

# AFB1 (Aflatoxin B1) ELISA Kit

## Cat: OPK-E008

For research use only. Not intended for diagnostic use.

## Technical specifications

Sensitivity: 0.02 ppb

Incubation temperature: 25°C

Incubation time: 15 ~ 30 minutes

## Detection limit:

Feed, rice, corn, peanut, tissue, edible oil :- 2ppb

## Cross-reaction rate:

Aflatoxin B1 :- 100%

Aflatoxin B2 :- 54.5%

Aflatoxin G1 :- 8.4%

Aflatoxin G2 :- 1.7%

Aflatoxin M1 :- 6.6%

## Recovery rate:

Feed, rice, corn, peanut, tissue, edible oil :- 95 + 35%

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## Principle

This test kit is based on the indirect competitive enzyme immunoassay for the detection of Aflatoxin B1. 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 the 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 sample has a negative correlation with the Aflatoxin B1 in the sample. This value is compared to the standard curve and the Aflatoxin B1 residues is subsequently obtained.

## Storage and Expiry date

Store the kit at 2-8°C. not frozen

Expiration date is on the packing box.

Item	Specifications	
Micro-well strip	12 strips with 8 removable wells each	
Standard A	0ppb	
Standard B	0.02ppb	
Standard C	0.06ppb	
Standard D	0.18ppb	
Standard E	0.54ppb	
Standard F	1.62ppb	
Enzyme Conjugate	7mL	Red cap
Antibody Working Solution	7mL	Blue cap
Substrate A	7mL	White cap
Substrate B	7mL	Black cap
Stop Solution	7mL	Yellow cap
20x Concentrated Washing Buffer	40mL	White cap
20x Concentrated Redissolving Solution	50mL	Transparent cap

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## Materials Required, Not Supplied

1. **Equipment:** ELISA Reader (450 nm/630nm), homogenizer, shaker, centrifuge, balance: 0.01g quantity sensitive, nitrogen-drying device, incubator, graduated pipettes, printer
2. **Micropipettes:**
  - a. single-channel 20 – 200µL, 100 – 1000µL
  - b. multi-channel 30 – 300µL
3. **Reagents:** Methanol, n-hexane.

## Sample pre-treatment

### Note

- 1) Only the disposable tips should be used for the experiments and the tips must be changed when used for absorbing different reagents;
- 2) Before the experiment, each experimental utensil 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) Sample Redissolving Solution

Use 1 part of 20X Concentrated Redissolving Solution and dilute 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 dilute with 3 parts of deionized water to obtain the ready to use Sample Extract Solution.

## Samples Preparation

### Preparation of tissue, feed, rice and corn sample

- 1) Take  $1.0 \pm 0.05$ g finely ground sample into 50mL centrifuge tube, add 5mL Sample Extract Solution, shake for 3 minutes, centrifuge at above 4000rpm at 20°C for 10 minutes.
- 2) Take 100µL supernatant, add 700µL Sample Redissolving Solution, shake vigorously.
- 3) Take 50µL to test.

**Dilution factor: 40**

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### **Preparation of edible oil sample**

- 1) Take  $1.0 \pm 0.05$ g edible oil sample into 50mL centrifuge tube; add 5mL Sample Extract Solution, then add 4mL n-hexane, shake for 3 minutes, centrifuge at above 4000rpm at 20°C for 10 minutes:
- 2) Discard the supernatant, take 100 $\mu$ L of the middle-layer liquid, add 700  $\mu$ L Sample Redissolving Solution, shake vigorously.
- 3) Take 50 $\mu$ L to test

**Dilution factor: 40**

### **Preparation of peanut sample**

- 1) Take  $1.0 \pm 0.05$ g ground peanut sample into 50mL centrifuge tube; add 5mL Sample Extract Solution, then add 4mL n-hexane, shake for 3 minutes, centrifuge at above 4000rpm at 20°C for 10 minutes.
- 2) Discard the supernatant, take 100 $\mu$ L of the middle-layer liquid, add 400 $\mu$ L Sample Redissolving Solution, shake vigorously.
- 3) Take 50 $\mu$ L to test

**Dilution factor: 25**

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## ELISA procedures

### Instructions

1. Bring reagents to room temperature (20- 25°C) before use.
2. Put ELISA reagents back to 2-8°C immediately after use.
3. The reproducibility of the test is largely depending on the proper washing of plate.
4. In all process of constant temperature incubation, avoid light exposure, seal the microplate with the cover membrane.

### Operation procedures

1. **Bring test kit to the room temperature** (20- 25°C) at least 30 minutes before the assay, note that each reagent must be mixed properly before use.
2. **Put the required number of micro-well strips into plate frames** Re-seal the unused microplate, stored at 2-8°C.
3. **Solution preparation:** Dilute 40mL 20x Concentrated Washing Buffer with deionized water at the ratio 1:19 (1-part 20x Concentrated Washing Buffer + 19 parts deionized water), or prepare the required volume as per the number of samples.
4. **Numbering:** number 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 corresponding wells in duplicates, followed by 50µL Enzyme Conjugate and 50µL Antibody Working Solution. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, incubate at 25°C for 30 minutes in the dark.
6. **Wash microplate:** Pour out the liquid of each well. Aspirate the solution and wash with 250µL of 1× Wash Buffer to each well and let it sit for 15 – 30 seconds. Repeat wash for 4 – 5 times.
7. **Coloration:** Add 50µL of Substrate A followed by 50µL Substrate B to each well. Mix gently by shaking the plate manually, and incubate at 25°C for 15 minutes in dark.
8. **Determination:** Add 50µL 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 within 5 minutes).

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## Result Assessment

There are two methods to judge the results; the first one is the qualitative determination, 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

### A. Qualitative determination

The concentration range (ppb) can be obtained by comparing the average absorbance value of samples with standards. Suppose absorbance value of Sample I is 0.3, Sample II is 1.0, and the standards are: 0ppb: 2.243

0.02ppb: 1.816

0.06ppb: 1.415

0.18ppb: 0.74

0.54ppb: 0.313

1.62ppb: 0.155.

Then the concentration of the Sample I is in the range of 0.54ppb ~ 1.62ppb; Sample II is 0.06ppb ~ 0.18ppb. The concentration range of aflatoxin B1 can be obtained by multiplying by the corresponding dilution of the sample.

### B. Quantitative Analysis

In order to calculate the concentration of samples, a standard curve should be made. Before standard curve is made, % absorbance should be known.

$$\text{Percentage of Absorbance} = \frac{B}{B_0} \times 100$$

B: Average OD value of the sample or the standard solution

B<sub>0</sub>: Average OD value of the 0 ng/mL standard solution (Blank)

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 aflatoxin B1 concentration [ug/L]. The aflatoxin B1 concentration in ug/L (ppb) corresponding to the absorbance of each sample can be read from the calibration curve.

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## Precautions

1. The room temperature below 25 °C or the temperature of the reagents and the samples being not returned to the room temperature (20-25 °C) will lead to a lower standard OD value.
2. 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.
3. Mix evenly before adding any reagents.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
5. 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.
6. Storage: store at 2-8 °C, 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.
7. 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))
8. The optimum reaction temperature is 25 °C, and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.

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