# Product information

User's Manual

Distribuito in ITALIA da
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# Aflatoxin B1 Rapid ELISA

Enzyme Immunoassay for the rapid quantitative determination of Aflatoxin B1 in cereals and beer / gyle

REF **DEAB1E03**

Σ **96**

Version 150217
DMC
Updated 150420

| | |
|---|---|
| Sensitivity | 0.5 – 1.4 ppb |
| Recovery (spiked samples) | 86 - 114% |
| Incubation Time | 20 min |

**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 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.

The **Demeditec Aflatoxin B1 RAPID ELISA** represents a highly sensitive detection system and is particulary capable of the rapid quantification of aflatoxin B1 contaminations in cereals and beer.

**Principle of the Test**

The **Demeditec Aflatoxin B1 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 B1 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 aflatoxin B1 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 10 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 10 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. 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).

## 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 B1 Standards (0; 1.5; 3; 6; 12; 24 ppb): 6 vials with 1 mL each, dyed red, ready-to-use. Because of the total dilution of 1:10 of the cereal 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 (rabbit): 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. Instruction Manual.

## 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

## Sample Preparation

### Cereals

- 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 sample diluent and test the sample in the ELI-SA.

### Beer / Gyle

- Dilute an adequate volume of sample diluent with 35% methanol.
- 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 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..

**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 the anti-aflatoxin antibody into each well.
5. Incubate for 10 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 10 minutes at room temperature.
9. Stop enzyme reaction by adding 100 µL of stop solution (1 N acidic solution) 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.

**Calculation of results**

The ready-to-use standards are prepared for a direct determination of cereal 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.

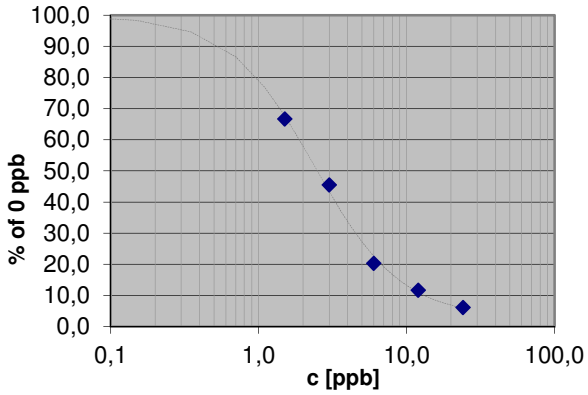
2. Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ppb on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis. Alternatively the evaluation can be carried 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 B1 in ppb from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be 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 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 B1 (ppb) | (% binding of 0 ppb) |
|---|---|
| 0 | 100 |
| 1.5 | 82 |
| 3 | 61 |
| 6 | 46 |
| 12 | 24 |
| 24 | 11 |




**Performance**

**Sensitivity**

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

Validation experiments with common matrices resulted in the following LODs [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 RAPID test** 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.

**Recovery**

| | |
|---|---|
| Wheat flour | 104% |
| Oats flour | 86% |
| Rye flour | 103% |
| Barley flour | 98% |
| Rice flour | 91% |
| Corn flour | 94% |
| Beer | 114% |

**Linearity**

The serial dilution of spiked samples (wheat, barley, rye, oats, rice, corn and beer) resulted in a dilution linearity of 82-115%.

**Precision**

| | |
|---|---|
| Intra-assay Precision | 3-6% |
| Inter-assay Precision | 5-11% |

**Cross-reactivity relative to Aflatoxin B1 (=100%)**

| | |
|---|---|
| Aflatoxin B2 | 29% |
| Aflatoxin G1 | 44% |
| Aflatoxin G2 | 5% |
| Aflatoxin M1 | 2% |

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