KRISHGEN BioSystems

QUALICHEK™ Total Aflatoxin ELISA

REF : KBFP1001

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

ISO9001:2016 ISO13485:2016

Ver 1.1

RUO

Quantitative testing of Total Aflatoxin in samples such as grain, peanut, formula feed, etc.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	Store At	**LOT**	**Batch Code**
	Manufactured By		**Biological Risk**
	Expiry Date		**Consult Operating Instructions**

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REF KBFP1001 Σ 96 tests

KRISHGEN BioSystems
For US/Europe Customers: toll free +1(888)-970-0827 | tel +1(562)-568-5005
For Asia/India Customers: +91(22)-49198700
Email: sales@krishgen.com | http://www.krishgen.com

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™ Total Aflatoxin ELISA is used for quantitative testing of Total Aflatoxin in sample, such as grain, peanut, formula feed, etc.

Principle:

The method employs the quantitative competitive enzyme immunoassay technique. The microtiter plate is pre-coated with coupled antigen. During the reaction, Aflatoxin in the samples or standard competes with coupled antigen on the microplate for sites of anti-Aflatoxin antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and TMB substrate is added. Color development is stopped by addition of stop solution. Absorbance is measured at 450 nm. There is a negative correlation between the OD value of samples and the concentration of Aflatoxin.

Materials Provided:

1. Total Aflatoxin Antigen Coated Microtiter plate - 1x 96 wells
2. Aflatoxin Standard, (1ml/vial) - 0, 0.02, 0.04, 0.08, 0.16 and 0.32 ppb
3. Aflatoxin:HRP Conjugate - 5.5 ml
4. Antibody Working Solution - 5.5 ml
5. TMB Substrate - 12 ml
6. Wash Buffer (20X) – 2 x 25 ml
7. Stop Solution - 12 ml
8. Instruction Manual - 1 no

Materials to be provided by the End-User:

1. Microplate reader
2. Printer
3. Homogenizer
4. Nitrogen Evaporator
5. Water bath
6. Centrifuge
7. Vortex mixer
8. Graduated pipette
9. Balance (sensibility 0.01 g)
10. Pipettes - single channel (20-200 ul, 100-1000 ul) and multichannel (300 ul)
11. Methanol
12. N-hexane
13. Trichloromethane
14. Dichloromethane.

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 research use only

Sample Preparation:

Bring all reagents and samples to room temperature before use.
Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

Sample Pre-treatment Notice:

Experimental apparatus should be clean; use disposable pipette tips to avoid cross-contamination during the experiment.

Pretreatment of cereals sample:

1. Homogenize the representative sample with a homogenizer and mix fully.
2. Weigh 2±0.05 g of homogenate sample into the 50 mL centrifuge tube, add 5 mL of **70% Methanol** (Solution 1), vortex for 5 min, centrifuge at 4000 r/min for 10 min at room temperature;
3. Take 0.5 mL of supernatant to another centrifuge tube, add 0.5 mL of deionized water, mix fully;
4. Take 50 uL for detection and analysis.

Note: Sample dilution factor: 5, minimum detection dose: 0.1 ppb

Pretreatment of Formula Feed sample:

1. Homogenize the representative sample with a homogenizer and mix fully.
2. Weigh 2±0.05 g of homogenate into the 50 mL centrifuge tube, add 10 mL of **70% Methanol** (Solution 1), vortex for 5 min, centrifuge at 4000 r/min for 10 min at room temperature;
3. Take 0.5 mL of supernatant to another centrifuge tube, add 0.5 mL of deionized water, mix fully;
4. Take 50 uL for detection and analysis.

Note: Sample dilution factor: 10, minimum detection dose: 0.2 ppb

(If aflatoxin content is higher in the sample, take the mixed liquid from step 2, diluted with 35% Methanol, the sample dilution multiple is the actual dilution multiple at the moment. For example: take the mixed liquid from step 2, diluted 10 times with 35% Methanol, the actual dilution multiple is 10×10=100, detection limit: 2 ppb)

Pretreatment of edible oil, peanut, high fat formula feed sample:

1. Homogenize the representative sample with a homogenizer and mix fully.
2. Weigh 2 g of homogenate sample into the 50 mL centrifuge tube, add 8 mL of N-hexane and 10 mL of 70% Methanol (Solution 1), vortex for 5 min, centrifuge at 4000 r/min for 10min at room temperature;
3. Discard the upper liquid, and take 0.5 mL of lower liquid to another centrifuge tube, add 0.5 mL of deionized water, mix fully;
4. Take 50 µL for detection and analysis.

