Note that this data sheet is not lot-specific. Please consult the vial label and the certificate of analysis for information on specific lots.

B-TeZ ELISA AFLA B1 Kit

Catalogue Number: BTAFEK-001 Package Size: 1 kit (96 wells)

1. Brief Description

The B-TeZ ELISA Aflatoxin B1 Kit is a competitive enzyme immunoassay for quantitatively detecting Aflatoxin B1 (AFLA B1) in food and feed crop (cereals, nuts, etc.). After a sample preparation to isolate the Aflatoxins, a maximum of 42 samples in duplicate can be tested in 45 minutes with the B-TeZ ELISA.

2. Kit components

1 Microplate (12 strips with 8 wells) coated with Anti-Aflatoxin-Antibody, 1 Sample dilution buffer, 6 calibrators for Aflatoxin B1 (AFB1 standards: 0, 0.05, 0.10, 0.25, 0.50, 1.20 ng/ml), 1 AFB1-HRP conjugate, 1 Wash solution, 10-fold concentrate, TMB reagent, Stopping solution (1M H_2SO_4)

3. Other materials and equipment required

Homogenizer, electronic balance, micropipettes, distilled water, vortexer, microplate shaker, washing device for microplates, microplate reader (450 nm / 620 nm)

4. Sample Preparation

Aflatoxins are extracted from a representative ground food or feed sample by shaking with a methanol/water mixture (70/30 v/v). The liquid and solid phases are separated by subsequent filtration. The filtrate must be diluted prior to testing in the ELISA with sample dilution buffer. The dilution factor for samples is 50.

5. Principle of the assay

The B-TeZ ELISA Aflatoxin B1 is a competitive enzyme immunoassay. The assay is carried out in a microplate, of which the cavities have already been pre-coated with a special anti-Aflatoxin antibody. To carry out the assay, Aflatoxin B1 (AFB1) standards and sample extracts respectively and Aflatoxin B1 peroxidase conjugate (AFB1-HRP conjugate) are pipetted into the cavities. The microplate is incubated for 30 minutes at room temperature.
Aflatoxin from the standard or sample now competes with the HRP labelled Aflatoxin on the antibody binding sites. The unbound components are removed by rinsing them off and then peroxidase substrate solution (3,3',5,5'-Tetramethylbenzidine, TMB) is pipetted into all cavities. The AFB1-HRP conjugate that is bound to the antibody reacts with the substrate solution by forming a blue colour. After 15 minutes, this reaction is terminated by adding the stop solution. The colour then changes from blue to yellow, which is detected photometrically. With the help of a microplate reader, the yellow colouring is measured as an optical density (OD) at a wavelength of 450nm (reference value 620nm).
The Aflatoxin B1 concentration is inversely proportional to the colour intensity. The higher the OD measurement, the lower is the concentration of Aflatoxin B1 in the standard or sample.

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6. Reaction scheme

1. Reaction step — *Incubation of AFLA B1-Standard 0-5 (0-1.2 ng/ml), diluted samples and the detection conjugate AFB1-HRP-Conjugate; each 50µl/well Incubation time: 30 min at 20-25°C*

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Washing step — 3 times *with 1:10 diluted Wash solution, 10-fold concentrate*

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2. Reaction step — *Enzymatic colour reaction with Tetramethylbenzidin for 15 min at 20-25°C*

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Stopping step — *Addition of stop reagent*

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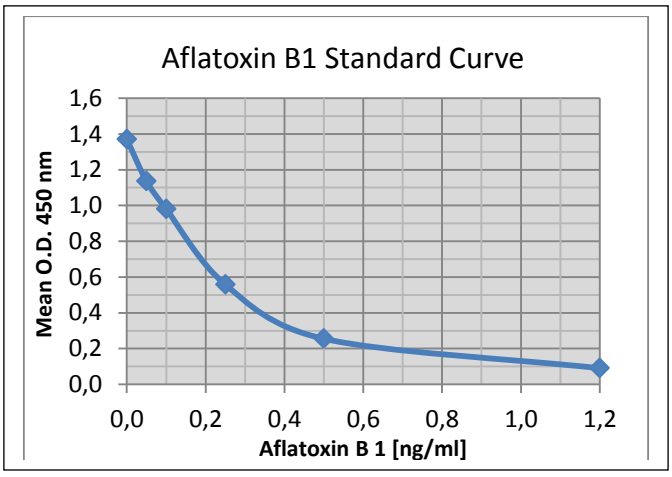
Measurement at 450 nm

7. Example of standard value and standard curve

AFLA B1 [ng/ml]	Mean O.D.	CV [%]	% O.D. [%]
0	1.371	2.1	100.0
0.05	1.135	2.9	82.8
0.1	0.980	2.6	70.5
0.25	0.558	1.8	40.7
0.5	0.255	2.8	18.6
1.2	0.091	2.3	6.6

Note: These values are only an example. The standard value of AFLA B1-Standards has to be measured in each test.



8. Test evaluation

The evaluation can be performed using commercial ELISA programs or Logit/Log. Using the mean optical density of the sample. the corresponding concentration of AFLA B1 [ng/mL] can be determined in a measured. diluted sample extract from the AFLA B1 standard curve. The recalculated AFLA B1 values must be further converted by the appropriate sample dilution factor.

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9. Assay Parameter

- Test format: microplate (12 strips with 8 wells)
- AFLA B1 standard range: 0. 0.05. 0.15. 0.25. 0.50. 1.20 ng/ml Aflatoxin B1 (for the calculation of diluted samples)
- Sample: food and feed
- Sample preparation: methanol/water (70/30 v/v) extraction
- Dilution factor: 50
- Incubation time: 45 min (30+15 min)
- Detection limit in matrices: ≤2 ppb
- Recovery rate: 80 – 110 %
- Cross reactivity: 100% Aflatoxin B1. 63% Aflatoxin B2. 65% Aflatoxin G1 19% Aflatoxin G2. 7% Aflatoxin M1
- Intra assay variation: ≤4%
- Inter assay variation: ≤6%
- Storage: 2-8°C
- Shelf Life: 12 month under storage conditions

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