Competitive Enzyme Immunoassay Kit for Quantitative Analysis of Ochratoxin A

1. Background

Ochratoxins are a group of mycotoxins produced by some Aspergillus species (mainly A). Ochratoxin A is known to occur in commodities such as cereals, coffee, dried fruit and red wine. It is considered a human carcinogen and is of special interest as it can be accumulated in the meat of animals. Thus meat and meat products can be contaminated with this toxin. Exposure to ochratoxins through diet can have acute toxicity to mammalian kidneys, and may be carcinogenic.

This kit is a new product for drug residue detection based on ELISA technology, which only costs 30min in each operation and can considerably minimize operation errors and work intensity.

2. Test Principle

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. Ochratoxin A in sample competes with the antigen coated on the microtiter plate for the antibody added. After the addition of enzyme conjugate, TMB substrate is used to show the color. Absorbance of the sample is negatively related to the ochratoxin A residue in it, after comparing with the Standard Curve, multiplied by the dilution factors, ochratoxin A quantity in the sample can be calculated.

3. Applications

This kit can be used in quantitative and qualitative analysis of ochratoxin A in feed.

4. Cross-reactions

Ochratoxin A…………………………………………..100%

5. Materials Required

5.1 Equipments

----Microtiter plate spectrophotometer (450nm/630nm)

----Vortex mixer

----Centrifuge

----Analytical balance (inductance: 0.01g)

----Graduated pipette: 10ml

----Rubber pipette bulb

----Glass test tube: 15 ml;

----Polystyrene centrifuge tube: 2ml, 50ml

----Micropipettes: 20μl-200μl.100μl-1000μl

250μl-multipipette

5.2 Reagents

----Methanol (AR)

----Deionized water

6. Kit Components

- Microtiter plate with 96 wells coated with antigen
- Standard solutions(6 bottles:1ml/bottle)

  0ppb, 0.4ppb, 0.8ppb, 1.6ppb, 3.2ppb, 6.4ppb
- Enzyme conjugate 7ml……………………..…..red cap
- Antibody solution 10ml…………………..green cap
- Substrate solution A 7ml……………………white cap
- Substrate Solution B 7ml…..…………………red cap
- Stop solution 7ml …………………………yellow cap
- 20×concentrated Wash solution 40ml

  ……………………………………..…transparent cap

7. Reagents Preparation

Solution 1: Wash solution

Dilute the concentrated wash solution with deionized water in the volume ratio of 1:19, which will be used to rinse the plates. The diluted wash solution can be conserved for a month at 4℃.

8. Sample Preparations

8.1 Notice and precautions before operation

(a) Please use one-off tips in the process of experiment, and change the tips when absorbing different reagent.

(b) Make sure that all experimental instruments are clean.

(c) Lean the tube when taking the supernate after centrifuge, which will avoid taking the impurities.

(d) Prepared sample should be used for assay within 4h.

8.2 Feed (feedstuff, compound and concentrated), etc

----Weigh 3.0±0.05g of ground sample into a 50ml polystyrene centrifuge tube, then add 10ml of methanol, shake fiercely for 5min and then centrifuge for separation: 10min / 3000g / ambient temperature;

----Take 500μl of the supernatant into a 2ml polystyrene

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centrifuge tube, mix with 1ml of deionized water completely.

Note: *Please avoid the impurities in sampling.*

----Take 20μl of the prepared solution for assay.

Dilution Factor: 10

9. Assay process

9.1 Notice before assay

9.1.1 Make sure all reagents and microwells are all at room temperature (20-25℃).

9.1.2 Return all the rest reagents to 2-8℃ immediately after use.

9.1.3 Washing the microwells correctly is an important step in the process of assay; it is the vital factor to the reproducibility of the ELISA analysis.

9.1.4 Avoid the light and cover the microwells during incubation.

9.2 Assay Steps

9.2.1 Take all reagents out at room temperature (20-25℃) for more than 30min, shake gently before use.

9.2.2 Get the microwells needed out and return the rest into the zip-lock bag at 2-8℃ immediately.

9.2.3 The diluted wash solution should be rewarmed to be at room temperature before use.

9.2.4 **Number:** Numbered every microwell positions and all standards and samples should be run in duplicate. Record the standards and samples positions.

9.2.5 **Add standard/sample, enzyme conjugate and antibody solution**: Add 20μl of standard solution(**Kit component**) or prepared sample to corresponding wells. Add 50μl of enzyme conjugate(**Kit component**), 80μl of antibody solution(**Kit component**). Mix gently by rocking the plate manually and incubate for 20min at 25℃ with cover.

9.2.6 **Wash**: Remove the cover gently and pure the liquid out of the wells and rinse the microwells with 250μl of diluted wash solution (**solution** 1) at interval of 10s for 4-5 times. Absorb the residual water with absorbent paper (*the rest air bubble can be eliminated with unused tip*).

9.2.7 **Coloration**: Add 50μl of solution A(**Kit component**) and 50μl of solution B(**Kit component**) to each well. Mix gently by rocking the plate manually and incubate for 10min at 25℃ with cover(*see 12.8*).

9.2.8 **Measure**: Add 50μl of the stop solution(**Kit component**) to each well. Mix gently by rocking the plate manually and measure the absorbance at 450nm (It's suggested measure with the dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution.)

10. Results

10.1 Percentage absorbance

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

$$\text{Absorbance}(\%) = \frac{B}{B_0} * 100\%$$

B ——absorbance standard (or sample)

B_0 ——absorbance zero standard

10.2 Standard Curve

----To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the ochratoxin A standards solution (ppb) as x-axis.

----The ochratoxin A concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

Please notice: *Software has been developed for data reduction, which can be provided upon request.*

11. Sensitivity, accuracy and precision

Test Sensitivity: 0.4ppb

Detection limit

Feed……………………………………………………5ppb

Accuracy

Feed……………………………………………………90±20%

Precision

Variation coefficient of the ELISA kit is less than 10%.

12. Notice

12.1 The mean values of the absorbance values obtained

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for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25℃).

12.2 Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tap the microwells holder.

12.3 Shake each reagent gently before use.

12.4 Keep your skin away from the stop solution for it is the 0.5M H_2SO_4 solution.

12.5 Don't use the kits out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.

12.6 Keep the ELISA kits at 2-8℃, do not freeze. Seal rest microwell plates; avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.

12.7 Substrate solution should be abandoned if it turns colors. The reagents may be turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm<0.5).

12.8 The coloration reaction needs 10min after adding solution A and solution B. And you can prolong the incubation time ranges to 15min if the color is too light to be determined, never exceed 20min, on the contrary, and shorten the incubation time properly.

12.9 The optimal reaction temperature is 25℃. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.

13. Storage

Storage condition: 2-8℃.

Storage period: 12months.

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