Note: Sample dilution factor: 10, detection limit: 0.2 ppb

Pretreatment of biscuit sample :

1. Homogenize the representative sample with a homogenizer and mix fully.
2. Weigh 2 g of homogenate sample into the 50 mL centrifuge tube, add 10 mL of **70% Methanol** (Solution 1), vortex for 5 min, centrifuge at 4000 r/min for 10 min at room temperature;
3. Take 2 mL of supernatant to another centrifuge tube, add 4 mL of **Trichloromethane** or **Dichloromethane,** vortex for 5 min, centrifuge at 4000 r/min for 10 min at room temperature;
4. Take the upper liquid to another centrifuge tube, keep the lower liquid for use (lower liquid A). Add 4 mL of **Trichloromethane or Dichloromethane** to the upper liquid, vortex sufficiently for 5 min, and centrifuge at 4000 r/min for 10 min at room temperature. Discard the upper liquid and keep the lower liquid (lower liquid B);
5. Mix lower liquid A and lower liquid B thoroughly ;
6. Take 2 mL of mixed lower liquid and dry with nitrogen evaporators or water bath at 50-60℃;
7. Add 0.5 mL of **70% Methanol** (Solution 1) to dissolve thoroughly, add 0.5 mL of deionized water, mix fully;
8. Take 50 µL for detection and analysis.

Note: Sample dilution factor: 10, detection limit: 0.2 ppb

Pretreatment of beer sample:

1 Stir beer thoroughly to remove CO2, take 2 mL of beer sample and add 1 mL of deionized water, then add 7 mL of **Methanol**, vortex for 5 min;
2 Take 0.5 mL of mixed sample liquid and add 0.5 mL of deionized water to another centrifuge tube, mix fully;
3 Take 50 µL for detection and analysis.

Note: Sample dilution factor: 10, detection limit: 0.2 ppb

Pretreatment of wine, soy sauce, vinegar sample:

1 Take 2 mL of sample and add 2 mL of deionized water, then add 10 mL of **Trichloromethane or Dichloromethane**, vortex for 5 min, centrifuge at 4000 r/min for 10 min at room temperature;
2 Remove all upper liquid. Take 1 mL of lower liquid to another centrifuge tube and dry with nitrogen evaporators or water bath at 50-60℃;
3 Add 0.5 mL of **70% Methanol** (Solution 1) to dissolve thoroughly, add 0.5 mL of deionized water, mix fully;
4 Take 50 µL for detection and analysis**.**

Note: Sample dilution factor: 5, detection limit: 0.1 ppb

Assay Procedure:

1. Number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. It is strongly recommended that all Controls and Samples be run in duplicates or triplicates. All steps must be performed at room temperature.

2. Add **50 uL** of **Standards or Samples** into respective wells.

3. Add **50 uL** of **Aflatoxin:HRP conjugate** into each well

4. Add **50 uL** of **Antibody working solution** into each well.

5. Cover the plate with plate sealer, oscillate for 5 s gently to mix thoroughly, incubate for 30min at 25℃ in shading light.

6. Aspirate and wash plate 4 times with **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. Add **100 uL** of **TMB Substrate** to each well, Incubate shading light for 15 min at room temperature 25℃

8. Add **100 uL** of **Stop solution** to each well.

9. Read the plate at 450 nm with a microplate reader. This step should be finished in 10 min after stop reaction.

Calculation of Results:

Absorbance (%) =A/A_0×100%

A: Average absorbance of standard or sample

A_0: Average absorbance of 0 ppb Standard

Drawing and calculation of standard curve

Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add average absorbance value of sample to standard curve to get corresponding concentration. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. For this kit, it is more convenient to use professional analysis form for accurate and fast analysis on a large number of samples.

Notes:

1. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25℃.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
5. **Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other with different lot numbers**.
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0)＜0.5 unit (A450nm＜0.5), it indicates the reagent may be deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. **For mentioned sample fast and efficient extraction methods are included in the kit description. Please consult technical support for the applicability if other sample need to be tested.**
11. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

Performance Characteristics of the Kit:
This kit has been validated. Please view the details herein below.

Sensitivity: 0.02 ppb (ng/mL)

Reaction mode: 25℃, 30 min, 15 min

Detection limit: Cereals---0.1 ppb; Formula feed---0.2 ppb; Edible oil, Peanut 0.2 ppb; Biscuit ---0.2 ppb; Beer---0.2 ppb; Wine, Soy sauce, Vinegar---0.1 ppb

Cross-Reactivity: Aflatoxin B1 (AFB1) ---100%, Aflatoxin B2 (AFB2) ---80%, Aflatoxin G1 (AFG1)--75%, Aflatoxin G2 (AFG2) ---45%, Aflatoxin M1 (AFM1) ---8%

Sample recovery rate: Cereals, Formula feed---85%±15%, Peanut---82%±15%, Edible oil---85%±15%, Biscuit---83%±15%;, Beer---84%±15%, Wine/Soy sauce/Vinegar---87%±15%

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