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LT92001AYSL

ELISA KIT
TOTAL AFLATOXIN

Research Use Only
For *in vitro* applications - not for consumption

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VERSION 1.2B

INTENDED USE

The kit is indirect competitive enzyme immunoassay for the detection of Total Aflatoxin in corn, rice, peanut, peanut butter, animal tissue and feed.

KIT CONTENTS

Micro-well strips [12 X 8 Strips]
Standards [0ppb, 0.02ppb, 0.06ppb, 0.18ppb, 0.54ppb, 1.62ppb]
Enzyme Conjugate [7ml]
Antibody Working Solution [7ml]
Substrate A [7ml]
Substrate B [7ml]
Stop Solution [7ml]
20x Concentrated Washing Buffer [40ml]
20x Conentrated Redissolving Solution [50ml]

Storage: Store at 2-8°C, not frozen.
Expiry date: 12 months; date mentioned on the box label.
Note: If the Vacuum package of microplate has leakage, it is still valid to use, do not affect the test result, be relax to use.

MATERIALS REQUIRED BUT NOT INCLUDED

Distilled water, single-channel and multi-channel pipettes and tips, ELISA reader, Eppendorf tubes, absorbent paper, homogenizer, shaker, centrifuge, balance, methanol, n-hexane.

SUMMARY AND PRINCIPLE

This kit is based on the principle of indirect competitive enzyme immunoassay. In brief, the coupling antigen is pre-coated on the micro-well strips. The Aflatoxin in the sample and the coupling antigens pre-coated on the micro-well strips compete for the anti-Aflatoxin 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 Aflatoxin in the sample. This value is compared to the standard curve and the Aflatoxin residues is subsequently obtained.

CHARACTERISTICS

Sample Type: Feed, corn, rice, peanut, peanut butter, animal tissues, edible oil

Minimum Detection Dose: 0.02ppb

Detection limit: Feed, rice, corn, peanut, tissue, edible oil……2ppb

Cross-reaction rate:

Aflatoxin B1…..... ….100%
Aflatoxin M1…..... …91.2%
Aflatoxin B2…..... ….68.4%
Aflatoxin G1…..... ….4.7%
Aflatoxin G2…..... ….2.7%

Recovery rate:

Feed, rice, corn, peanut, tissue, edible oil……95±35%

SOLUTION PREPARATION

1) Sample redissolving solution

Use 1 part of 20x concentrated redissolving solution and dissolve with 19 parts of deionized water to obtain the ready to use sample redissolving solution.

2) Sample extract solution

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

SAMPLE PREPARATION

Preparation of tissue, feed, rice and corn 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 100ul supernatant, add 700ul Sample redissolving solution, shake to evenly;
3) Take 50μl to test

Preparation of edible oil sample

1)Take 1.0±0.05g edible oil 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 700ul Sample redissolving solution, shake to evenly;
3)Take 50μl to test

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

PROCEDURE

1. Bring test kit to the room temperature (20-25 ℃) 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-sealed the unused microplate, store at 2-8 ℃, not frozen.

3. **Solution preparation:** Take the 40ml 20× concentrated washing buffer, dissolve with deionized water (1 part 20× concentrated washing buffer + 19 parts deionized water), or prepare as quantity needed.

4. **Labeling:** Label 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 50μL of the sample or the standard solution into separate duplicate wells, then add enzyme conjugate, 50μL/well; then antibody working solution, 50μL/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 membrane, pour liquid out of microwell; add 250μL/well of washing buffer, wash fully for 4-5 times, 15-30s each time, then take out and flap to dry with absorbent paper.(Use unused spear to pierce bubble after dry).

7. **Coloration:** Add 50μL of substrate A solution then 50μL substrate 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. **Result Measurement:** 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 min).

RESULT AND RESULT INTERPRETATION

There are two methods to interpret 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 Aflatoxin B1 in the sample.

1. Qualitative Determination

The concentration range (ppb) can be obtained by comparing 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; 0.02ppb of 1.816; 0.06ppb of 1.415; 0.18ppb of 0.74; 0.54ppb of 0.313; 1.62ppb of 0.155. Then the concentration of the sample one is in the range of 0.54ppb ~ 1.62ppb; Sample Two is 0.06ppb ~ 0.18ppb. The concentration range of Total Aflatoxin in the samples can be obtained by multiplying with the corresponding dilution of the sample.

2. Quantitative Analysis

In order to calculate the concentration of samples, a standard curve should be made. Before standard curve is made, the concept of % absorbance should be known.
Calculation of % absorbance:
Percentage of absorbance value = (B/B0)X100%

B—the average OD value of the sample or the standard solution
B0—the average OD value of the 0ng/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 Total Aflatoxin concentration [ng/L]. The Total Aflatoxin concentration in ng/L (ppb) corresponding to the absorbance of each sample can be read from the calibration curve.
A special software for result analysis of ELISA will facilitate double or multiple determinations.

TROUBLESHOOTING

We understand research is challenging as it is but it can get very frustrating when experiments themselves don't go as they should. That is the precise reason why we strive to ensure that all our products work for the application shown in their respective data sheets.

Still if you have any concerns please feel free to write to us at customerservice@lifetechindia.com

PRECAUTIONS

- 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.

- In all process of constant temperature incubation, avoid light exposure, seal the microplate with the cover membrane.

- 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.

- 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.

- The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.

- 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.

- 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.

- 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.

- 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.

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