AccuDiag™ Aflatoxin Total ELISA Kit

REF 5156-8

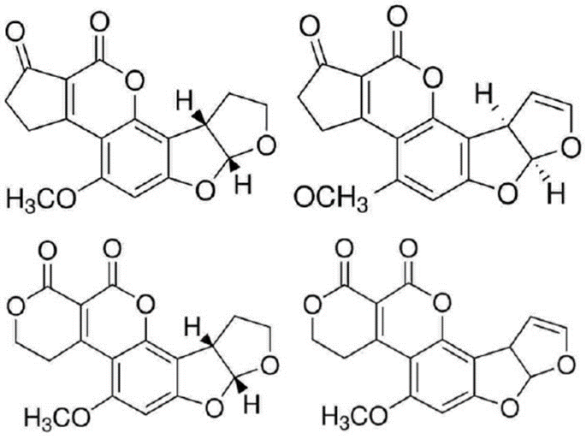
See external Label 2°C 8°C Σ 96 Tests

Test	Aflatoxin Total ELISA
Recovery	87 - 121%
Total Time	~ 45 min.
Sensitivity	0.015 ng/mL

INTENDED USE

The **Diagnostic Automation, Inc.** Aflatoxin Total ELISA is an enzyme immunoassay for the quantitative determination of Aflatoxins in Food

SUMMARY



Aflatoxins belong to the class of mycotoxins. Chemically they are defined as difuranocyclopentanocu-marines 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 environ-mental reactions. 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 Diagnostic Automation, Inc. (DAI) Aflatoxin Total ELISA represents a highly sensitive detection system and is particular capable of the quantification of total aflatoxin contaminations in cereals, beer, nuts, dry fruits, oils and chili. The detection and quantification in further spices and coffee in combination with the DACD Aflatoxin Immunoaffinity Column is also possible.

TEST PRINCIPLE

The DAI Aflatoxin Total ELISA is based on the principle of the enzyme-linked immunosorbent assay. An antibody binding protein is coated on the surface of a microtiter plate. Aflatoxins 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 30 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 15 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 inversely proportional to the colour intensity of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the test kits

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** (0; 0.05; 0.1; 0.25; 0.5; 1.5 ng/mL): 6 vials with 1 mL each, dyed red, ready-to-use.
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. **Sample Diluent (PBS):** 2 x 60 mL, dyed red, ready-to-use.
8. **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.
9. Plastic bag to store unused microtiter strips.
10. Insert

Materials required but not provided

Instrumentation

1. 50, and 100 µL-micropipettes
2. ELISA reader (450 nm)
3. Centrifuge
4. Ultra-Turrax, mixer, vortex

Reagents

1. Methanol
2. Hexan (Spices only)
3. Double-distilled water

Sample Preparation

Solid Samples (Cereals, Nuts, Dry Fruit)

- Grind sample to pass through a 20-mesh sieve and thoroughly 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 100 µL of filtrate/supernatant with 600 µL of sample diluent and test the sample in the ELISA.
  Dilution factor = 35

Spices

- Grind sample to pass through a 20-mesh sieve and thoroughly 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.
- To 1 mL of filtrate add 2 mL of Hexan and mix for 5 min, then separate the upper hexan layer.
- Dilute 100 µL of the lower layer with 600 µL of sample diluent and test the sample in the ELISA.
  Dilution factor = 35

Liquid Samples (Beer)

- Depending on the number of samples dilute an adequate volume of sample diluent with 10% methanol.
- Carbonized samples should be preliminarily de-gassed by moderate heating.
- Cloudy samples (such as beer brewed from wheat) / gyle should preliminarily be sterile-filtered.
- Dilute 100 µL sample with 900 µL sample diluents/methanol dilution and test the diluted sample in the ELISA.
  Dilution factor = 10

Oil Samples

- Dilute 20 mL of oil with 100 mL of 70% methanol and mix for 5 min in a separating funnel.
- Discard the oil layer.
- Dilute 100 µL of the methanol layer with 600 µL of sample diluent and test the sample in the ELISA.
  Dilution factor = 35

In case of too highly concentrated samples, an adequate volume of sample diluent is diluted with methanol to a concentration of 10% methanol. The sample extracts have to be further diluted with this dilution.

PRECAUTION

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 (micro pipets, ELISA reader etc.).

Health and safety instructions

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

ASSAY PROCEDURE

1. Prepare samples as described above.
2. Pipet 100 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 anti-aflatoxin antibody into each well.
5. Incubate for 30 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 absorbances.
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 15 minutes at room temperature.
9. 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.
10. After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

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 ng/mL 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-para-meter method should be preferred.
3. The diluted samples must be further converted by the appropriate sample dilution factor for calculating the sample concentration in ppb. The factors for each sample matrix are listed in the sample preparation section.

Examples:

A wheat sample prepared as described above results in 0.2 ng/mL. The concentration of the sample is calculated as follows:

$$C_{sample} = 0.2 \text{ (ng/mL)} * 35 \text{ (ppb*ml/ng)} = 7 \text{ ppb}$$

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.

Aflotoxin (ng/mL)	(% binding of 0 ng/mL)
0	100
0.05	74
0.1	63
0.25	32
0.5	21
1.5	6

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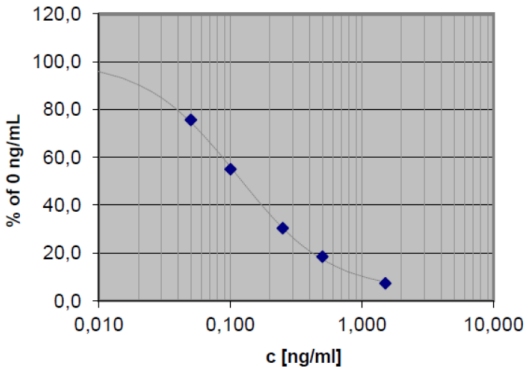

PERFORMANCE CHARACTERISTICS

Sensitivity
The limit of detection (LOD) of the DAI Aflatoxin Total ELISA is 0.015 ng/mL for the standard curve.
The limit of quantification (LOQ) of the DAI Aflatoxin Total ELISA is 0.046 ng/mL for the standard curve.
Validation experiments with common matrices result-ed in the following LODs and LOQs [ppb].

Matrix	LOD	LOQ
Wheat	0.9	1.9
Rye	0.8	1.8
Barley	0.7	2.1
Oats	0.8	1.7
Sorghum	1.3	2.0
Rice	0.9	2.3
Corn	1.1	2.5
Beer	0.4	0.7
Hazelnut	1.4	2.6
Peanut	0.9	3.0
Almond	1.2	2.4
Dry Fruit - Sultana	2.8	3.9
Dry Fruit - Fig	1.2	2.4
Chili	3.0	4.4
Oil	0.4	1.4

Recovery

Wheat	99%
Rye	92%
Barley	92%
Oats	85%
Sorghum	101%
Rice	102%
Corn	92%
Beer	101%
Hazelnut	87%
Peanut	101%
Almond	93%
Dry Fruit - Sultana	103%
Dry Fruit - Fig	114%
Chili	92%
Oil	121%

Linearity
The serial dilution of spiked samples (as seen in the table above) resulted in a dilution linearity of 91-119%.

Precision
The intra-assay precision is 4.7%; the intra-assay precision is 6.6%.

Reactivity

Aflatoxin B_1	100%
Aflatoxin B_2	73%
Aflatoxin G_1	74%
Aflatoxin G_2	49%
Aflatoxin M_1	60%

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ISO 13485
ISO 9001

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