# Aflatoxin Total Elisa Kit

**DEIA051-2**

## 1. Background

Aflatoxins are naturally occurring mycotoxins that are produced by many species of Aspergillus, a fungus, the most notable ones being Aspergillus flavus and Aspergillus parasiticus. 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. 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 compared with the standard curve, multiplied by the dilution factor, aflatoxin B1 residue in sample can be calculated.

## 3. Applications

This kit can be used for qualitative and quantitative analysis of aflatoxin total in edible oil, peanut, cereal etc.

## 4. Cross reactions

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

## 5. Materials Required

### 5.1 Equipment

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

### 5.2 Reagents

----Acetonitrile ($C_2H_3N$, AR)
----Chloroform ($CHCl_3$, AR)
----n-hexane ($C_6H_{14}$, 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(*e.g. 10ml of 2×concentrated extraction solution + 10ml of deionized water*). This solution can be conserved for 1month at 4℃.

**Solution** 2: Wash solution

Dilute 20×concentrated wash solution with deionized water in the volume ratio of 1:19(*e.g. 2ml of 20×concentrated wash solution + 38ml of deionized water*), 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,*


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*and change the tips when absorbing different reagent.*

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

*(c) Make sure do not pipet the impurity when taking the supernate after centrifuge.*

*(d)Peanut sample: Avoid taking the oil droplet when transfer the supernatant for drying*

*(e)Cooked beans: Emulsification would occur when extracting, avoid fierce vortex.*

*(f)Treated sample can't be conserved*

**8.2 Edible oil (peanut oil, blend oil, corn oil, bean oil etc)**

----Take 200μl of the edible oil sample to 2ml centrifuge tube;

----Add 1ml of extraction solution(**solution 1**), 0.5ml of n-hexane($C_6H_{14}$), shake for 5min;

----Centrifuge for separation: 3000g / 5min/ at room temperature;

----Remove the upper layer of n-hexane phase, take 50μl of the lower layer per well for assay

***Sample dilution factor: 5***

**8.3 Peanut**

----Homogenize the peanut sample

----Weigh 2.0±0.05g of the homogenizer in a 50ml centrifuge tube.

----Add 3.0ml of the acetonitrile($C_2H_3N$), 3.0ml deionized water, shake for 5min;

----Centrifuge for separation: 3000g / 5min/ at room temperature;

----Take 3ml of the upper layer orgnic phase to a 50ml centrifuge tube, add 4.5ml of chloroform, vortex for 5min.

----Centrifuge for separation: 3000g / 5min/ at room temperature;

----Remove the upper layer liqid, take 3ml of the lower layer orgnic phase to dry clean 10ml glass tube, dry under 50-60℃ water bath nitrogen flow.

----Add 1ml n-hexane($C_6H_{14}$), vortex for 30s to dissolve the dry leftover, add 1ml extraction solution(**solution 1**), vortex for 30s;

----Centrifuge for separation: 3000g / 5min/ at room temperature;

----Remove the upper layer of n-hexane phase, take 50μl of the lower layer per well for assay

***Sample dilution factor: 2***

**8.4 Cereal (wheat flour, soy flour, rice flour, corn flour etc.)**

----Homogenize the cereal sample

----Weigh 2.0±0.05g of the homogenizer in a 50ml centrifuge tube.

----Add 4.0ml of the acetonitrile($C_2H_3N$), 2.0ml deionized water, shake for 5min;

----Centrifuge for separation: 3000g / 5min/ at room temperature;

----Take 3ml of the upper layer orgnic phase to a 50ml centrifuge tube, add 6ml of chloroform, vortex for 5min.

----Centrifuge for separation: 3000g / 5min/ at room temperature;

----Remove the upper layer liqid, take 4ml of the lower layer orgnic phase to a dry clean 10ml glass tube, dry under 50-60℃ water bath nitrogen flow.

----Add 1ml n-hexane($C_6H_{14}$), vortex for 30s to dissolve the dry leftover, add 1ml extraction solution(**solution 1**), vortex for 30s;

----Centrifuge for separation: 3000g / 5min/ at room temperature;

----Remove the upper layer of n-hexane phase, take 50μl of the lower layer per well for assay

***Sample dilution factor: 2***

**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.


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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 solution**: Add 50µl of the standard solution(**kit provided**) or prepared sample to corresponding wells. Add 50µl of enzyme conjugate(**kit provided**), 50µl of antibody solution(**kit provided**), 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(**kit provided**) and 50µl of solution B(**kit provided**) to each well. Mix gently by shaking the plate manually and incubate for 15min at 2**5**℃ 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 (It's suggested that measure with dual-wavelength of 450/630nm. Read the result within 5min after adding stop solution)

**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.

**11. Sensitivity, accuracy and precision**

**Sensitivity:** 0.02ppb

**Detection limit**

Edible oil……0.1ppb

Peanut……0.2 ppb

Cereal……0.05ppb

**Accuracy**

Edible oil……90±15%

Peanut……90±15%

Cereal……90±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 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 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 the addition of solution A and solution B. And you can prolong the incubation time ranges to 15min if the color


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is too light to be determined. Never exceed 20min, 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.


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