Aflatoxin B1 ELISA Kit

Enzyme Immunoassay for the quantification of Aflatoxin B1 in cereals and beer/gyle.

Catalog number: ARG81029

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MANUFACTURED BY:

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INTRODUCTION

Aflatoxins belong to the class of mycotoxins. Chemically they are defined as difuranocyclopentanocumarines or difuranopentanolidocumarines, i.e. aflatoxins contain a dihydrofuran or a tetrahydrofuran ring, to which a substituted cumarin system is condensed. Out of about 20 known aflatoxins, the moulds Aspergillus flavus and A. parasiticus produce exclusively aflatoxin B1, B2, G1 and G2, and all the other aflatoxins are derivates of these four.

The derivates are developed either by metabolism in humans, animals and microorganisms or by environmental reactions. Aflatoxins belong to the strongest mycotoxins, which act primarily in a hepatotoxic and carcinogenic way. The four main aflatoxins show a different toxicity. B1 is without doubt the most toxic aflatoxin, followed by G1, B2 and G2. Aflatoxin B1, however, does not show a direct toxic action. In the process of biotransformation in the liver, the lipophilic toxin is epoxidated and transformed into an active derivative, the so-called aflatoxin B1-2,3-epoxid. This highly reactive epoxid is able to react with nucleophilic regions of macromolecules. Amongst other this metabolite of aflatoxin B1 binds covalently to the N-7 atom of the guanine bases of DNA. This covalent bond causes an inhibition of the DNA replication, the RNA synthesis and mutations.

Both chronical and acute intoxications are effected by aflatoxins. There are only few documented reports about acute intoxications, which are caused by uptake of mycotoxins. Of special importance for human beings are the chronical intoxications by aflatoxins. To the diseases, which develop after such chronical intoxications, belong primary liver carcinoma, hepatitis, Reye´s syndrome and Kwashiorkor. Besides the generation of primary liver carcinoma,

aflatoxins are presumably also responsible for other sorts of tumors, like intestinal cancer. Contaminations with aflatoxins occur mostly with nuts and grain. In most cases aflatoxins penetrate the human body via the food. Aflatoxins are stable to heat and are only partly destroyed by boiling. In order to protect people against aflatoxin-induced diseases, there is a need for the quantitative and qualitative control of endangered foodstuff, besides appropriate hygienic precautions, which avoid the formation of aflatoxins.

In the European Union the limits for aflatoxin B1 are 2 – 12 ppb for regular food products. Thus a monitoring of food and feed with respect to the concentration of aflatoxin B1 is obligatory.

PRINCIPLE OF THE ASSAY

This Aflatoxin B1 ELISA kit is an Enzyme Immunoassay and represents a highly sensitive detection system and is particularly capable of the rapid quantification of aflatoxin B1 contaminations in cereals and beer.

This assay employs the competitive quantitative enzyme immunoassay technique. An antibody binding protein has been pre-coated onto a microtiter plate. The antibody binding protein can bind to the primary antibody which recognizes Aflatoxins. The primary antibodies in the kit will be competitively bound by both HRP-conjugated Aflatoxin B1 peptides and Aflatoxin B1 peptides in standards or targeted Aflatoxin B1 peptides in samples. After washing away any unbound peptide or antibody, a substrate solution (TMB) is added to the wells and color develops in inverse-proportion to the amount of Aflatoxin B1 present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of

450nm±2 nm. The concentration of Aflatoxin B1 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Microplate (antibody binding protein coated)	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standards 0-5 (0; 1.5; 3; 6; 12; 24 ppb)	6 vials (1ml/vial)	4°C
Anti-Aflatoxins antibody	6 ml (Ready-to-use)	4°C
HRP-Conjugated Aflatoxin	6 ml (Ready-to-use)	4°C
Sample diluent	2 X 60ml (Ready-to-use)	4°C
10X Wash buffer	60ml	4°C
TMB substrate	15ml (Ready-to-use)	4°C (Protect from light)
STOP solution	15ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Methanol
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 22-25°C) 20 min before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 10X Wash buffer, warm it up to 37°C until the crystals are completely dissolved.
- Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
- Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

- Cereals:

Grind sample to pass through a 20 mesh sieve and thoroughly mix prior to sub-sampling.

1. Suspend 20 g of sample in 100 mL of 70% methanol.
2. Mix suspension for 5 minutes.
3. Filter through Whatman #1 filter or alternatively centrifuge at a minimum of 3000 g for 5 minutes.
4. Dilute 500 µL of filtrate/supernatant with 500 µL of sample diluent and test the sample in the ELISA.

- Beer / Gyle:

1. Dilute an adequate volume of sample diluent with 35% methanol.
2. Carbonized beer samples should be preliminarily degassed by moderate heating.
3. Cloudy beers (such as beer brewed from wheat) / gyle should preliminarily be sterile-filtered.
4. Dilute 100 µL beer / gyle with 900 µL sample diluents/methanol dilution.

In case of too high concentrated samples, an adequate volume of sample diluent is diluted with 35% methanol. The sample extracts have to be further diluted with this dilution.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. E.g. 60 ml of 10X Wash buffer + 540 ml of distilled water.
- Standards:

  The ready-to-use standards are prepared for a direct determination of cereal sample concentrations. Thus no further calculation after analysis is necessary.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards and samples into respective wells.
3. Add 50 µL of HRP-conjugated aflatoxin into each well.
4. Add 50 µl of anti-Aflatoxins antibody into each well.
5. Cover wells and incubate for 10 mins at RT with shaking.
6. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (350µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
7. Add 100 µl of TMB substrate to each well. Incubate for 10 minutes at room temperature (in dark).

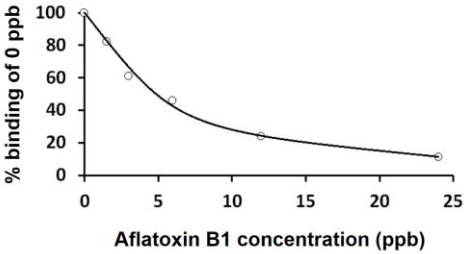
8. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow. Gently tap the plate to mix the contents of the microplate wells thoroughly.
9. Read the OD with a microplate reader at 450nm (optional: reference wavelength 620 nm), immediately. It is recommended read the OD within 30 min after adding STOP solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The ready-to-use standards are prepared for a direct determination of cereal sample concentrations.
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. The binding is calculated as percentage of absorption at 0 ppb.



QUALITY ASSURANCE

Sensitivity

The limit of detection (LOD) of the Aflatoxin is 0.5 ppb.

Validation experiments with common matrices resulted in the following LODs.

matrices	ppb
Wheat	0.7
Rye	1.1
Barley	0.9
Oats	0.7
Corn	1.4
Rice	1.1
Beer	0.9

The limit of quantification (LOQ) of the Aflatoxin B1 is 1.5 ppb.

Due to the variety of sample matrices and their influence on the blank, results less than the LOQ should be treated as negative.

Specificity

Cross-reactivity	Relative to Aflatoxin B1 (=100%)
Aflatoxin B2	29%
Aflatoxin G1	44%
Aflatoxin G2	5%
Aflatoxin M1	2%

Intra-assay precision

The CV value of intra-assay precision was 3-6% and Inter-assay was 5-11%.

Recovery

86-114%