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Product no AS20 4397

Aflatoxin B1 | ELISA quantitation kit

Background

This ELISA assay utilizes the principle of competitive binding to measure the concentration of Aflatoxin B1 in analyzed samples. The Aflatoxin B1 has been pre-coated on the surface of the reaction wells. Samples, containing an unknown amount of Aflatoxin B1, or standards are added to the appropriate plate wells together with Aflatoxin B1 specific antibodies and Horseradish Peroxidase (HRP) conjugated antibody. During incubation, the competitive inhibition reaction occurs between pre-coated Aflatoxin B and Aflatoxin B1 in standards and samples and Aflatoxin B1 antibodies. Substrate solution is added to the wells and the color develops in opposite to the amount of Aflatoxin B1 in the sample or standards. Reaction is stopped and the intensity of the color is measured at 450 nm.

Aflatoxins are toxic metabolites of fungaus *Aspergillus flavus* and *Aspergillus parasiticus* and display strong carcinogenic activity (Category I cardinogen). Aflatoxins are commonly found in cereal, nuts, cotton seed, human blood and animal feed and contamination may occur in the field, during growing, harvest or processing, storage and transport. Therefore, timely detection of pollution source is a good way to prevent aflatoxin contamination.

The Aflatoxin M1 ELISA kit reagents

Assay plate	1 (96 wells)
Standard	5 x 1 ml
HRP-conjugate	1 x 7 ml
Antibody	1 x 7 ml
TMB Substrate	1 x 12 ml
Stop Solution	1 x 10 ml
Sample Diluent	1 x 50 ml
Wash Buffer (10x concentrate)	1 x 30 ml

Sensitivity: ≥ 1 5 µg/kg
Detection range: 0.15-4.05 µg/kg
Recovery rate: Edible oil: 80-120%, feedstuff/feed/grain: 70-100%
Limit of detection: Edible oil: 1 µg/kg, feedstuff/feed/grain: 2 µg/kg
Detection wavelength: 450 nm
Intra-assay precision (within assay): CV%<10%
Intra-assay precision (between assays): CV%<10%
Storage: 2-8°C
Cross-reactivity: Aflatoxin B1 100%
Sample type: edible oil, feedstuff, feed, grain

Not provided in the kit but required to conduct the test

Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm **|** Incubator with stable incubation temperature up to 25°C **|** Squirt bottle, manifold dispenser or automated microplate washer **|** Centrifuge, vortex mixer **|** Analytical balance, 2 decimal place **|** Single-channel micropipette (20-200 µl, 100-1000 µl, 1000-5000 µl) **|** Multi-channel micropipette (300 µl) **|** Deionized or distilled water **|** Pipette and pipette tips **|** Methanol **|** Ethanol **|** NaOH

Important notes

The kit should not be used beyond the expiration date on the kit label. Reagents from different lots should not be mixed. In case analyzed samples are generating values which are higher than the highest standard, samples should be diluted, and the assay repeated. The assay may vary due to factors such as operator, pipetting technique, washing technique, incubation time, temperature, and kit expiry date. This assay is designed to eliminate the interference caused by soluble receptors, binding proteins, and other components present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

Samples: Samples should be added very carefully to avoid foaming and touching the well wall. For each step in the procedure, the total dispensing time for the addition of reagents or samples to the assay plate should not exceed 10 min. Cross contamination should be avoided by changing pipette tips between additions of each standard, sample and reagent.

Incubation: Proper adhesion of plate sealers during each incubation step must be applied to ensure accurate results. Strips should not be let dry at any time. Incubation temperature and time must be kept constant with the optimal temperature being 25°C.

Washing: This step is critical, and the complete removal of liquid at each step is essential for a good performance of this assay. Any drops and fingerprints must be removed from a bottom of the plate. Insufficient washing will result in poor precision. In case of automated washing, addition of a 30 s soak period, following the addition of wash buffer and plate rotation by 180 degrees between each wash step, may improve assay precision.

Reaction development: Substrate should change from colorless or light blue to gradations of blue. In case the color is too intense Stop Solution should be added. Substrate solution can be easily contaminated. The liquid should remain colorless or light blue until added to the plate. Keep this solution away from any light.

Stop solution: The reaction is developed correctly if color development in the wells is from blue to yellow upon addition of the Stop Solution. Green color indicates that the Stop Solution was not thoroughly mixed with the substrate.

Safety measures

The Aflatoxin B1 ELISA quantitation kit Stop Solution is an acidic solution. Please use suitable protective gear for your eyes, hands, face, and clothes when handling this item.

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Procedure instructions

Please read the whole manual carefully before proceeding with your experiment.

Before starting

As a recommendation, all samples and standards should be made in duplicates for this assay. Prepared samples can be stored at 2-8°C for up to one day. The sample concentration needs to be estimated before the assay. Do not exceed handling time of 10 minutes per step. Always use a plate cover during all steps. Do not let the strips dry at any time during the assay.

70 % Ethanol: transfer 700 ml of ethanol into 300 ml of deionized or distilled water, mix well.
40 % Ethanol: transfer 400 ml of ethanol into 600 ml of deionized or distilled water, mix well.

