Biorex food diagnostics

AFLATOXIN TOTAL V2 ELISA

PRODUCT CODE: BXEFT02A

QUALITY MANAGEMENT SYSTEM

ISO 13485 CERTIFIED COMPANY

BXEFT02A
96 TESTS (12x8 STRIP WELLS)
STORE AT 2-8°C
INSTRUCTIONS FOR USE
FOR USE IN THE ANALYSIS OF FOOD ONLY. Not for use in diagnostic procedures.

IVD For In Vitro Diagnostics Use Only
LOT Lot Number
REF Catalogue Number
Storage Temperature
Expiry Date (Year / Month)
Warning, Read Enclosed Documents
Instructions For Use
Manufactured By

Total Aflatoxin ELISA

Introduction
Enzyme-Linked Immunosorbent Assay for the Determination of total aflatoxins in corn/other grains and nut samples

Importance of Aflatoxins determination
Aflatoxins are a naturally occurring Mycotoxin produced by two types of mold: Aspergillus flavus and Aspergillus parasiticus. At least 13 different types of aflatoxin are produced in nature with aflatoxin B1 considered as the most toxic. Most mycotoxins and their modified forms tend to be mainly concentrated in the bran fractions or outer layers of the grains. Other parts of the cereal structure that produce fractions such as white flour or maize grits are usually contaminated with lower concentrations of mycotoxin than the outer layers that are present in the original whole grain. Since aflatoxins are known to be genotoxic and carcinogenic exposure through food should be kept as low as possible.

Samples
Cereals/Grains and nuts.

Sample Preparation
Dilution in alcohol/water followed by agitation, centrifugation, filtration and dilution.

Assay Time
15mins

Sensitivity/Cut off/Limit of detection
The sensitivity of the Total Aflatoxin test is 0.4 ng/mL (real concentration).

Cereals/Grains – 2ppb
Nuts – 1ppb

Specificity
This test has the ability to detect the sum of Aflatoxin B1, B2, G1, G2.

Test Principle
The assay is performed in plastic microwells that have been coated with anti-aflatoxins antibody. In the premixing wells the enzyme labelled aflatoxin and the standard solutions or samples are mixed and then transferred into the anti-aflatoxins microtiter plate. During the first incubation, free aflatoxins in the standard solution /sample and enzyme-labelled aflatoxin compete for the anti-aflatoxins antibody binding sites on the solid phase. Any unbound enzyme conjugate and aflatoxin molecule is then removed in a washing step. The bound enzyme activity is determined adding a fixed amount of a chromogenic substrate. The enzyme converts the colourless chromogen into a blue product. The addition of the stop reagent leads to a colour change from blue to yellow. The absorbance is measured with a microplate reader at 450 nm. The colour development is inversely

Reagents Provided

Code / Size	Component / Description
FT02-01 **FT02-02** **FT02-03** **FT02-04** **FT02-05** (5 vials with 1.5mL each)	STANDARD A – 0.0 ng/ml STANDARD B – 2.0 ng/ml STANDARD C – 8.0 ng/ml STANDARD D – 30 ng/ml STANDARD E – 80 ng/ml Ready-to-use (Note: Because of the 1:5 dilution of the sample in the extraction step, the calibrators actually contain 1/5th of the stated value. No further correction back to the concentration in the original sample is required.)
FT02-08 (1x96 wells) 8×12/12×8 well per plate	**MICROTITER PLATE** consisting of 12 strips with 8 breakable wells each, vacuum-packed in aluminized pouch with indicating desiccant.
FT02-21 (1x96 wells)	**Premixing Plate:** Non-coated microtiter plate for premixing of reagents.
WS-009 (1x20ml)	**WASH BUFFER:** Wash Buffer Solution (50X), Concentrate 20ml. If using full bottle add 980ml double de-ionised water and mix.

DILUTE BEFORE USE!	(Detergent Tween-20)
FT02-10 (1x8ml)	**STOP SOLUTION:** Colorless liquid in a clear vial with red screw cap. Ready to use as supplied. (Caution! Diluted Acid; Handle with care.)
FT02-11 (1x14ml)	**SUBSTRATE SOLUTION:** Liquid, ready-to-use.
FT02-12 (1x14ml)	**CONJUGATE:** Aflatoxin-HRP Enzyme Conjugate., Ready-to-use, light sensitive
1x	**PLATE COVER** To cover the strips during the incubation.
1x	**IFU** (Instruction Manual)

Please note all reagents are supplied ready to use apart from the wash buffer solution.

The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

Materials required but not provided

Instrumentation

- 50, 100, 500 and 1000 µL-micropipets
- ELISA reader (450 nm)
- Centrifuge
- Ultra-Turrax, or alternative mixer
- Filter paper, coffee filter (larger volumes) or Whatmann 1 filter
- Glassware
- Timer
- Paper towels or equivalent absorbent material.

