test kit to the room temperature (20-25 °C) for at least 30 min, note that each reagent must be shaken evenly before use;
2. Put the required micro-well strips into plate frames. Re-seal the unused microplate, stored at 2-8 °C, not frozen.
3. Solution preparation: take the 15ml 20× concentrated washing buffer, dissolve

with deionized water at 1:19 (1 part 20X concentrated washing buffer + 19 parts deionized water), or prepare as quantity needed.

4. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions.

5. Add standard/sample: Add 50 µl of the sample or the standard solution into separate duplicate wells, then add enzyme conjugate, 50 µl/well; then antibody working solution, 50 µl/well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, incubate at 25 °C for 30 min in the dark.

6. Wash microplate: Carefully open the cover membrance, pour liquid out of microwell; add 250 µl/well of washing buffer, wash fully for 4-5 times, 15-30 s each time, then take out and flap to dry with absorbent paper (Use unused spear to pierce bubble after dry).

7. Coloration: add 50 µl of substrate A solution then 50 µl B solution into each well. Mix gently by shaking the plate manually, and incubate at 25 °C for 15min in the 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 of every well (Recommend to read the OD value at the dual-wavelength 450/630 nm within 5 min).

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 before adding any reagents.

4. The stop solution is the 2M 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 lot numbers to use.

6. Storage: store at 2-8 ℃, not frozen. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance 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(450/630nm) of the 0 standard solution (0 ppb) of less than 0.5 (A450nm<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 AND EXPIRY DATE

Storage: store at 2-8 ℃, not frozen.

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