



Aflatoxin B1 ELISA Assay Kit

(For Research Use Only)

Instructions

**Competitive enzyme immunoassay for the
quantitative detection of Aflatoxin B1**

Catalog number: BTAFEK-001

BioTeZ Berlin-Buch GmbH
Robert-Rössle-Str. 10
13125 Berlin, Germany
Tel.: +49 (0) 30 9489 2130
Fax: +49 (0) 30 949 4509
E-mail: info@biotez.de
www.biotez.de



Aflatoxin B1 ELISA Assay Kit

Competitive enzyme immunoassay for quantitative detection of Aflatoxin B1 in food and feed.

For research use only

96 Test cavities

Assay time: 30+15 min

Detection limit (matrices): ≤ 2 ppb

Recovery rate: 80 – 110 %

Cross reactivity:

Aflatoxin B1: 100 %

Aflatoxin B2: 63 %

Aflatoxin G1: 65 %

Aflatoxin G2: 19 %

Aflatoxin M1: 7 %

Storage: 2 – 8 °C

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Please read the directions for use carefully before carrying out the test!



Introduction

Aflatoxins are a type of mycotoxin (toxic mould fungus). They are formed as poisonous metabolites from the *Aspergillus* (A.) species, such as *A. flavus*, *A. parasiticus*, and *A. nomius*.

Aflatoxins enter into the human food chain via contamination of plant-based foods. Grains and oil-containing seeds and nuts, such as corn, rice, peanuts, pistachios, sesame seeds, cotton seeds, dried fruits, spices, and cocoa beans are particularly affected. Climate conditions have a big influence on contamination. *Aspergillus* species are thus spread particularly in humid regions. Unfavourable harvesting and storage conditions can lead to increased Aflatoxin exposure.

Aflatoxins can cause chronic and acute cases of poisoning. Aflatoxin B1 exhibits the highest toxicity of the discovered Aflatoxins and it is one of the strongest of all the mycotoxins. It has a strong genotoxic and carcinogenic effect, to which the liver is particularly susceptible. In addition to Aflatoxin B1, Aflatoxins G1, B2, and G2 are also amongst important fungi of the group that often occur together. M1 and M2 derivatives which are often found in milk are also important.

To protect consumers from illnesses caused by Aflatoxins, there are statutory limits in the European Union for Aflatoxin B1 (2 ppb), as well as for the overall amount of Aflatoxins (4 ppb).

Purpose of use

The Aflatoxin B1 ELISA Assay Kit allows the quantitative determination of Aflatoxins in food and feed crop (cereals, nuts, etc.). For further applications the user is encouraged to verify the validity of the tests themselves or contact the manufacturer.

The test is easy to use, requires no complex instrumental equipment, and delivers the sensitive results quickly. After a sample preparation to isolate the Aflatoxins, a maximum of 42 samples in duplicate can be tested in 45 minutes with the Aflatoxin B1 ELISA Assay Kit. The sample extraction with methanol/water is described in the following. Alternatively, Aflatoxins can also be isolated with the help of immunoaffinity chromatography.

Principle of the method












The Aflatoxin B1 ELISA Assay Kit 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. Aflatoxin from the standard or sample now competes with the HRP labelled Aflatoxin for the antibody binding sites. After an incubation period of 30 minutes, 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 concentration is inversely proportional to the colour intensity. The higher the OD measurement, the lower is the concentration of Aflatoxin in the standard or sample.



Kit components

No.	Components	Code	Amount	Colour of the top cap	Condition *
01	Microplate; coated with anti-Aflatoxin-antibody	AFL-PLATE	12 Strips á 8 well	-	G
02	Sample dilution buffer	AFB-SAMPLE-BUF	2 x 30.0 mL	white 	G
03	AFB1-Standard 0 (0 ng/mL)	AFB1-0	1 x 1.0 mL	red 	G
04	AFB1-Standard 1 (0.05 ng/mL)	AFB1-1	1 x 1.0 mL	red 	G
05	AFB1-Standard 2 (0.10 ng/mL)	AFB1-2	1 x 1.0 mL	red 	G
06	AFB1-Standard 3 (0.25 ng/mL)	AFB1-3	1 x 1.0 mL	red 	G
07	AFB1-Standard 4 (0.50 ng/mL)	AFB1-4	1 x 1.0 mL	red 	G
08	AFB1-Standard 5 (1.20 ng/mL)	AFB1-5	1 x 1.0 mL	red 	G
09	AFB1-HRP-Conjugat	AFB-HRP	1 x 6.0 mL	green 	G
10	Substrate solution (TMB)	TMB	1 x 12.0 mL	brown 	G
11	Stop solution (1M H ₂ SO ₄)	STOP-H ₂ SO ₄	1 x 4.0 mL	white 	G
12	Wash solution, 10-fold concentrate	WASH-10x	1 x 30.0 mL	white 	R
13	Adhesive foil for microplate	-	1 piece	-	G
14	Instructions	-	1 piece	-	-

