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User's Manual

Citrinin ELISA Kit

REF **DEIASL218**

Σ **96T**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Citrinin ELISA Kit is a competitive ELISA for the quantitative analysis of Citrinin for food safety purposes. (example: grains, fermented bean curd, red yeast rice and chili.)

General Description

Citrinin is a widespread mycotoxin commonly found in grains, rice, health foods, etc., posing a significant threat to human and animal health. Scholars have confirmed that Citrinin can damage the kidneys, liver, intestines, and reproductive systems of animals. As our understanding of its toxic effects deepens, based on recent research, focuses on detection methods and toxicological studies to aid future research on this and other natural mycotoxins, and to provide a theoretical basis for food safety and livestock health issues caused by mycotoxins. This kit enables both qualitative and quantitative detection of the citrinin in grains, fermented bean curd, and red yeast rice samples

Principles of Testing

The kit uses indirect competitive ELISA. The microplate wells are pre-coated with the Citrinin antigen. The citrinin in the sample and this antigen compete for the citrinin antibody. The antibody then binds to the enzyme - labelled secondary antibody. After colour development, the absorbance is inversely proportional to the citrinin level in the sample. Comparison with the standard curve, adjusted for dilution, gives the citrinin content.

Reagents And Materials Provided

1. 96 - well microplates (12 strips × 8 wells)
2. Standard solutions (Standard 1 - 5)× 5 bottles: (1 mL/bottle): 0 ng/mL, 0.2 ng/mL, 0.6 ng/mL, 2 ng/mL, 6 ng/mL
3. Antibody solution: 8 mL (ready - to - use)
4. Enzyme - labelled conjugate: 8 mL (ready - to - use)
5. Substrate solution: 15 mL (ready - to - use)
6. Stop solution: 7 mL (ready - to - use)
7. Concentrated wash buffer (10 - fold): 60 mL (if crystals form during storage, heat at 37 °C for 15 min before use)
8. Concentrated sample extraction solution (15 - fold): 100 mL
9. Sample diluent: 50 mL (ready - to - use)
10. Plate sealer(s): 2 pieces
11. Instructions: 1 copy
12. Bags: Those for packaging the plate frame and instructions can be used to store unused wells.

Materials Required But Not Supplied

1. 15 mL centrifuge tubes
2. Balance 0.01g
3. 100 mL measuring cylinder
4. Shaker or equivalent
5. Deionised or distilled water
6. Qualitative filter paper
7. Centrifuge with tubes
8. 20 - mesh filter funnel
9. pH meter or equivalent
10. 1 M HCl or NaOH
11. 1 - 2 mL centrifuge tubes
12. Single - channel pipettes (20 - 200 µL, 100 - 1000 µL) and tips,
13. Multi - channel pipettes (30 - 300 µL) and tips,
14. Reagent troughs (for 8 - channel pipettes)
15. Absorbent paper
16. Timer
17. Microplate reader with 450 nm and 630 nm filters

Storage

2-8°C (for sealed box), please do not freeze! See kit label for expiry date.

Specimen Collection And Preparation

Note: The optimal pH range of the sample solution is 6.0 - 8.0. Adjust the pH with 1 M NaOH or 1 M HCl if necessary.

For grains (wheat, corn, rice, etc., low - fat grains) and fermented bean curd (dilution factor:30):

1. Grind the sample, pass through a 20 - mesh sieve, mix thoroughly, and subsample.
2. Weigh 1.0 g of the sample, add 5 mL of sample extraction solution, and shake for 5 min.
3. Filter with qualitative filter paper or centrifuge at 4000 r/min for 5 min.
4. Take 100 µL of the filtrate or supernatant, add 500 µL of sample diluent, mix well, and the sample is ready for testing.

For red yeast rice and chili (dilution factor: 60):

1. Grind the sample, pass through a 20 - mesh sieve, mix thoroughly, and subsample.
2. Weigh 1.0 g of the sample, add 10 mL of sample extraction solution, and shake for 5 min.
3. Filter with qualitative filter paper or centrifuge at 4000 r/min for 5 min.
4. Take 100 µL of the filtrate or supernatant, add 500 µL of sample diluent, mix well, and the sample is ready

for testing.

If the toxin level in the sample exceeds the detection range of the kit, further dilute the sample extraction solution and recalculate the dilution factor for the results.

Reagent Preparation

1. **Wash solution**: Dilute the concentrated wash buffer with deionised water at a 1:9 ratio (1 part buffer + 9 parts water).
2. **Sample extraction solution**: Dilute the concentrated sample extraction solution with deionised water at a 1:14 ratio (1 part extraction solution + 14 parts water).

Assay Procedure

Pre - testing Notes

1. Before use, bring all reagents and microplate strips to room temperature (20 - 25 °C).
2. After use, immediately return all reagents to 2 - 8 °C.
3. Washing consistency is crucial for ELISA reproducibility. Proper washing is a key step in the ELISA procedure.
4. During all incubation steps, avoid light exposure and cover the microplate with the sealer.

Testing Procedure

1. Prepare the sample solution as described above.
2. Take out the required number of microplate wells. Add 50 µL/well of standard solution or sample solution, with two parallel wells for each sample.
3. Add 50 µL/well of enzyme - labelled conjugate and 50 µL/well of antibody solution. Mix well, cover with the sealer, and react at room temperature for 30 min.
4. Wash three to four times as follows: Remove the sealer, discard the well liquid, add 300 µL/well of wash solution, wash for 30 s, and discard the liquid. Repeat three to four times, and dry the wells with absorbent paper after the last wash. Incomplete washing can lead to poor precision and increased absorbance.
5. Add 100 µL/well of substrate solution, cover with the sealer, and react in a light - protected place (such as a drawer or cabinet, as the substrate is light - sensitive) for 15 min.
6. Add 50 µL/well of stop solution to terminate the reaction, changing the colour from blue to yellow.
7. Gently mix the contents and read the absorbance at 450 nm (with 630 nm as the reference wavelength) on a microplate reader within 10 min, during which the colour remains stable.

Detection Limit

6 µg/kg for grains and fermented bean curd; 12 µg/kg for red yeast rice and chili.

Specificity

N/A

Precautions

1. Follow laboratory standard operating procedures to ensure reliable results.
2. Bring all reagents to room temperature (20 - 25 °C) before starting the experiment.
3. Gently invert or vortex mix all reagents before use to avoid foaming.
4. Once the experiment starts, complete all subsequent steps within the recommended time.
5. Close all reagent bottles immediately after use. Do not interchange bottle caps between different reagents.
6. Use a separate disposable pipette tip for each sample to avoid cross - contamination.
7. Process samples and standards simultaneously to ensure consistent experimental conditions.
8. Do not mix reagents from different batches.
9. Do not use expired test kits.
10. Calibrate laboratory equipment and instruments (such as pipettes and microplate readers) before use.