Reagents

- Double distilled water
- Methanol, ACS grade
- Sodium Chloride

Safety Precautions

1. Do not smoke or eat or drink or pipet by mouth in the laboratory.
2. Wear disposable gloves whenever handling patient specimens.
3. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
4. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

Handling and Storage Instructions

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
5. Use a separate disposable tip for each specimen to prevent cross-contamination.
6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.
5. Aflatoxin is a very toxic substance. Dispose of all liquids in a plastic container containing household bleach (minimum 10%). All lab ware should be soaked for at least 1 hour in a 10% solution of household bleach. Avoid contact of skin and mucous membranes with reagents and sample extracts by wearing gloves and protective apparel. If exposure of skin and mucous membranes to liquids should occur, immediately flush with water.
6. The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water
7. Do not mix components from different batches.
8. Do not use reagents after expiration date.
9. Check both precision and accuracy of the laboratory equipment used during the procedure (micropipets, ELISA reader etc.).

Sample Preparation

Cereals and grains

1. Grind a representative sample weight to pass a 20 mesh sieve and thoroughly mix prior to sub-sampling. Samples not being immediately analyzed should be stored refrigerated.
2. Weigh 5 g ground sample and add 25 mL of sample extraction buffer* to the sample tube.
3. Vortex for 1 minute and shake/rotate for 10mins.
4. Centrifuge at 3000g for 10minutes or filter through a Whatmann 1 filter and apply to microtiter plate.

*To prepare cereal sample extraction buffer (70% Methanol + 4% NaCl), dissolve 8g NaCl in 40ml of double deionised water, add 140ml of methanol then make volume up to 200ml using deionised water.

Alternatively if a larger sample size is more efficient to the laboratory testing regimen the same sample preparation can be used just by multiplying the weights and volumes eg 50g sample + 250ml of sample extraction buffer.

Nuts

1. Grind a representative sample weight to pass a 20 mesh sieve and thoroughly mix prior to sub-sampling. Samples not being immediately analyzed should be stored refrigerated.
2. Weigh 5 g ground sample and add 1g NaCl and 25ml 60% Methanol/40% water solution to the sample tube.
3. Vortex for 1 minute and shake/rotate for 10mins.
4. Centrifuge at 3000g for 10minutes or filter through a Whatmann 1 filter and apply to microtiter plate.

Assay Procedure

1. Allow reagents and sample extracts to reach room temperature prior to running the test.

2. Add 100 µL of enzyme conjugate solution to every individual premixing wells successively using a multi-channel pipette or a stepping pipette.
3. Add 50 µL of the standard solutions or samples (sample extracts) into the appropriate pre mixing wells of the test strips according to the working scheme given. We recommend using duplicates.
4. Using a multi-channel, draw up 100ul of the well contents 3 times to mix the reagents.
5. Directly pipette 100ul of the mixed reagents to the same corresponding wells of the microtiter plate coated in anti-aflatoxin antibodies.
6. Continue to carry out steps 3 and 4 for each strip of 8 wells.
7. Incubate for exactly 10mins at room temperature (darkened).
8. After incubation, remove the covering and vigorously shake the contents of these wells into a sink. Wash the strips three times using the 1X washing buffer solution (wash bottle can be used) Use at least a volume of 250 µL of washing buffer for each well and tap the plate for 10 seconds with every wash. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
9. Add 100 µL of substrate solution to the wells. Cover the wells with the plate sealer and mix the contents by moving the strip holder in a circular motion on the benchtop for about 30 seconds.
10. Incubate the plate for exactly 5 minutes at room temperature. Protect the strips from direct sunlight.
11. Add 50 µL of stop solution to the wells in the same sequence as for the substrate solution.
12. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution

Recommended working template

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

(Note: Running calibrators and samples in duplicate will improve assay precision and accuracy.)

Calculation of results

1. Semi-quantitative results can be derived by simple comparison of the sample absorbance's to the absorbance of the calibrator wells: Sample containing less color than a calibrator well have a concentration of aflatoxin greater than the concentration of the calibrator. Samples containing more color than a calibrator well have a concentration less than the concentration of the calibrator.
2. Quantitative interpretation requires graphing the absorbances of the calibrators (Y axis) versus the calibrator concentration (X axis). Either 4-paramerter or LOG-LOGIT curve fit can be used. If your plate reader software doesn't provide these curve fits, a spreadsheet that will perform the

Example of standard curve

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 0 ng/mL standard. These values are only an example and should not be used instead of the standard curve which has to be measured in each new test.

Total Aflatoxin (ppb)	% binding B/B0
0	100
2	89
8	60
30	29
80	18

Results Evaluation

The ELISA result has already taken into consideration the dilution of both sample preparation therefore no dilution factor needs applied to the samples.