* Condition: G = ready for use; R = reconstitution required

Additional equipment, not included in delivery

Micropipettes with their corresponding tips (range of 10-100 µL, 100-1000 µL)

Mortar or laboratory mill

Laboratory scales

Erlenmeyer flask or plastic centrifuge tube

100mL measuring cylinder

Funnel

Stand

Filter (e.g. Whatman No. 1)

Collection containers for sample extracts

Containers for diluted sample extracts, e.g. 1mL volume

Microplate shaker

Microplate washer or multi-channel pipette for washing

Microplate reader (450 nm / 620 nm)

Methanol

Distilled water



Storage and shelf life

The Aflatoxin B1 ELISA Assay Kit has to be stored at 2-8°C (Do not freeze!). After the expiry date has passed, the quality of the kits can no longer be guaranteed.

With a lower number of samples, individual microplate strips can also be used. The remaining strips must be put back in the foil bag with desiccant and securely closed for further storage.

Opened reagents must be used within 6 weeks and stored at 2-8°C in the meantime.

The substrate solution can no longer be used if the normally colourless solution shows a markedly blue colour. Another indication of the deterioration of the reagents would be very little or no staining at all in the cavities of maximum binding (AFB1-Standard 0).

Special health information

The Aflatoxin B1 ELISA Assay Kit contains the toxic Aflatoxin B1. It is present as a solution in the concentration range of 0.05 – 1.2 ppb (0.05 – 1.20 ng/mL) in the standards (see Kit Components, AFB1-1 to AFB1-5). The standard solutions also contain 7 % methanol. To prevent inhalation of toxin- or methanol-containing aerosols, these containers must only be opened under a fume hood and pipetted there on the microplate. Contact with skin must also be avoided. Therefore protective gloves and laboratory suits are recommended.

Solutions and materials containing Aflatoxin must be disposed of or cleaned professionally. For decontamination of the solutions and glass containers containing Aflatoxin a sodium hypochlorite solution (10 % (v/v), pH = 7) is recommended, which should take effect overnight.

The stop solution (see Kit Components, STOP-H₂SO₄) contains 1M of sulphuric acid. Avoid contact with skin and mucus membranes. If you come into contact with it, wash it off with plenty of water. Protective gloves must be worn!

General information for the test procedure

- Kit Components from different lots should not be mixed.
- The reagents should be warmed to room temperature (20-25°C) before beginning the test.
- Neither the reagents nor the microplate may be exposed to direct sunlight.
- The substrate solution (TMB) must always be kept in the dark.
- All reagents have to be mixed carefully before use. Avoid foaming!
- Components that are not delivered ready for use must be freshly prepared before use. This concerns the washing solution that is included in the kit as a 10-fold concentrate (WASH-10x).
- Samples should always be tested at the same time as the standards. The standards should be included in each test run to check the quality of the results.
- The interior of the cavities should not be touched while pipetting to ensure the coating is not damaged.
- To avoid cross-contamination, a new pipette tip must be used for each sample. The tips should be pre-saturated with the solution to be pipetted.
- Close the kit reagent containers immediately after use. Screw caps should not be mixed up.
- All cavities of the microplate strips should be uniformly washed at the same time before the colour reaction. The washing process is critical. Insufficient washing can lead to inaccurate results.
- Do not allow the microplate to dry after washing.
- The test should be carried out as per the manufacturer's instructions. The specified times must be adhered to. Work should be carried out quickly.



- Check the precision and accuracy of the laboratory equipment that you use during the process.

Sample preparation

- Solid samples should be present in crushed, homogeneous form as a fine to medium-fine powder. If necessary, the solid sample must be crushed in a mortar or with a laboratory mill.
- 5g of the crushed, homogeneous sample is weighed in a suitable container, such as an Erlenmeyer flask or a plastic centrifuge tube and mixed with 25mL of methanol/water (70/30 v/v). This suspension is shaken intensively for 3 minutes to extract the Aflatoxin. The suspension is then filtered via a folded filter for quantitative analysis. It is recommended to let the solids shortly settle before filtering.
- The filtrate (sample extract) is diluted in a new container with a 1:10 ratio with the sample diluent (see Kit Components, AFB-SAMPLE-BUF):

1 part filtrate + 9 parts AFB-SAMPLE-BUF, e.g. 100 µL filtrate + 900 µL AFB-SAMPLE-BUF
- The diluted filtrate can now be tested with the Aflatoxin B1 ELISA Assay Kit. 1 mL of diluted filtrate equals 0.1 g solid sample = dilution factor 50.
- Note: if higher Aflatoxin concentrations are expected, then the filtrate must be diluted at a higher ratio than 1:10, while the ratio of the methanol/water mixture (70/30, v/v) must be maintained.

