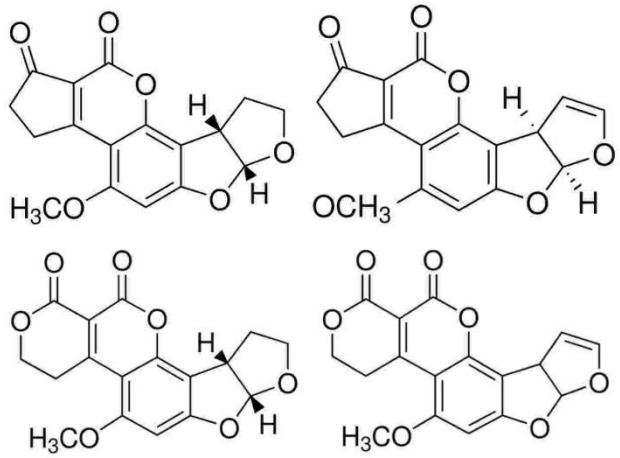
SENSI*Spec* ELISA Aflatoxin Total RAPID 96 Tests

Enzyme Immunoassay for the Rapid Quantitative Determination of Total Aflatoxin in Food and Feed (Cat.nr. HU0030086)

Sensitivity	0.6 – 1.5 ppb
Recovery (spiked samples)	82 - 100%
Recovery (natur. contaminated samples)	92 – 113%
Incubation Time	10 min

1. 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 B_1, B_2, G_1 and G_2, 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.

In the European Union the limits for total aflatoxins are 4 – 15 ppb for food. Thus a monitoring of food and feed with respect to the concentration of aflatoxins is obligatory.
The **SENSI*Spec* Aflatoxin Total RAPID ELISA** represents a rapid and sensitive detection system and is particulary capable of the quantification of total aflatoxin contaminations in cereals, beer, nuts, dry fruits, DDGS and cottonseed.

2. PRINCIPLE OF THE TEST

The **SENSI*Spec* Aflatoxin Total RAPID** quantitative test is based on the principle of the enzyme-linked immunosorbent assay. An antibody binding protein is coated on the surface of a microtiter plate. Aflatoxin containing samples or standards, an aflatoxin-peroxidase conjugate and an antibody directed against aflatoxins are given into the wells of the microtiter plate. The conjugate competes with the aflatoxins of samples/standards for the limited number of antibody sites. Simultaneously the anti-aflatoxin antibody is bound to the antibody-binding protein coated on the microtiter plate. After 5 minutes incubation at room temperature the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated for 5 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 aflatoxins is indirectly proportional to the colour intensity of the test sample.

3. 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.).

4. HEALTH AND SAFETY INSTRUCTIONS

1) Do not smoke or eat or drink or pipet by mouth in the laboratory.

Eurofins Technologies Hungary Kft., Fóti út 56., 1047 Budapest, Hungary
E-mail: technologies.hu@eurofins.com
Website: www.eurofins-technologies.com

2) 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.

3) Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

5. 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 antibody-binding protein.

2) Aflatoxin Standards, 10/1/1/1 mixture of B1/B2/G1/G2 (0; 2; 5; 10; 20; 50 ppb) according to GIPSA guidelines: 6 vials with 1 mL each, dyed red, ready-to-use. Because of the total dilution of 1:10 of the samples in the extraction step, the calibrators contain 1/10th of the stated value. Thus no further calculation after analysis is necessary.

3) Anti-Aflatoxin Antibody (mouse): 6 mL, dyed blue, ready-to-use.

4) Conjugate (Aflatoxin-Peroxidase): 6 mL, dyed red, ready-to-use.

5) Substrate Solution (TMB): 15 mL, ready-to-use.

6) Stop Solution (0.5 M H_2SO_4): 15 mL, ready-to-use.

7) Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate. 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.

8) Plastic bag to store unused microtiter strips.

9) Instruction Manual.

6. ADDITIONAL INSTRUMENTATION AND REAGENTS (not provided)

Instrumentation

- 50 and 100 µL- micropipets
- ELISA reader (450 nm)
- Centrifuge
- Ultra-Turrax, mixer, vortex

Reagents

- Double distilled water
- Methanol

7. SAMPLE PREPARATION

Solid Samples

- Grind sample to pass through a 20 mesh sieve and thoughly mix prior to sub-sampling.
- Suspend 20 g of sample in 100 mL of 70% methanol.
- Mix suspension for 5 minutes.
- Filter through Whatman #1 filter or alternatively centrifuge at a minimum of 3000 g for 5 minutes.
- Dilute 500 µL of filtrate/supernatant with 500 µL of double distilled water and test the sample in the ELISA.
- If clouds appear during final dilution with double distilled water it is recommended to filter through Whatman #1 filter or alternatively centrifuge at a minimum of 3000 g for 5 minutes another time and test the filtrate/supernatant in the ELISA

In case of DDGS and other acidic matrices, the pH has to be adjusted to 7.0 before final dilution with double distilled water.

If analyzing very swelling samples like cottonseed 20 g of samples should be suspended in 200 mL of 70% methanol. The thus generated additional diltution factor of 2 has to be accounted for when calculating final result.

Liquid samples

- Carbonized beer samples should be preliminarily degassed by moderate heating.
- Cloudy beers (such as beer brewed from wheat) / gyle should preliminarily be sterile-filtered.
- Dilute 1 mL of liquid sample with 9 mL of 35% methanol and test the sample in the ELISA.

