Total Aflatoxin Fast ELISA (Catalog Number: TBS21131)

One-step and one hour Elisa for determination of aflatoxin concentrations in foods, herbs, cannabis, animal feeds and plants.

Introduction

Aflatoxins are a class of structurally similar mycotoxins produced by Aspergillus species. 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.

Tribioscience's Total Aflatoxin ELISA Kit can **quickly, sensitively, and accurately** determine the presence of total aflatoxin in plants, herbs, foods, and animal feeds. It provides a vital tool to prevent consumption of food tainted with this toxic chemical.

Intended Use

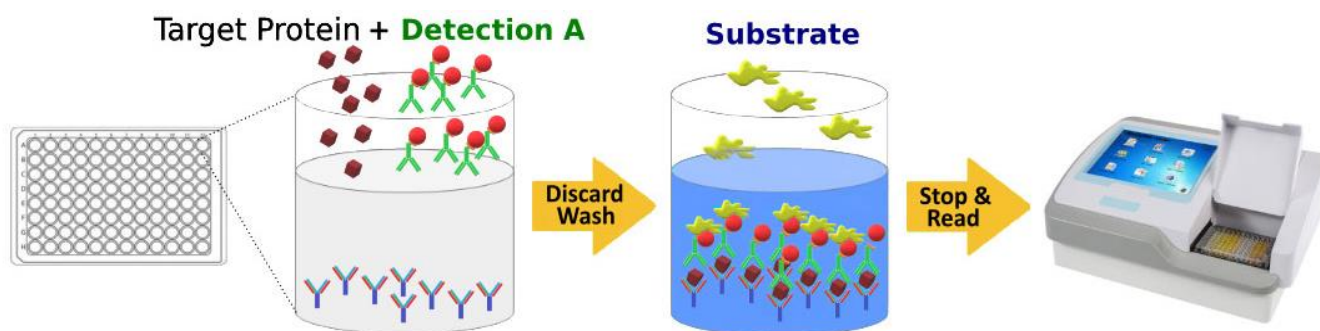
The Tribioscience's Total Aflatoxin ELISA Kit utilizes competitive ELISA technologies for the quantitative analysis of total aflatoxin in grains, nuts, cottonseeds, cereals, cannabis plant, herbs, and other commodities including animal feeds. The limit of detection (LOD) of Total Aflatoxin in ELISA Kit is 0.2ng/ml (1 ppb).

Assay Principle

Tribioscience's Total Aflatoxin ELISA Kit is a competitive enzyme-labeled immunoassay (Fig. 1). 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 each well, 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 incubation. After plate wash, an ultra-sensitive 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.

The kit main feature is that the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate and fast. The measurement can be finished in 1 hour, not need 4-5 hours.

Fig. 1. Simple Procedures



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Pre-coated Microplate	TBS21131A	96 well microplate (12 strips of 8 wells) coated with an antibody specific for total aflatoxin.	Return unused wells to the foil pouch. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Total Aflatoxin Standard	TBS21131B	80 µl of total aflatoxin (80ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS21131C	80 µl of HRP- aflatoxin conjugate (100x).	May be stored for up to 6 months at 2-8 °C.
Assay Diluent	TBS3000D	25 ml of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 ml of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 ml of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 ml of 1 N sulfuric acid.	

Store the unopened kit at 2-8 ℃. Do not use past kit expiration date.
The kit contains sufficient materials to run an ELISA on one 96-well plate.

Sample preparation for ELISA assay

A sample to be tested should be collected according to accepted sampling techniques. The sample should be ground and thoroughly mixed prior to proceeding with the extraction. Store samples at 2-8 ℃ until analyzed.

NOTE: Wheat, barley, oats, rice, and flour samples must be extracted in 70% methanol/water solution for maximum recovery. All other commodities should be extracted with 50% methanol/water solution.

1. Prepare the Extraction Solution: 50% methanol/water solution made by mixing 1 part of distilled deionized water with 1 part of methanol (reagent grade) for each sample to be tested, or prepare 70% methanol by mixing 3 part of distilled deionized water for each sample to be tested.
2. Grind a representative sample to the particle size of fine instant coffee.
3. Weigh out a 5 g ground portion of the sample and add 25 mL of the Extraction Solvent (70% methanol for Wheat, barley, oats, rice and flour samples, 50% Methanol for other commodities).

Note: The ratio of sample to extraction solvent is 1:5 (w/v). If preparing samples for other different weigh, adjust extraction solvent volume accordingly.

4. Mix by shaking vigorously in a sealed container for 5 minutes or in a high-speed blender for 2 minutes.
5. Centrifuge 5500rpm for 5 min at room temperature. Collect the upper layer to be tested. Or filter the extract through a Whatman #1 filter paper (or equivalent) and collect the filtrate to be tested. The sample is now ready to be assayed.

