

Sensitivity	5 pg/mL
Recovery (spiked samples)	>80%
Incubation Time	140 min

Test Instruction

Aflatoxin B₁

96 Tests

Enzyme Immunoassay
for the Quantitative
Determination of
Aflatoxin B₁ in Food

Cat.-No.: HU0030082

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General Information

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 derivatives of these four. The derivatives 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 chronic 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 chronic intoxications by aflatoxins. To the diseases, which develop after such chronic 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. The **SENSISpec Aflatoxin B₁ ELISA** is a quick, economical and sensitive method to detect aflatoxin B₁ in food. After an appropriate sample preparation, 40 samples can be tested in duplicate within 140 minutes.

Principle of the Test

The **SENSISpec Aflatoxin B₁** quantitative test is based on the principle of the enzyme linked immunosorbent assay. An aflatoxin conjugate is bound on the surface of a microtiter plate. Aflatoxin B₁ containing samples or standards and an antibody directed against aflatoxin B₁ are given into the wells of the microtiter plate. Immobilized and free aflatoxin B₁ compete for the antibody binding sites. After one hour incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugate against the antibody is given into the wells and after another hour incubation, the plate is washed again. Then a substrate solution is added and incubated for 20 minutes, resulting in the development of a blue colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of aflatoxin B₁ is indirectly proportional to the colour intensity of the test sample.

Precautions

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
5. Use a separate disposable tip for each specimen to prevent cross-contamination.
6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.
7. Do not mix components from different batches.
8. Do not use reagents after expiration date.
9. Check both precision and accuracy of the laboratory equipment used during the procedure (micropipets, ELISA reader etc.).

Health and safety instructions

1. Do not smoke or eat or drink or pipet by mouth in the laboratory.
2. Wear disposable gloves whenever handling patient specimens.
3. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
4. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).
5. Aflatoxins are very toxic substances. They can cause cancer or irreversible damages of the genetic substance. Aflatoxins are toxic after inhalation, swallowing or dermal contact. Appropriate protective clothing must be worn.

Reagents

The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

1. Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with aflatoxin conjugate.
2. Aflatoxin B₁ Standards (0; 100; 400; 1000; 4000; 10000 pg/mL): 6 vials with 0.5 mL each in methanol as 10x concentrate. Dilute 1+9 with sample/standard diluent.
Note: The concentrations above refer to the 10x concentrated standards.
3. Anti-Aflatoxin B₁ Antibody (rabbit): 6 mL, dyed red, ready-to-use.
4. Conjugate (anti-rabbit-IgG-HRP): 15 mL, dyed red, ready-to-use.
5. Substrate Solution (TMB): 15 mL; ready-to-use.
6. Stop Solution (0.5 M H₂SO₄): 15 mL; ready-to-use.
7. Sample/Standard Diluent (PBS): 60 mL, ready-to-use.
8. Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate, dyed blue. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.

9. Two plastic foils to cover the strips during the incubation.
10. Plastic bag to store unused microtiter strips.
11. Instruction Manual.

Additional Instrumentation and Reagents (not provided)

Instrumentation

- 50, 100, 500 and 1000 μL -micropipets
- Microtiter plate shaker
- ELISA reader (450 nm)
- Mortar or mixer
- Horizontal shaker or magnetic stirrer
- Centrifuge

Reagents

- Methanol
- Double-distilled water

Sample Preparation

- An appropriate amount of grain or nuts is crushed in a mortar or mixer to produce a fine to medium-fine powder. If flour is used, the first step can be omitted.
- 2 g of this powder are extracted with 10 mL methanol / double-distilled water (70/30 v/v). This mixture is agitated for 30 minutes on a horizontal shaker (120 / minute). Alternatively also a magnetic stirrer can be used.
- The mixture is centrifuged afterwards for 5 minutes at 3000 g.
- The aqueous phase is diluted 1:10 in standard/sample diluent, before being assayed in the **SENSISpec Aflatoxin B₁ ELISA**.

Alternatively also immunoaffinity columns (Cat.-No.: AFB-A01) can be employed for the extraction. When using such columns care must be taken, that the eluate which is given into the **SENSISpec Aflatoxin B₁ ELISA** does not contain more than 5% organic solvent (methanol, acetone). In that case, the eluate must be further diluted with sample diluent.

Reagent Preparation

Because the standards are concentrated 10x, they have to be diluted by the enclosed standard/sample diluent 1:10 (e.g. 50 μL standard + 450 μL diluent), before using them in the assay procedure.

Procedure

1. Prepare samples as described above.

2. Pipet 100 μL diluted (1:10) standards or prepared samples in duplicate into the appropriate wells of the microtiter plate. Immediately add 50 μL aflatoxin B₁ antibody into each well.
3. Cover the microtiter plate with a plastic foil and incubate for 60 minutes at room temperature on a microtiter plate shaker (or 90 minutes without shaker).
4. Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 μL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbencies.
5. Pipet 100 μL of conjugate (anti-rabbit-IgG-HRP) into each well.
6. Cover the microtiter plate with a plastic foil and incubate for 60 minutes at room temperature on a microtiter plate shaker (or 90 minutes without shaker).
7. Wash the plate as outlined in 4.
8. Pipet 100 μL of substrate solution into each well.
9. Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 20 minutes at room temperature.
10. Stop enzyme reaction by adding 100 μL of stop solution (0.5 M H₂SO₄) into each well. The blue colour will turn yellow upon addition.
11. After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

Calculation of results

1. Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.
2. Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in pg/mL on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis.

- Using the mean optical density value for each sample, determine the corresponding concentration of aflatoxin B₁ in pg/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
- The diluted samples must be further converted by the appropriate dilution factor (50 for the above described extraction). The factor is dependent on the sample preparation procedure employed.

Typical Standard Values

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 0 pg/mL standard. These values are only an example and should not be used instead of the standard curve which has to be measured in every new test.

Aflatoxin B ₁ (pg/mL)	(% binding of 0 ng/mL)
0	100
10	90
40	85
100	70
400	40
1000	25

Performance

Sensitivity

The sensitivity of the **SENSISpec Aflatoxin B₁ ELISA** is 5 pg/mL (based on the standard curve).

Recovery

The recovery of spiked samples was determined to >80%.

Intra-assay Precision

The intra-assay variation of the aflatoxin B₁ test was determined to 3%.

Cross-reactivity relative to aflatoxin B₁ (=100%)

Aflatoxin G ₁	26%
Aflatoxin B ₂	6%
Aflatoxin G ₂	3%
Aflatoxin M ₁	2%
Sterigmatocystin	0.02%

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