Total Aflatoxin ELISA Kit

Cat. #: T21131 (1 x 96-well plate)

Ship and Storage: ship and store at 4°C **Shelf Life:** 12 months

Product Description

Aflatoxins are a class of structurally similar mycotoxins produced by Aspergillus species, with the most of total aflatoxinble being Aspergillus flavus. Currently, 17 different types of aflatoxins have been discovered, including aflatoxins B1 and B2 (produced by A. flavus and A. parasiticus), aflatoxins G1 and G2 (produced by A. parasiticus), and aflatoxins M1 and M2, metabolites of B1 and B2 respectively. Total Aflatoxin, the most common form of aflatoxin originated in naturally contaminated food, has acute toxicity to animals and has been classified as a Group 1 carcinogen by IARC of World Health Organization.

This 101Bio Total Aflatoxin ELISA Kit can **quickly, sensitively, and accurately** determine the presence of total aflatoxin in animal feed and tissue samples of animals, providing a vital tool to prevent consumption of food tainted with this toxic chemical.

Intended Use

The Total Aflatoxin ELISA Kit utilizes competitive ELISA for the quantitative and qualitative analysis of Total Aflatoxin in animal feed and tissue samples of farm animals. The limit of detection (LOD) of Total Aflatoxin in ELISA Kit is 0.2ng/ml (0.2ppb).

Assay Principle

101Bio Total Aflatoxin ELISA Kit is a competitive enzyme-labeled immunoassay. Each well of the 96-well microtiter plate is pre-coated with an anti-Total Aflatoxin antibody. During the assay, Total Aflatoxin standard solution or samples are added to test wells, followed by adding horse radish peroxidase (HRP)-Total Aflatoxin conjugate, which will compete with Total Aflatoxin in standard or sample for binding to antibody during the 30-minute incubation. After plate wash, a clear HRP substrate is added to the wells leading to a colored product only in the presence of HRP, and optical density is inversely related to Total Aflatoxin concentrations in the samples. The accurate concentration of Total Aflatoxin can then be determined by interpolation using the standard curve constructed in the same run.

Product Components:

The reagents included in the kit are sufficient for performing 96 measurements (including standards and samples).

1) 1 microtiter plate containing 12 test strips of 8 wells sealed in an aluminized pouch with desiccant.
2) 6 vials each containing 0.5 mL of Total Aflatoxin standard with 0, 0.2, 0.5, 1, 2, 4 ng/mL of Total Aflatoxin, respectively.
3) 1 vial containing 0.1 mL Total Aflatoxin-HRP conjugate (100×).

4) 1 bottle containing 12ml sample diluent buffer (10 x).
5) 1 bottle containing 50 mL microtiter plate wash solution (20×).
6) 1 bottle containing 12 mL TMB Ultra-Sensitive substrate (1×).
7) 1 bottle containing 12 mL stop solution (1×).
8) 2 microtiter plate sealers.
9) 1 booklet of instruction.

Safety Instructions

To receive complete safety information on this product, contact 101Bio, and request Material Safety Data Sheets. Stop solution is 1N sulfuric acid. Handle with care.

Materials required but not provided

- ✓ Microplate reader with 450 nm filter.
- ✓ Pipet capable of dispensing 20-200 µl.

Protocol

Assay Procedure

- ✓ Equilibrate kit components at room temperature (20-25 °C) for at least 30 min prior to running the test, and thoroughly mix all liquid components before use.
- ✓ Use test strips as needed on the frame, and store unused strips in the resealable bag at 2 -8 °C.
- ✓ Number standards and samples according to positions on microtiter plate. All standards and samples need duplicate measurement for accuracy.

Sample preparation

Samples need to be processed as followings before ELISA assay:

For Meat (including fish and shrimp) samples:

1. Homogenize sample
2. Weight 1.0 g homogenized sample
3. Add 2ml Ethyl Acetate, mix by vortex thoroughly
4. Centrifuge 4000rpm for 10 min at room temperature
5. Take 1 ml supernatant
6. Evaporate to dryness in a nitrogen evaporator
7. Add 0.5 ml hexane to reconstitute
8. Add 0.5 ml of sample diluent
9. Vortex vigorously
10. Centrifuge 4000rpm for 10 min at room temperature
11. Discard upper layer, take 50 µl bottom aqueous phase to ELISA plate.

For animal feed:

1. Weigh out 2.0 g homogenized sample
2. Add 2ml ethyl acetate and mix by vortex thoroughly

3. Centrifuge at 4000 rpm for 10 min at room temperature
4. Take 1 mL supernatant
5. Evaporate to dryness in a nitrogen evaporator
6. Add 1 mL hexane to reconstitute
7. Add 1 mL of sample diluent
8. Vortex vigorously
9. Centrifuge at 4000 rpm for 10 min at room temperature
10. Discard upper layer, and transfer 50 µl bottom aqueous phase to ELISA plate
11. Sample processed in this method has dilution factor of 2

