QUALICHEK™ Aflatoxin B1 ELISA

REF : KBFP1002-2

Designed and Developed as per AOAC Official Methods of Analysis Guidelines (Method 971.22)

ISO9001:2016 ISO13485:2016

Ver 1.1

IVT

Enzyme Immunoassay for Quantitative Determination of Aflatoxin B1 in samples such as feedstuff and animal feed

IVT	**For In-Vitro Test Only**	**REF**	**Catalog Number**
	Store At	**LOT**	**Batch Code**
	Manufactured By		**Biological Risk**
	Expiry Date		**Consult Operating Instructions**

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REF KBFP1002-2 Σ 96 tests

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Introduction:

Aflatoxins are a family of toxins produced by certain fungi that are found on agricultural crops such as maize (corn), peanuts, cottonseed, and tree nuts. The main fungi that produce aflatoxins are Aspergillus flavus and Aspergillus parasiticus, which are abundant in warm and humid regions of the world. Aflatoxin-producing fungi can contaminate crops in the field, at harvest, and during storage.

Intended Use:

The QUALICHEK™ Aflatoxin B1 ELISA is used for quantitative testing of Aflatoxin B1 in sample, such as feedstuff and animal feed.

Principle:

The QUALICHEK™ Aflatoxin B1 ELISA is a competitive inhibition enzyme immunoassay. AFB1 antigen is pre-coated on microtiter plate. Aflatoxin B1 in the samples or standard competes with coupled antigen on the microplate for binding of Biotinylated Anti-AF B1. Then Horseradish Peroxidase (HRP) conjugate is added to the wells, followed with TMB substrate for color development. The intensity of the color developed is inversely proportional to the amount of Aflatoxin found in the sample.

Materials Provided:

1. Aflatoxin B1 Antigen Coated Microtiter plate (8x12 wells) - 1 no
2. Aflatoxin B1 Standard, (1 ml per vial) - 0, 0.15, 0.45, 1.35, 4.05 ppb
3. Anti-Aflatoxin B1:HRP Conjugate - 7 ml
4. Biotinylated AFB1 Antibody - 7 ml
5. Sample Diluent – 50 ml
6. TMB Substrate - 12 ml
7. (10X) Wash Buffer - 30 ml
8. Stop Solution - 10 ml
9. Instruction Manual - 1 no

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water / Distilled Water (DW)
4. Absolute Ethyl Alcohol
5. Wash bottle or automated microplate washer
6. Clean tubes and Eppendorf tubes
7. Precision single and multi-channel pipette and disposable tips.
8. 37°C incubator
9. Timer.

Handling/Storage:

1. All reagents should be stored at 2ºC to 8ºC for stability.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For In-Vitro Use Only

Sample Preparation:

Note: Sample needs to be pulverized or ground and passes 20 mesh screen (particle size smaller than 1mm). Then mix well for storage (Ground sample shall avoid moisture absorption. It is better measure moisture content before storage and regularly measure moisture content in storage).

Corn, Wheat and other cereals:

1. Weigh 5.00 ±0.05 gms well mixed sample.
2. Add 25 ml 40% Ethanol Water
3. Vibrate for 2 minutes
4. Centrifuge at 4000rpm for 5 minutes
5. Take 200 ul supernatant of mixed solution, then add 600 ul sample diluent. Mix well.
6. Take 50 ul mixed solution

Sample Dilution: 20X
For fermentation sample or samples whose pH value below 6, we recommend to use NaOH solution to adjust sample pH between 6 & 7

Reagent Preparation:

1. **70% Ethanol:** To make 70% Methanol, add 700ml Absolute Ethyl Alcohol in 300ml Distilled Water.
2. **40% Ethanol:** To make 40% Ethanol, add 400ml Absolute Ethyl Alcohol in 600ml Distilled Water.
3. **Wash Buffer (1X)**: To make (1X) Wash Buffer, add 30 ml (10X) Wash buffer in 270 ml Distilled Water.

Assay Procedure:

1. Bring all reagents to room temperature for 30 min before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming..

2. Add **50 ul Standards** or **prepared Sample** into respective well. It is recommended that all samples and standards be assayed in duplicate.

3. Add **50 ul Anti-Aflatoxin B1:HRP Conjugate** to all wells.

4. Add **50 ul Biotinylated Aflatoxin B1 Antibody** to all wells. Seal plate with adhesive strip and gently shake for 30s.

5. Incubate at room temperature 25°C for 15 minutes.

6. Aspirate and wash plate 4 times with diluted **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

7. Pipette **100 ul TMB Substrate** to all wells.

8. Incubate the plate at **25°C** for **5 minutes**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

9. Pipette **50 ul** of **Stop Solution** to all wells. The wells should turn from blue to yellow in color.

10. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Standard graph paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points.

To determine the unknown Aflatoxin B1 concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Aflatoxin B1 Concentration. If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit is best recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.
- If the absorbance value is equivalent or higher than the 4.05 ppb standard.

Performance Characteristics of the Kit:

Sensitivity:
Limit of Detection (LOD): It is defined as the lowest concentration that could be differentiated from zero. It was determined by mean O.D value of 20 replicates of zero standards added by their three standard deviations.
20 replicates of '0' standards were evaluated and the LOD was found to be less than 0.15 ppb.

Specificity:
The antibodies used in the kit are antibodies specific for Aflatoxin.B1
Aflatoxin B1 (AFB1): 100%

Assay Range:
0.15 ppb – 4.05 ppb

Recovery Rate:
80% - 120%

Precision:
Intra-Assay: CV%≤ 10%
Inter-Assay: CV%≤ 10%

Safety Precautions:

- This kit is In-vitro Test Use only and not for In-Vitro Human Diagnostics. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
  - Do not smoke, eat or drink while handling kit material
  - Always use protective gloves
  - Never pipette material by mouth
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not

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