ELISA Procedures

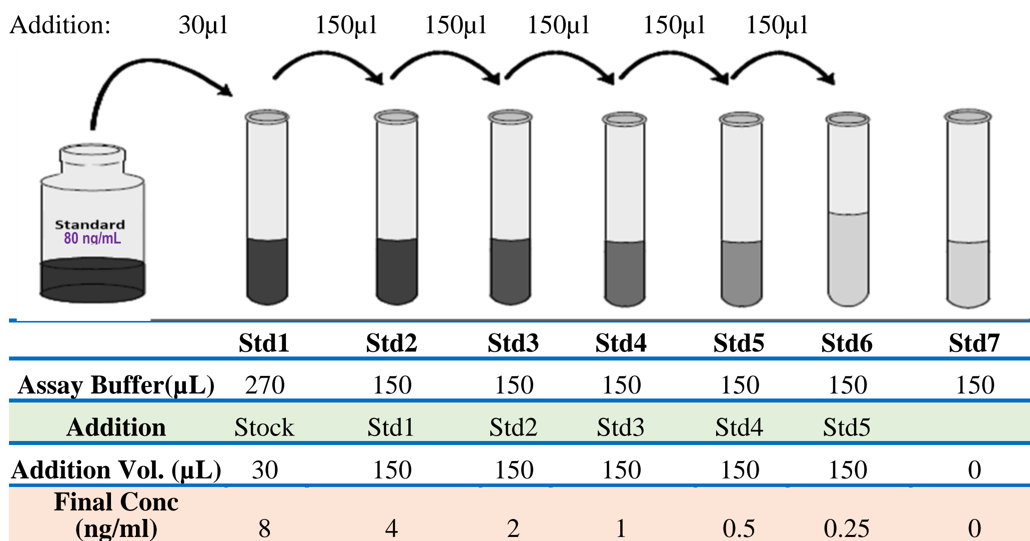
Bring all reagents to room temperature before use.

Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer.

Total Aflatoxin Standard Preparation: Label test tubes as #1 through #7. Pipet 270 µL of 1x Assay Diluent into tube #1, and 150 µL into tubes #2 to #7 **as diagram below.**

1. Add 30 µL of the total aflatoxin Standard stock solution (80ng/mL) to tube #1 (8 ng/mL) and mix.
2. Make 2x serial dilutions of the standard using the Tube#1(8 ng/mL standard solution) from Tube #2 through #6 with sequential transfer of 150 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 6 will be 8, 4, 2, 1, 0.5, and 0.25 ng/mL. Tube# 7 is Standard 0.

Fig.2 Diagram for standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7
Assay Buffer(µL)	270	150	150	150	150	150	150
Addition	Stock	Std1	Std2	Std3	Std4	Std5	
Addition Vol. (µL)	30	150	150	150	150	150	0
Final Conc (ng/ml)	8	4	2	1	0.5	0.25	0

Assay Procedures:

1. Add 50 µL of standard, sample, or control per well.
2. Add 50 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 30min**.
3. Aspirate each well, and wash for 3 times by filling each well with 200 µL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue**.**
5. Add 100 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to

ensure thorough mixing).

6. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Quantitative Calculation of Total Aflatoxin Concentration

a) Calculate B/B0

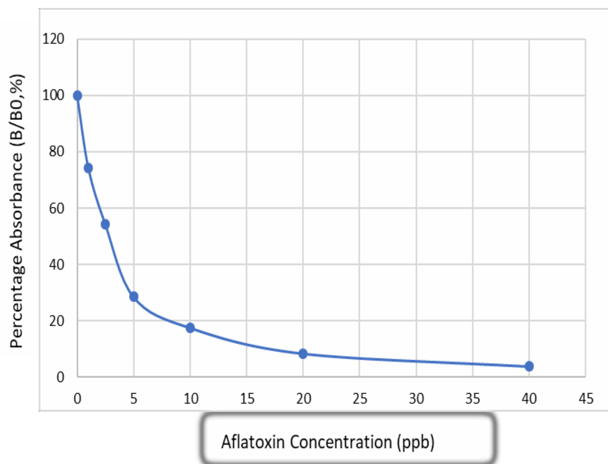
Dividing average absorbance of each standard and sample (B) by absorbance of the standard 0 ng/mL total aflatoxin concentration, B0) to obtain percentage absorbance as below:
Percentage absorbance (%) = 100% x (B/B0)
B: Average absorbance of a standard or sample
B0: Average absorbance of 0 ng/mL standard

b) A standard curve Calculation: A standard curve is obtained by graphing the percentage absorbance of standards (Y axis) versus their corresponding concentration (X axis), 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.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

Fig3. Total Aflatoxin Standard Curve



Note: the sample has been diluted at a 5:1 ratio with extraction solution (dilution Factor=5). Thus, the level of aflatoxin shown by the standard must be multiplied by 5 in order to indicate the ng of aflatoxin per gram of commodity (ppb) as follows:

Standard ng/mL	Commodity (ppb)
0.0	0.0
0.2	1.0
0.5	2.5
1.0	5.0
2.0	10.0
4.0	20.0
8.0	40.0

If a sample contains aflatoxins at greater than the highest standard, it should be diluted appropriately in extraction solution and retested. The extra dilution step should be factored in when expressing the result.

Range of Standard Curve: 0 – 40 ppb Assay
Quantitative Range: 1 – 40 ppb Assay
Recovery: 70-130%

Limit of Detection (LOD):
Food and feed (after calculation of dilution factor): 1 ppb
Cannabis plant, herbs (after calculation of dilution factor): 1 ppb

Sensitivity (defined as the average of absorbance from 6 zero-standards minus 3 times of standard deviation): 1ppm.

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℃ and avoid freezing conditions; unused test strips should be sealed in reclosable bag. The substrate is sensitive to light so prolonged exposure to light needs to be avoided.
2. Reagents should be brought to room temperature (20-25℃) prior to use. A room temperature of lower than 20℃ 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℃storage immediately after use.
3. Reagents need to be thoroughly mixed to improve reproducibility.
4. During all incubation steps, avoid light and seal plate with sealer.

Storage and Expiration Date

Storage: All components of the kit should be stored at 2-8℃. Expiration
Date: This kit expires 6 months after receipt.

Technical Assistance

For ordering or technical assistance regarding this kit, or for additional information about TribioScience products, please email: support@tribioscience.com or call (408) 498-0197, or 833-697-8998 (Toll Free).

For research use only.