Test procedure

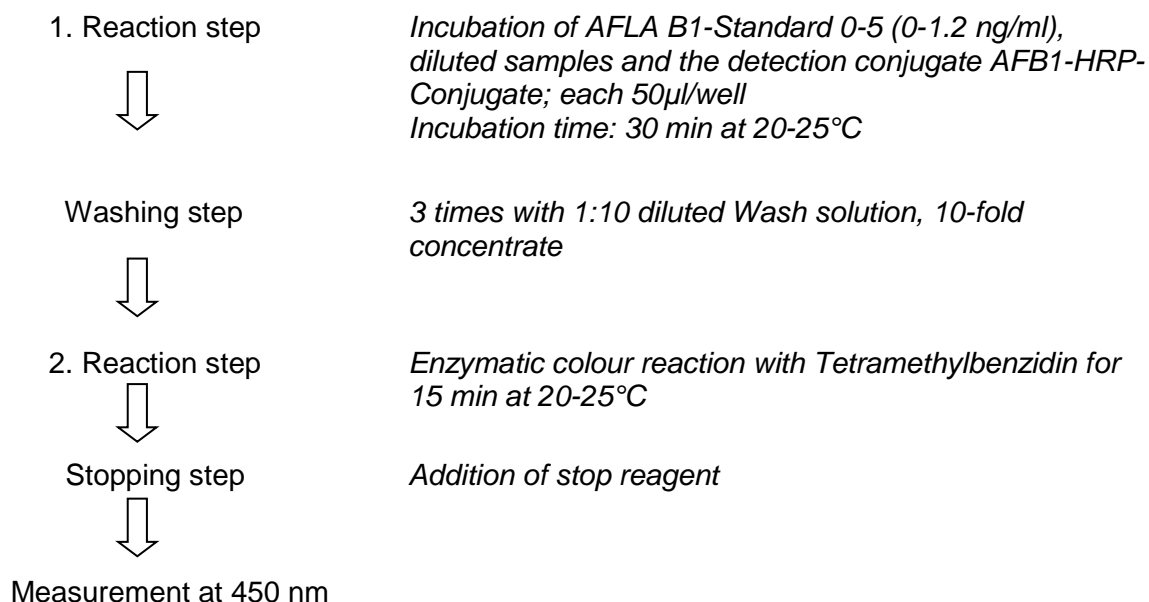
Note: It is recommended to test both the standards and the samples in duplicate and further, that no more than 18 samples and 6 standards (maximum of 6 strips) are measured in one run.

- The microplate (AFL-PLATE) is already ready for use; it must only be taken from the foil bag and can be used immediately without washing.
- The washing solution comes in a 10-fold concentration (WASH-10x) and must be diluted with distilled or deionised water: fill 25mL WASH-10x with 225mL of water at a volume of 250mL.
- We recommend testing both the standard and the samples in duplicate. Generally, standards and samples are pipetted first. To finish, Aflatoxin B1 peroxidase conjugate is added.
- 50 µL of the standards (AFB1-0 to AFB1-5) and the prepared sample extract are pipetted into the corresponding cavities.
- Finally, the 50µl AFB1-HRP conjugate (AFB-HRP) is pipetted into all cavities.
- The microplate is now covered with adhesive foil and briefly shaken on the microplate shaker and incubated for 30 minutes at room temperature, protected from light.
- All cavities are aspirated or shaken out for washing. Then 300 µL of reconstituted washing solution is pipetted into each cavity and again aspirated out. This process is repeated twice.
- 100 µL of substrate solution (TMB) is then pipetted into all cavities for the colour reaction.
- Cover the microplate with adhesive foil again, briefly shake it and leave it to incubate for 15 minutes at room temperature, protected from light.



- To stop the process, 25 µL of stop solution (STOP-H₂SO₄) is pipetted into each cavity.
- The OD value measurement of the 96 cavities is undertaken by using a microplate reader at 450nm (reference wave length 620nm).

