DATA SHEET

Aflatoxin(AFT) ELISA Kit

Catalog No. CSB-E09923o

(96)

This test kit can be used to detect total aflatoxins in maize, corn, feed, edible oil and other samples qualitatively and quantitatively.

Expiration date six months from the date of manufacture

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PRINCIPLE OF THE ASSAY

This test kit is based on the competitive enzyme immunoassay for the detection of aflatoxins in the sample. The coupling antigens are pre-coated on the microtiter plate wells. The aflatoxins in the sample and the precoated antigens compete for the anti-aflatoxins antibody. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The color develops in opposite to the amount of aflatoxins bound in the initial step. The concentration of aflatoxins in the samples is then determined by comparing the O.D. of the samples to the standard curve.

RECOVERY RATE

Peanuts	95±15%
Animal feed	85±15%
Edible oil	95±15%
Corn	90±15%

CROSS-REACTION RATE

Aflatoxin B2----------100%
Aflatoxin B1----------97%
Aflatoxin G2----------98%
Aflatoxin G2----------101%

DETECTION RANGE

0.25 ng/ml-8 ng/ml.

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium

SENSITIVITY

The minimum detectable dose of aflatoxins is typically less than 0.25ng/ml.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.

MATERIALS PROVIDED

Reagent	**Quantity**
Assay plate	1
Standard	2
Antibody	1 x 6 ml
HRP-conjugate	1 x 6 ml
Wash Buffer (25×concentrate)	1 x 20 ml
TMB Substrate	1 x 10 ml
Stop Solution	1 x 10 ml

Standard	S 1	S 2	S 3	S 4	S5	S6	S7
Concentration (ng/ml)	0	0.25	0.5	1	2	4	8

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag. The test kit may be used throughout the expiration date of the kit, provided it is stored as prescribed above. Refer to the package label for the expiration date.
2. Opened test plate should be stored at 2-8°C in the aluminum foil bag with desiccants to minimize exposure to damp air. The kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

1. **Wash Buffer** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely

dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 500 ml of Wash Buffer.
Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

OTHER SUPPLIES REQUIRED

_ Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
_ Pipettes and pipette tips.
_ Deionized or distilled water.
_ Squirt bottle, manifold dispenser, or automated microplate washer.
_ An incubator which can provide stable incubation conditions up to 37°C±0.5°C.

SAMPLE COLLECTION AND STORAGE

_ Grind the samples.
_ Weight 10 g of sample and add 50 ml of 33% Methanol Solution, Vortex for 2 minutes.
_ Let to settle for 15 minutes at room temperature.
_ Filter the extract throught a Whatman N°1 fi lter paper (or equivalent).
_ Dilute 1:2 the clear supernatant with 33% Methanol Solution (i.e. 1ml + 1ml) and test the sample. For feed samples, a further dilution 1:10 with 33% Methanol Solution (i.e. 1ml + 9ml) is required befor testing.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate.
All the reagents should be added directly to the liquid level in the well. The pipette should avoid contacting the inner wall of the well.

1. Add 50µl of Standard, Blank or Sample per well.
2. Add 50µl of Antibody, 50µl of HRP-Conjugate to each well.
3. Cover with the adhesive strip. Incubate for 20 minutes at 37°C.
4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash: Fill each well with Wash Buffer (200µl) and let it stand for 2 minutes, then remove the liquid by flicking the plate over a sink. The remaining drops are removed by patting the plate on a paper towel. Complete removal of liquid at each step is essential to good performance.
5. Add 90µl of **TMB Substrate** to each well. Incubate for 10-30 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.

6. Add 50µl of **Stop Solution** to each well when the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

The unknown values for Total Afl atoxin concentration in samples are determined from a calibration curve.
1. Calculate the mean absorbance value for Blank and subtract it from the absorbance value of all other wells.
2. Calculate the mean absorbance value for the Maximum Binding, the standards and the samples.
3. Divide the mean absorbance value of standards and samples (B) by the mean absorbance value of the Maximum Binding (Bo) and multiply by 100. Maximum binding is thus made equal to 100% and the absorbance values are quoted in percentages:

$$\frac{\text{Absorbance standard (or sample)}}{\text{Absorbance Maximum Binding}} \times 100 = \frac{B}{Bo} \ (\%)$$

4. Enter the B/Bo (%) values calculated for each standard in a semi-logarithmic system of coordinates against the Total
5. Afl atoxin standard concentration; draw the standard curve.
6. Take the B/Bo (%) value for each sample and interpolate the corresponding concentration from the calibration curve.

LIMITATIONS OF THE PROCEDURE

_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute reagents with those from other lots or sources.
_ It is important that the Standard Diluent selected for the standard curve be consistent with the samples being assayed.
_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Standard Diluent and repeat the assay.
_ Any variation in Standard Diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of

interference cannot be excluded.

TECHNICAL HINTS

_ Centrifuge vials before opening to collect contents.
_ When mixing or reconstituting protein solutions, always avoid foaming.
_ To avoid cross-contamination, change pipette tips between

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium