Aflatoxin Total elisa kit

1. Principle

This test kit is based on the competitive enzyme immunoassay for the qualitative,quantitative detection of Aflatoxin B1 in the Corn, rice, wheat, beans, peanuts, peanut butter, cooking oil.The coupling antigen is pre-coated on the micro-well stripes. The Aflatoxin B1 in the sample and the coupling antigens pre-coated on the micro-well stripes compete for anti-Aflatoxin B1 antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the testing sample has a negative correlation with the Aflatoxin B1 concentration in the sample. This value is compared to the standard curve and the Aflatoxin B1 concentration is subsequently obtained.

2. Technical specifications

Sensitivity : 0.1ppb

Detection limit:

Peanuts, peanut butter; corn, wheat and other grains; cookies, honey 2ppb
Feed 2.4ppb/1.25ppb
Cooking oil 1.5ppb
Soy sauce, vinegar, wine 1ppb
Dry miso, hot pot materials 1ppb

Recovery rate:

Peanuts..... 95±15%
Corn..... 90±15%
Feed ..... 85±15%
Cooking oil..... 95±15%

Cross-reaction rate:

Aflatoxin B2..... 100%
Aflatoxin B1..... 97%
Aflatoxin G1 ..... 98%
Aflatoxin G2 ..... 101%

Precision:

Intra-assay CV<10%;
Inter-assay CV<10%

3. Components

1） Micro-well strips:96wells(12 strips with 8 removable wells each)
2） 5× standard solution (1 mL each):0 ppb,0.1ppb, 0.3ppb,0.9ppb,2.7ppb,8.1ppb
3） Spike standard 100ppb(1ml)
4） Anti-AFT monoclonal antibody solution(6ml) ..... ready for use directly
5） Enzyme conjugate (9 mL) ..... ready for use directly
6） Substrate solution A (9 mL) ..... ready for use directly
7） Substrate solution B (9 mL)..... ready for use directly

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8） Stop solution (6 mL) ..... ready for use directly

9） 10× concentrated washing buffer (40 mL) ..... dilute for use

4. Materials required but not provided

1) **Equipments:** microplate reader, oscillator,shaker,balance(a reciprocal sensibility of 0.01 g), printer, measuring pipets,centrifuge,water-bath

2) **Material** : graduated pipettes washing ear ball, funnel, **separatory funnel,** beaker,centrifuge tube, rapid qualitative filter paper

3) **Micropipettors:** single-channel 20~200 µL, 100~1000 µL; and multi-channel 250 µL;

3) **Reagents:**Methanol (AR), deionized water or distill water,Petroleum ethe or N-hexane

5. Sample 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 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

1) Solution 1, washing buffer:dilute 40 mL of the concentrated washing buffer (10 × concentrated) with the distilled or deionized water at 1: 9 to 400 mL (or just to the required volume) for use. washing buffer Can be in good condition for 30days in 4℃.

2) Solution 2,extract solution1,dissolve Methanol (AR) in the deionized water at 3:2 (3 mL Methanol (AR) + 2 mL deionized water).

3) Solution 3,extract solution 2,dissolve Methanol (AR) in the deionized water at 7:3 (7 mL Methanol (AR) + 3 mL deionized water).

Sample treatment:

5.1 Peanuts

1)Take 10g smashed sample into centrifuge,add 30 ml extract solution1,shake severely for 5 min

2) Filter at rapid qualitative filter paper or centrifuge at 3500r/min for 5min

3) Take 1ml filtrate,add 5ml deionized water for diluting

4) Take 50 µL for further analysis.

Fold of dilution of sample :18

5.2 feed,corn

Method 1:

1) Take 3g smashed sample into centrifuge,add 15ml extract solution 2, shake severely for 10 min

2) Filter at rapid qualitative filter paper or centrifuge at 3500r/min for 5min

3) Take 1ml filtrate or supernatant in 6ml deionized water

4) Take 50 µL for further analysis.

Fold of dilution of sample :35

5.3 Cooking oil

1)Take 5g cookingoil into Small beaker

2) With 10mL petroleum ether (or hexane) split the sample transferred to a 125mL separatory funnel

3)Add 15ml extract solution 1,cover,shake for 5min, standing for stratification,release the lower methanol extract;

4)Take 1ml methanol extract;dilute with 5ml deionized water

5) Take 50 µL for further analysis.

Fold of dilution of sample :18

6. ELISA procedures

Tips:

1)Be careful when using the standard solution,there are AFT in them.

2)The time of adding samples each time should be finished in 5 mins.

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; put the required micro-well strips into plate frames. Re-sealed the unused microplate, stored at 2-8 ℃, not frozen.

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

3 Add 30 µL of the sample and standard solution to separate duplicate wells, then add 70 µL enzyme conjugate,then add 50 µL AFT monoclonal antibody solution to each well,seal the microplate with the cover membrane,and incubate for 20min

4 Abandon the liquid of the microplate,wash the microplate with the washing buffer at 300 µL/well for five times. Each time soak the well with the washing buffer for 30s,after the last time, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips);

5 Coloration: add 75 µL of the substrate A solution into each well, 75 µL of the substrate B solution into each well Mix gently by shaking the plate manually, and incubate for 15-20 min in the dark for coloration;

6 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)

7. 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 content of Aflatoxin Total.

7.1 Qualitative determination

The concentration range (ng/mL) of AFT 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 0.659, and that of the sample Ⅱ is 1.525, the OD value of standard solutions is: 2.101 for 0 ppb, 1.738 for 0.1ppb, 1.313for 0.3ppb, 0.831for 0.9 ppb, 0.469 for 2.7ppb, 0.262 for 8.1ppb, accordingly the concentration range of the sample Ⅰ is 0.9 to 2.7 ppb, and that of the sample Ⅱ is 0.1 to 0.3 ppb.

7.2**Quantitative determination**

The mean values of the absorbance values is equivalent to the percentage of the average OD value (B) of the testing 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

$$\text{Percentage of absorbance value} = \frac{B}{B_0} \times 100\%$$

B—the average (double wells) OD value of the testing sample or the standard solution

B_0—the average OD value of the 0ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solutions and the semilogarithmic values of the Aflatoxin B1 elisa kitstandard solutions (ng/mL) as Y- and X-axis, respectively.Read the corresponding concentration of the testing 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 Aflatoxin B1 concentration in the sample.

Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software)

8. Precautions

1 Bring all reagents and micro-well strips to the room temperature (20-25℃).

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 the procedures of ELISA.

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.

5 The room temperature below 20℃ or the temperature of the reagents and the testing samples being not returned to the room temperature (20-25℃) will lead to a lower standard OD value.

6 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.

7 Mix evenly, otherwise there will be the undesirable reproducibility.

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

9 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.

10 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.

11 Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of standard solution 1（0 ppb）of less than 0.5 indicates its degeneration.

12 Colouration time is about 20 min, if the color is light, prolong the time of colouration but don't exceed 30 min.

13 The optimum reaction temperature is 37 ℃, and too high or low temperatures will result in the changes in the detecting sensitivity and OD values.

9. Storage and expiry date

Storage: stored at 2-8 ℃, not frozen.

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

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Green Earth depends on everyone' efforts

"Build of green Earth needs the cooperation of you and me"

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