Reaction scheme



Test evaluation

- First, the mean values of all duplicate results for the optical density (O.D. 450nm) are calculated.
- The results can be evaluated easily and quickly by creating a standard curve. For this, the mean optical density of the standards is plotted against their concentration [ng/mL] semi-logarithmic. (x-axis: log Aflatoxin B1 [ng/mL]; y-axis: mean optical density).
- Using the mean optical density of the sample, the corresponding concentration of Aflatoxin [ng/mL] can be determined in a measured, diluted sample extract from this standard curve. The content of the sample is calculated in consideration of the factors for the sample dilution. When using the above-described sample preparation, the dilution factor is 50. Thus the Aflatoxin B1 concentration from the sample is obtained in ppb [ng/g or µg/kg].
- A Logit/log evaluation is also an option. For this procedure, the values for the mean optical density of the standards and samples are divided by the mean value of the zero standard (AFB1-0). Multiplying by 100 then gives an O.D. value percentage in reference to the zero standard (100%):

$$\frac{\text{O. D. (Standard or Sample)}}{\text{O. D. Standard 0}} \times 100 = \% \text{ O. D.}$$

- The Logit/log evaluation takes place by using the O.D. value percentage as well as the logarithmic concentration value. This returns a linear standard curve. Implementing a linear regression is thus an option.
- Is it possible to perform a non-linear regression, the following function is proposed:



$$Y = pr1/(1+\exp(-pr2*X1))+pr3.$$

Example of standard value and standard curve

AFLA B1 [ng/ml]	Mean O.D.	CV [%]	% O.D. [%]
0.0	1.371	2.1	100.0
0.05	1.135	2.9	82.8
0.10	0.980	2.6	70.5
0.25	0.558	1.8	40.7
0.50	0.255	2.8	18.6
1.20	0.091	2.3	6.6

Table 2: Example of standard value with AFLA B1 Standard 0-5

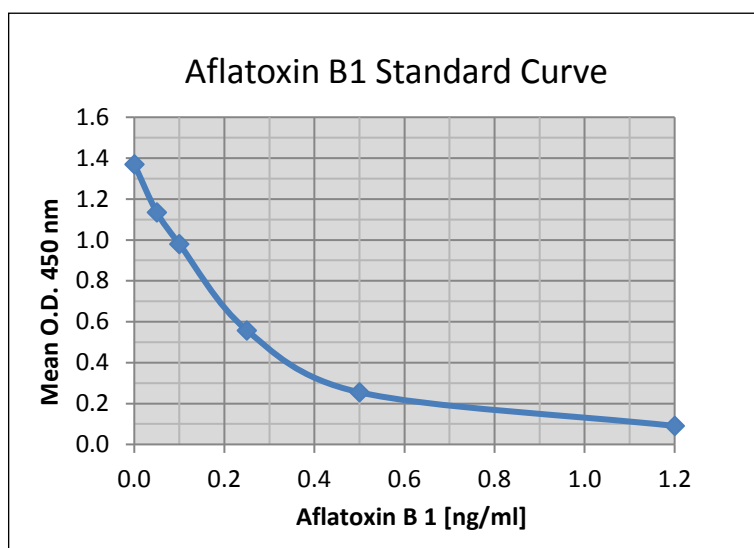


Fig. 1: Example of standard curve with AFLA B1 Standard 0-5

Note: The standards must be run with each test.

Performance features of the method

Accuracy

The limit of detection (LOD) of the Aflatoxin B1 ELISA Assay Kit is ≤ 2 ppb in matrices. Different commodities can have an influence on the LOD due to their "matrix effects". As a result, the LOD may be dependent on the matrix and should be measured for each different commodity.

Recovery

The recovery of spiked samples was found to be 80% to 110%

Linearity

The assay should be carried out with a filtrate dilution of 1:10. With dilutions of 1:20 to 1:40, the linearity of the assay results is given as their dilution.



Intra-Assay Accuracy

The intra-assay variation of the Aflatoxin B1 test has been determined as $\leq 4\%$.

Inter-Assay Accuracy

The inter-assay variation of the Aflatoxin B1 test has been determined as $\leq 6\%$.

Specificity

Specificity studies have shown the following cross-reactivity:

Aflatoxin B1:	100 %
Aflatoxin B2:	63 %
Aflatoxin G1:	65 %
Aflatoxin G2:	19 %
Aflatoxin M1:	7 %

Table 3: cross-reactivity





BIOTEZ

Manufactured by:

BioTez Berlin-Buch GmbH
Robert-Rössle-Str. 10, 13125 Berlin Germany
Phone +49 30 9489-2130 • Fax +49 30 9494-509
e-mail info@biotez.de • www.biotez.de

distributed in the US/Canada by:



EAGLE
BIOSCIENCES

EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 • FAX: 617-419-1110
WWW.EAGLEBIO.COM • info@eaglebio.com

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