ELISA assay procedures

1. Prepare Wash Solution by diluting 1 part of Wash Solution Concentrate (20x) with 19 parts of distilled water to Wash Solution (1x).
2. Prepare Sample Diluent Buffer by diluting 1 part of Sample Diluent Buffer (10x) with 9 parts of distilled water to Sample Diluent Buffer(1x).
3. Prepare HRP working solution by diluting 1 part of HRP conjugate (100x) with 99 parts of sample diluent buffer(1x) to HRP working solution (1x).
4. Add Total Aflatoxin standard sample, or unknown samples, 50 µl/well in duplicate
5. Add HRP working solution 50 µl/well, gently shake plate by hand for 1 min, and incubate at room temperature for 30 min.
6. Wash plate 4 times with wash solution (1x), 200 µl/well wash buffer each time.
7. Add 100 µl/well TMB Ultra-Sensitive Solution, and incubate plate at room temperature for 15 min in dark place.
8. Add **100 µl stop solution** to each well and mix by shaking gently. Measure absorbance of the wells at 450 nm (OD_{450} value) with microplate reader.

Quantitative Calculation of Total Aflatoxin Concentration

a) Calculate B/B0

Dividing average absorbance of each standard and sample (B) by absorbance of first standard (the standard with 0 ng/mL Total Aflatoxin concentration, B0) to obtain percentage absorbance.

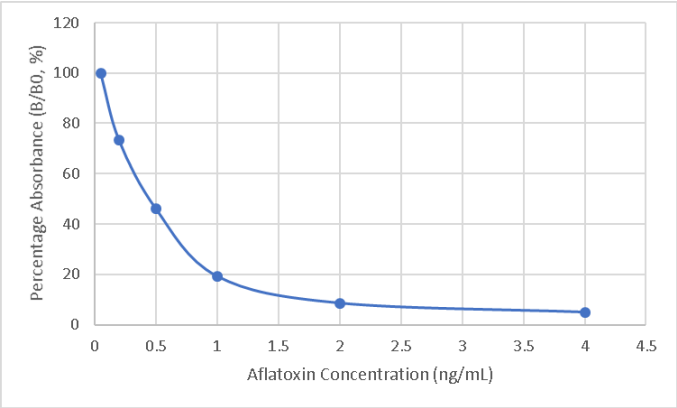
percentage absorbance (%) = B/B0 x 100%

B — average absorbance of a standard or sample

B0 — average absorbance of 0 ng/mL standard

b) A standard curve is obtained by graphing the percentage absorbance of standards (Y axis) versus their corresponding concentration (X axis) on semi-log graph paper (Example as below), and sample concentration can be read from this standard curve. Alternatively, Total Aflatoxin concentration in the samples can be calculated with regression equation correlating percentage absorbance to Total Aflatoxin concentration.

Graphing software can also be used for quick analyses of large number of samples.



Performance Data

Range of Standard Curve:	0 - 4 ng/ml
Assay Quantitative Range:	0.2 – 4 ng/ml
Assay Time:	50 min

Limit of Detection (LOD):

Meat (after calculation of dilution factor):	0.2 ppb
Feed (after calculation of dilution factor):	0.2 ppb

Recovery: 70-130%

Sensitivity (defined as the average of absorbance from 6 zero-standards minus 3 times of standard deviation): 0.2 ng/ml

Precision:

Intra-assay CV	<12%
Inter-assay CV	<15%

Cross reactivity:

Aflatoxin B1: 100%

Aflatoxin B2: 80%

Aflatoxin G1: 64%

Aflatoxin G2: 25%

Precautions

1. Assay kit should be stored at 2-8°C and avoid freezing conditions; unused test strips should be sealed in resealable bag; colorless substrate is sensitive to light so prolonged exposure to light needs to be avoided.
2. Reagents should be brought to room temperature (20-25°C) prior to use. A room temperature of lower than 20°C or failure to equilibrate reagents or samples to room temperature could result in low OD readings for all samples. All reagents should be put back into 2-8°C storage immediately after use.
3. Adhere to assay protocol on reaction temperature and time, and use pipet to add components whenever

possible. Results are solely based on OD450 readings from plate/strip reader.

4. Reagents need to be thoroughly mixed to improve reproducibility.
5. During all incubation steps, avoid light and seal plate with sealer.
6. If wells are dried out during plate wash steps, linearity of standard curve will be negatively affected, and reproducibility will be poor. Therefore, substrate addition should be carried out immediately after tapping the plate dry (following the last wash).
7. The stop solution is 1N sulfuric acid. Avoid contact with skin or clothing. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.
8. Do not use reagents beyond expiration date. Dilution or adulteration of reagents or samples not called for in the procedure may result in adverse changes in sensitivity and OD reading. Do not substitute reagents from kits with different lot numbers.
9. Obvious color in substrate suggests expiration and it should be discarded. When absorbance of zero-standard is lower than 0.8, the reagents may have expired.

General Limited Warranty

101Bio warrants its manufactured products against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product's printed expiration date. 101Bio makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose. The warranty provided herein and the data, specifications and descriptions of 101Bio products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of 101Bio. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and, if given, should not be relied upon.

In the event of a breach of the foregoing warranty, 101Bio's sole obligation shall be to repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies 101Bio promptly of any such defect. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as 101Bio is willing and able to repair or replace any nonconforming 101Bio product or part. 101Bio shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by a customer from the use of its products. However, in some states the purchaser may have rights under state law in addition to those provided by this warranty.

-- The end --