Sample preparations

Edible oil

Add 25 ml of Ethanol (70%) to 5.00 ± 0.05 g of edible oil. Vortex for 2 min, and then centrifuge for 5 min at 4000 rpm. Move 100 µl of the supernatant into a clean centrifugal tube. Add 100 µl of Sample Diluent and shake well. Use 50 µl of the sample for further analysis. Dilution factor of the sample: 10.

Feedstuff/feed/grain

Add 25 ml of Ethanol (40%) to 5.00 ± 0.05 g of homogenized sample and vortex for 2 min. Centrifuge for 5 min at 4000 rpm. Move 200 µl of the supernatant into a clean centrifugal tube. Add 600 µl of Sample Diluent and shake well. Use 50 µl of the sample for further analysis. Dilution factor of the sample: 20. If the pH is below 6, adjust the pH to 6-7 using NaOH.

Prepared samples can be stored for up to one day at 2-8°C.

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Manual

1. Bring all reagents and samples to room temperature before use (keep in RT for at least 30 min).

2. Centrifuge the samples.

3. Prepare the reagents and samples.

    a) Prepare the Washing Buffer (1x). In case crystals have formed, warm the concentrate to room temperature and mix gently until the crystals have dissolved. Prepare 100 ml of Wash Buffer (1x) by mixing 10 ml of Washing Buffer Concentrate (10x) with 90 ml of deionized or distilled water.

    b) Prepare the Standards. Centrifuge the standard vials at 6000-10000 rpm for 30 s. The most concentrated standard, S5, is at 4.05 µg/kg, while the zero standard, S0, is at 0 µg/kg.

Tube	S1	S2	S3	S4	S5
µg/kg	0	0.15	0.45	1.35	4.05

4. Determine the number of wells to be used. Unused wells should be put back into the Ziplock pouch and stored at 4°C.

5. Add 50 µl of standard or sample to each well. Add samples gently to avoid foaming and be careful not to touch the well walls. Add 50 µl of HRP-conjugate and 50 µl of Antibody to each well. Incubate the microtiter plate at 25°C for 15 min, covered by an unused adhesive strip.

6. Aspirate the wells and wash the plate by filling the wells with Wash Buffer (1x, 250 µl in each well) using a squirt bottle, multi-channel pipette or auto-washer. Let it stand for 30 s, remove the liquid, and repeat the washing process four times for a total of five washes. After the fifth wash, remove all liquid and invert the plate against clean paper towels. Remove water and fingerprint on the bottom of the plate to avoid falsely reading results. The wash procedure is critical. Complete removal of liquid at each step is essential.

7. Add 100 µl of TMB Substrate to each well. Mix well and incubate at 25°C for 5 min, protected from light.

8. Add 50 µl of Stop Solution to each well in the same order as the substrates and mix thoroughly. The color will change from blue to yellow.

9. Read the optical density of each well within 5 minutes, at 450 nm. OD value should be read at the dual wavelength of 450/630 nm within 5 minutes.

Sample	µg/kg	Average
S4	4.05	0.259
S3	1.35	0.711
S2	0.45	1.248
S1	0.15	1.679
S0	0	1.926

Example of Aflatoxin B1 standard curve. Note that developed color is inversely proportional to the amount of Aflatoxin B1 in the sample.

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Results

There are two methods to judge the results: the first one (A) is the rough judgment, while the second (B) is the quantitative determination.

Note that the OD value of the sample has a negative correlation with Aflatoxin B1 in the sample.

A: Compare the sample average absorbance values with standards values, the Aflatoxin B1 concentration in the samples can be concluded. For example, the absorbance value of sample 1 is 0.653, the absorbance value of sample 2 is 1.419; absorbance values of standard are: 1.926, 1.679, 1.248, 0.711, 0.259 and the corresponding concentrations are: 0 µg/kg, 0.15 µg/kg, 0.45 µg/kg, 1.35 µg/kg and 4.05 µg/kg, then the Aflatoxin B1 in sample 1 and sample 2 are 1.35-4.05 µg/kg and 0.15-0.45 µg/kg. Lastly, the reader is multiplied by the corresponding dilution factor of each sample and the actual concentration of sample is obtained.

B: The software offered together will facilitate the calculation process, it is suitable for accurate and fast analysis of large-scale samples. Please contact us for more information. Note: Discard substrate with any color that indicates the degeneration of this solution. When the absorbance value of standard solution 0 is less than 0.5, this indicates its degeneration. The optimum reaction temperature is 25°C. A temperature that is too high or too low will result in changes in the absorbance value and detecting sensitivity.

Related products

Cis-zeatin riboside | Cytokinin ELISA quantitation kit

AS12 1844| Reactivity: Cis-zeatin riboside

IAA | Auxin ELISA quantitation kit

AS11 1749| Reactivity: IAA-indole-3-acetic acid (C1')

N6-benzyladenosine | Cytokinin ELISA quantitation kit

AS12 1846| Reactivity: N6-benzyladenosine