# Aflatoxin B1 ELISA Kit

*Cat.No: DEIA051*
*Lot. No. (See product label)*

## Size

96T

## Intended use

This kit can be used for qualitative and quantitative analysis of aflatoxin B1 in feed.

## General Description

Aflatoxin is toxic chemical which always contaminates cereal, corn and peanut, etc. Strict residue limit has been established for aflatoxin 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 15min in one operation, which can considerably reduce operation error and work intensity.

## Principle Of The Test

This kit is based on indirect-competitive ELISA. The microtiter wells are coated with coupling antigen. Aflatoxin 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 concentration in it, After comparing with the standard curve, multiplied by the dilution factor, aflatoxin B1 residue quantity in the sample can be calculated.

## Reagents And Materials Provided

_ Microtiter plate precoated with antigen, 96 wells
_ StandardSolution×5bottle(1ml/bottle): 0ppb, 3ppb, 8ppb, 25ppb, 70ppb
_ Enzyme conjugate 12ml, red cap
_ Substrate A: 7ml, whitecap
_ Substrate B: 7ml, red cap
_ Stopsolution: 7ml, yellowcap

## Materials Required But Not Supplied

- Microtiter plate spectrophotometer (450nm/630nm)
- Shaker
- Vortex mixer
- Analytical balance (inductance: 0.01g)
- ···Graduated pipette: 10ml


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- Rubber pipette bulb
- Conical flask (250ml)
- Funnel
- Whatman#1 filter paper
- Volumetric flask: 100ml
- Polystyrene centrifuge tubes: 2ml, 50ml
- Micropipettes: 20μl-200μl, 100μl-1000μl, 250μl-multichanel
- Methanol (AR)
- Deionized water

## Storage

Storage condition: 2-8°C.
Storage period: 12 months.

## Specimen Collection And Preparation

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.

**Feed Sample**
1. Method 1
-Homogenize the sample.
-Weigh 20.0±0.05g homogenate into a conical flask(or jar which is clean and sealable). Add 100ml of 70% methanol(Solution 1), shake for 5min, and then keep still for 10min;
-Filter the supernate with the Whatman#1, collect the filtrate.
-Dilute the filtrate with deionized water in the volume ratio of 1:3(e.g. 0.5ml of filtrate + 1.5ml of deionized water), mix completely.
-Take 20μl per well for assay.
Note: The pH of the filtrat should range from 5 to 9, deviant pH will interfere the result. Use HCl / NaOH to adjust the pH if needed.

2. Method 2
-Homogenize the sample.
-Weigh 5.0±0.05g homogenate into a 50ml polystyrene centrifuge tubes. Add 25ml of 70% methanol(Solution 1), shake fiercely for 5min, then centrifuge for separation: 3000g / 5min / ambient temperature.
-Dilute the supernate with deionized water in the volume ratio of 1:3(e.g. 0.5ml of filtrate + 1.5ml of deionized water), mix completely.
-Take 20μl per well for assay.
Note: The pH of the supernate should range from 5 to 9, deviant pH will interfere the result. to adjust the pH if needed.

## Reagent Preparation

Solution 1: 70% methanol
Mix the methanol and deionized water in the volume ration of 7:3 completely.

## Assay Procedure


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1 Make sure all reagents and microwells are all at room temperature (20-25°C).
2 Return all the rest reagents to 2-8°C immediately after used.
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.
4 Avoid the light and cover the microwells during incubation.
5 Please make the assay not more than 48 tests if use multichannel pipette or not more than 24 tests if use single-chanel micropipette for the operation time is short.

**Assay Steps**
1 Take all reagents out at room temperature (20-25°C) for more than 30min, homogenize before use.
2 Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.
3 The wash solution should be rewarmed to room temperature before use.
4 Number: number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
5 Add standard solution/sample, enzyme conjugate and antibody: Add 20μl of standard solution or prepared sample to corresponding wells. Add 100μl of enzyme conjugate, mix gently by shaking the plate manually and incubate for 10min at 25°C with cover.
6 Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250μl of deionized water 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).
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 5min at 25°C with cover (see Precautions 8).
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)

## Calculation

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%.
Absorbance (%) = B/B0*100%
B ——absorbance of standards or samples
B0 ——absorbance of zero standard

## Typical 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.

## Precision

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

## Detection Range


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3-70ppb (Dilute the sample if the residue concentration exceeds 70ppb)

## Detection Limit

3ppb

## Sensitivity

0.15ppb

## Specificity

Cross reactions
Aflatoxin B1: 100%
Aflatoxin B2: 56%
Aflatoxin G1: 68%
Aflatoxin G2: 25%

## Precautions

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°C).
2. Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tap the microwells holder.
3. Shake each reagent gently before using.
4. Keep your skin away from the stop solution for it is the 0.5M $H_2SO_4$ solution.
5. Don't use the kits out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.
6. Keep the ELISA kits at 2-8°C, do not freeze. Seal rest microwell plates, Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.
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).
8. The coloration reaction needs 5min after the addition of solution A and solution B; But you can prolong the incubation time ranges to 7min or more if the color is too light to be determined, never exceed 10min, On the contrary, shorten the incubation time properly.
9. The optimal reaction temperature is 25°C. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.


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