

Instructions on use of

DOA-Aflatoxin ELISA Test Kit



Exclusive Distributor: Thai Global Complex Co., Ltd., Tel. 02 375 2455 -58, Fax 02 375 2499
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Collection of toxin from sample

Weigh 20 g. of sample. Put it in a flask or blender. Add 100 mg. of 70 methanol.

With an agitator having a speed of 300 rpm. shake for a few minutes.

Let it stand 5 minutes.

Put the sample in No.4 filter to let the clear part run through.

Keep the remainder. (ratio: 1:5)

Mix the extract 1 ml. with 3 ml. of buffer (ratio: 1:20).

The extract will be used for ELISA test.

Test of Aflatoxin with DOA-Aflatoxin ELISA Test Kit

Drop 50 microliters of toxin (0, 4, 10, 20, 40 ppb) in test hole that is covered with antibody, and drop 50 microliters of the extract in the rest of the holes.

Add about 50 microliters of enzyme conjugate (1 vial + 1ml conjugate buffer). Let the mixture stand for about 30 minutes at 37° C.

Remove the mixture from test holes, and wash the holes 3 times with 0.01 M phosphate buffer saline (washing buffer).

Put 100 microliters of substrate in all the holes, and leave them to stand in dark place for 10 minutes.

During chemical reaction the color will turn blue. Through visual inspection the results can be determined by comparison of toxin at different level of concentration.

Add 100 micrograms of 0.5 M phosphoric acid. The reaction will cause the color to turn yellow.

Read the concentration of color at wave range of 450 nanometers, and determine the concentration of toxin in ppb,.

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Aflatoxins

Aflatoxins are naturally occurring mycotoxins that belong to the secondary metabolites produced by many species of *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*. Crops (food and feed) which are frequently affected include cereals (maize, sorghum, rice, wheat, barley, Job's tears), oilseeds (sunflower, peanut and other nuts), desiccated coconut, dried chili, dried shrimps, dried fish, spices, herbs and coffee. The food processed from contaminated raw materials is also infected.

Aflatoxins can colonize and contaminate crops before harvest or during storage and transportation. Host crops are particularly susceptible to infection by fungi following prolonged exposure to a high humidity environment. The ambient temperature in the range of 28° -35° C is favorable for Aflatoxin growth.



The 4 major naturally occurring Aflatoxins are AFB1, AFB2, AFG1 and AFG2. AFB1 is most frequently found. It is most toxic and carcinogenic to human beings and animals. Exposure to only a small amount of AFB1 is a high risk of liver cancer. There are also Aflatoxin M1 and M2, which are the derivatives of the B1 and B2. Milk is can be contaminated with Aflatoxins which are resistant to heat up to 280°C. Aflatoxins remain in acidic condition,

decomposes in alkaline condition and is slightly soluble. When they enter the body they will be discharged with urine, feces and milk; certain parts of Aflatoxins will turn into metabolites. The most toxic ones are Aflatoxins B1, B2 and B3. Chronic exposure also leads to a high risk of developing liver cancer, as the metabolite Aflatoxin can intercalate into DNA and RNA and alkylate the bases through its epoxide moiety. At present, Aflatoxins are listed by the International Association Research Cancer (IARC) as Class I carcinogens.





Not only are Aflatoxins hazardous to health, they also raise local and international trade issues. For its own benefit, each country sets a limit on each type of Aflatoxin. The Thai Ministry of Public Health Notification No.98, 1986, imposes a limit of 20 micrograms of Aflatoxin in kg. of food or 20 ppb.



Therefore, protective and control measures must be introduced. The best way of prevention is to avoid the intake of food with Aflatoxin contamination.



Aflatoxins are colorless, odorless and tasteless. To find out if the crops are contaminated with Aflatoxins or not, food manufacturer, service provider and consumer must be able to perform fast, efficient and accurate tests. When the tests results are known decision on crops handling can be made in time.



The Department of Agriculture specialists have developed the DOA-Aflatoxin ELISA Test Kit through enzyme-linked immunosorbent assay (ELISA) technology, using Direct competitive ELISA. This kit is capable of detection of Aflatoxins in samples with 82 -100% accuracy, which is as efficient as the imported ones, but it is much cheaper.



Advantages of DOA-Aflatoxin ELISA Test Kit:

- 1. Samples can easily be collected for tests without any clean up.**
- 2. Many samples can be tested simultaneously.**
- 3. Tests can be conducted easily, conveniently and safely.**
- 4. The analysis is fast: only one hour after sample preparation.**
- 5. Highly sensitive. Detection of as low concentration as 0.4 ppb is possible.**
- 6. The cost of test per sample is quite high as compared with the chemical method.**
- 7. It is non-pollutant; not dangerous chemicals are used.**
- 8. Qualitative and quantitative results of the analysis are given.**
- 9. Can be used for detection of crops, food and feed.**

Useful applications:

The kit can be used for testing crops at any stage of food production chain to ensure safety. In the crop processing industries such as food and feed production within the government or private sector, the kit can be used to detect Aflatoxins in raw materials prior to food processing or production of feed for animals to ensure that the products are free from contamination,



which will help reduce animal deaths or disabilities. Using the kit, the products will meet the standards for local consumption and export. Most important of all, consumers will feel safe because the risk of cancer is reduced; Eating Aflatoxin free food is the way to ensure better health and good living.