In case of too high concentrated samples, the sample extracts have to be further diluted with 35% methanol.

8. PROCEDURE

1) Prepare samples as described above.

2) Pipet 50 µL standards or prepared samples in duplicate into the appropriate wells of the microtiter plate.

3) Add 50 µL of aflatoxin-peroxidase conjugate into each well.

4) Add 50 µL of the anti-aflatoxin antibody into each well. In order to avoid time drifts it is recommended to use a stepper (multipette) for this step.

5) Incubate for 5 minutes at room temperature.

6) 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.

7) Pipet 100 µL of substrate solution into each well.

8) Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 5 minutes at room temperature.

9) Stop enzyme reaction by adding 100 µL of stop solution (0.5 M H_2SO_4) into each well. The blue colour will turn yellow upon addition.

10) After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

9. CALCULATION OF RESULTS

The ready-to-use standards are prepared for a direct determination of the sample concentrations. The dilution of samples in the extraction process as described in the above stated sample preparation procedure is already considered. Additional dilution due to high sample concentration has to be accounted for.

1) Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.

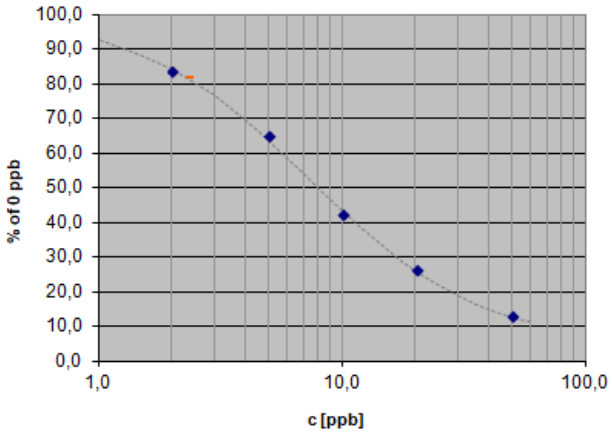
2) Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ppb on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis. Alternatively the evaluation can be carried out by software. In this case the 4-parameter method should be preferred.

3) Using the mean optical density (OD) value for each sample, determine the corresponding concentration of aflatoxin in ppb from the standard curve. Depending on experience and / or the availability of computer capability, other methods of data reduction may be employed.

10. 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 ppb standard. These values are only an example and should not be used instead of the standard curve which has to be measured in each new test.

Aflatoxin (ppb)	(% binding of 0 ppb)
0	100
2	83
5	64
10	43
20	26
50	12



11. PERFORMANCE

Sensitivity

The limit of detection (LOD) of the **SENSI*Spec* Aflatoxin Total Rapid Test** is 0.6 ppb for the standard curve.

The limit of quantification (LOQ) of the **SENSI*Spec* Aflatoxin Total Rapid Test** is 2.1 ppb for the standard curve.

Validation experiments with common matrices resulted in the following LODs and LOQs [ppb].

Matrix	LOD	LOQ
Wheat	0.8	2.8
Oats	1.2	3.7
Barley	1.5	3.0
Rice	1.2	2.8
Corn	1.3	2.2
Beer	1.2	2.4
Peanut	1.3	2.5
Pistachio	1.5	3.5
DDGS	1.2	3.0
Raisin	1.2	3.6
Hazelnut	1.3	2.0
Cottonseed	1.1	1.8

11.1. Cross-reactivity *relative to 10/1/1/1 aflatoxin standard mixture (=100%)*

Aflatoxin B1	110%
Aflatoxin B2	74%
Aflatoxin G1	69%
Aflatoxin G2	47%

11.2. Precision

Intra-assay Precision	3-4%
Inter-assay Precision	6-11%

11.3. Linearity

The serial dilution of spiked samples (wheat, oats, barley, rice, corn, beer, peanut, pistachio, DDGS, raisin, hazelnut, cottonseed) resulted in a dilution linearity of 83-107%.

11.4. Recovery *(spiked samples)*

Wheat	91%
Oats	88%
Barley	100%
Rice	92%
Corn	92%
Beer	93%
Peanut	90%
Pistachio	92%
DDGS	82%
Raisin	96%
Hazelnut	94%
Cottonseed	96%

11.5. Recovery (naturally contaminated samples)

Trilogy A-C-2223, Corn, 5.2 ppb	113%
Trilogy A-C-2215, Corn, 21.0 ppb	101%
Trilogy A-C-292, Corn, 51.6 ppb	92%
Trilogy A-C-2225, Corn, 91.2 ppb	103%
Trilogy A-C-2229, Corn, 300.0 ppb	99%

12. REFERENCES

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2) Siahi SMR, et al. (2012) – Determination of aflatoxins in nuts of Tabriz confectionaries by ELISA and HPLC methods. Adv Pharm Bull, 2(1):123-126

3) Tsung-Che T, et al. (1992) – Preparation and characterization of a monoclonal antibody against aflatoxins B_1, B_2, G_1, G_2. Bot Bull Acad Sin, 33:369-374

4) Zhang D, et al (2009) – Production of ultrasensitive generic monoclonal antibodies against major aflatoxins using a modified two-step screening procedure. Anal Chim Acta, 636(1):63-69

Eurofins Technologies Hungary Kft., Fóti út 56., 1047 Budapest, Hungary
E-mail: technologies.hu@eurofins.com
Website: www.eurofins-technologies.com