Competitive enzyme immunoassay Kit for Quantitative analysis of Aflatoxin B1

1. Background

Aflatoxin B1 is a toxic chemical which always contaminates cereal, corn and peanut, etc. Strict residue limit has been established for aflatoxin B1 in animal feed, food and other samples. This product is based on indirect competitive ELISA, which is rapid, accurate and sensitive compared with conventional instrumental analysis. It needs only 45min in one operation, which can considerably reduce operation error and work intensity.

2. Test Principle

This kit is based on indirect-competitive ELISA. The microtiter wells are coated with coupling antigen. Aflatoxin B1 in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme conjugate, chromogenic substrate is used to show the color. Absorbance of the sample is negatively related to the aflatoxin B1 concentration in it, After comparing with the standard curve, multiplied by the dilution factor, aflatoxin B1 residue quantity in the sample can be calculated.

3. Applications

This kit can be used for qualitative and quantitative analysis of aflatoxin B1 in edible oil, peanut, cereal, grains, soy sauce, vinegar and feed (raw feed, mixed batch materials and concentrated materials)

4. Cross reactions

Aflatoxin B1……..100%
Aflatoxin B2……81.3%
Aflatoxin G1……62%
Aflatoxin G2……22.3%

5. Materials Required

5.1 Equipments

-----Microtiter plate spectrophotometer (450nm/630nm)
-----Shaker
-----Vortex mixer
-----Analytical balance (inductance: 0.01g)
-----Graduated pipette: 10ml
-----Rubber pipette bulb
-----Glass tube: 10ml
----Volumetric flask: 100ml, 500ml
-----Polystyrene centrifuge tube: 2ml, 50ml
-----Micropipettes: 20μl-200μl, 100μl-1000μl, 300μl-multichanel

5.2 Reagents:

------Methanol (AR)
------Acetonitrile(AR)
------Trichloromethane(Chloroform, AR)
------n-hexane(AR)
------Deionized water

6. Kit components

- Microtiter plate precoated with antigen, 96 wells
- Standard Solution ×6 bottle (1ml/bottle)

0ppb, 0.02ppb, 0.06ppb, 0.18ppb, 0.54ppb, 1.62ppb

- Enzyme conjugate 7ml……red cap
- Antibody solution 7ml……green cap
- Substrate A 7ml……white cap
- Substrate B 7ml……red cap
- Stop solution 7ml……yellow cap
- 20×concentrated wash solution 40ml ……transparent cap
- 2×concentrated extraction solution 50ml ……blue cap

7. Reagents Preparation

Solution 1: Extraction solution

Dilute 2×concentrated extraction solution with deionized water in the volume ratio of 1:1, which will be used to sample extraction. This solution can be stored for 1 month at 4℃.

Solution 2: Wash solution

Dilute 20×concentrated wash solution with deionized water in the volume ratio of 1:19, which will be used to wash the plates. This diluted solution can be stored for 1 month at

4℃.

8. Sample Preparation

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.

8.2 Edible oil (Peanut oil, blending oil, corn oil, soybean oil)

-----Weigh 200ul of edible oil sample into a 2ml polystyrene centrifuge tube. Add 1ml of extraction solution(Solution 1), 0.5ml of n-hexane, shake fiercely for 5min;

-----Centrifuge at room temperature (20-25℃/68-77℉) for 5min, at least 3000g;

-----Remove the supernatant organic phase, take 50µl of the substrate water phase for assay.

8.3 Peanut sample

-----Homogenate the peanut sample;

-----Weigh 2.0±0.05g of the peanut sample into a 50ml polystyrene centrifuge tube. Add 3ml of acetonitrile, then add 3ml of deionized water, shake for 5min;

-----Centrifuge at room temperature (20-25℃/68-77℉) for 5min, at least 3000g;

-----Take 3ml of the supernatant into a 50ml polystyrene centrifuge tube, add 4.5ml trichloromethane, shake for 5min; centrifuge at room temperature (20-25℃/68-77℉) for 5min, at least 3000g;

-----Remove the upper liquid, take 3ml substrate organic phase into a 10ml clean glass tube, dry with 50-60℃ nitrogen gas flow;

-----Add 1ml n-hexane, whorl for 30s with vortex mixer, then add 1ml of extraction solution (**solution 1**), vortex for 30s with vortex mixer to mix completely; centrifuge at room temperature (20-25℃/68-77℉) for 5min, at least 3000g;

-----Remove the supernatant organic phase, take 50µl of the substrate water phase for assay.

8.4 Cereal sample(soybean, rice, maize, wheat etc.)

-----Homogenate the cereal sample;

-----Weigh 2.0±0.05g cereal sample into a 50ml polystyrene centrifuge tube. Add 4ml of acetonitrile, then add 2ml of deionized water, shake for 5min;

-----Centrifuge at room temperature (20-25℃/68-77℉) for 5min, at least 3000g;

-----Take 3ml of the supernatant into a 50ml polystyrene centrifuge tube, add 6ml of trichloromethane, shake for 5min; centrifuge at room temperature (20-25℃/68-77℉) for 5min, at least 3000g;

-----Remove the upper liquid, take 4ml of substrate organic phase into a 10ml clean glass tube, dry with 50-60℃ nitrogen gas flow;

-----Add 1ml of n-hexane, whorl for 30s with vortex mixer, then add 1ml extraction solution (**solution 1**), whorl for 30s with vortex mixer to mix completely; centrifuge at room temperature (20-25℃/68-77℉) for 5min, at least 3000g;

-----Remove the supernatant organic phase, take 50µl of the substrate water phase for assay.

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 used.
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, homogenize 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 wash solution should be rewarmed to room temperature before use.

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

9.2.5 **Add standard solution/sample, enzyme conjugate and antibody**: add 50µl of standard solution or prepared sample to corresponding wells. Add 50µl of enzyme conjugate, 50µl of antibody solution, mix gently by shaking the plate manually and incubate for 30min at 25℃ with cover.

9.2.6 **Wash**: remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250µl wash solution (**solution 2**) 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 and 50µl of solution B to each well. Mix gently by shaking the plate manually and incubate for 15min at 25℃ with cover (see 12.8).

9.2.8 **Measure**: add 50µl of stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm against an air blank (It's suggested that measure with the dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution. We can also observe by sight without stop solution in short of the ELISA reader)

10. Results

10.1 Percentage absorbance

The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

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

B ——absorbance of standards or samples

B_0 ——absorbance of zero standard

10.2 Standard Curve

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

---The aflatoxin 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.

Notice:

Special software has been developed for result calculation, which can be provided on request.

Sample dilution factor:

Edible oil sample: 2

Peanut: 2

Cereal: 2

11. Sensitivity, accuracy and precision

Sensitivity: 0.05ppb

Detection limit

Edible oil sample.....0.1ppb

Peanut.....0.2ppb

Cereal.....0.05ppb

Accuracy

Edible oil sample.....80±15%

Peanut.....80±15%

Cereal.....80±15%

Precision

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

12. Notice

12.1 The mean values of the absorbance values obtained 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 using.

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 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 15min after the addition of solution A and solution B; But you can prolong the incubation time ranges to 25min or more if the color is too light to be determined., never exceed 30min, On the contrary, 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 condition and storage period

Storage condition: 2-8℃.

Storage period: 12 months.