Helica® MycoTox Total Aflatoxin ELISA

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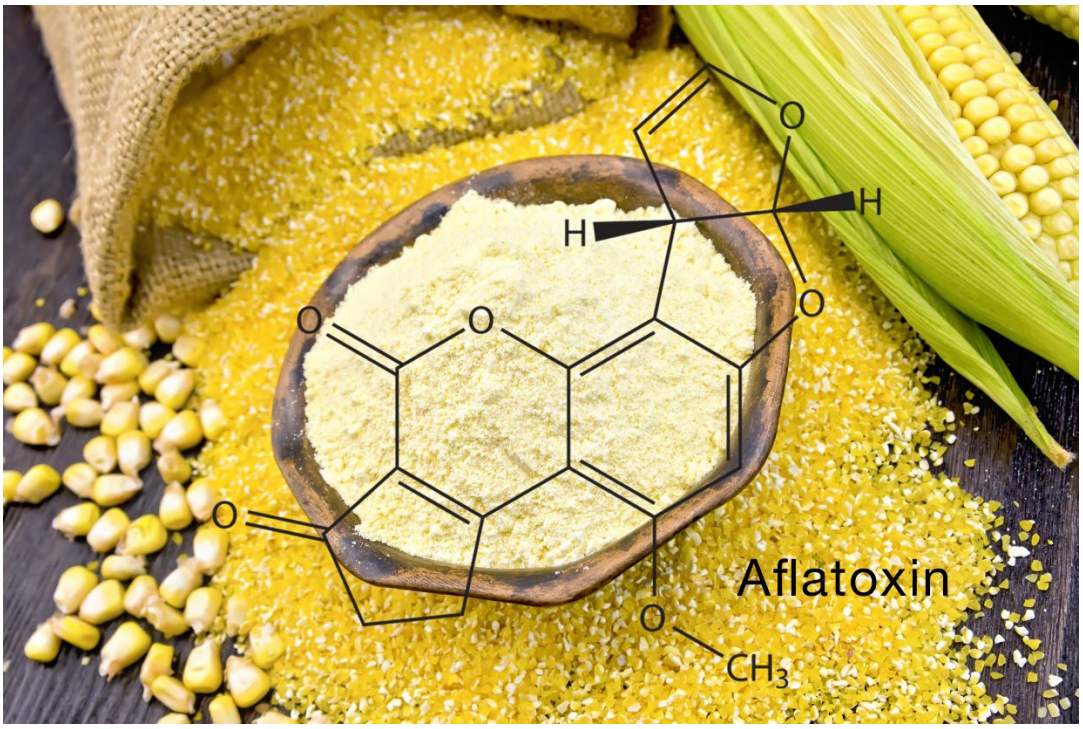

Helica® MycoTox Total Aflatoxin ELISA

For the quantitative detection of total aflatoxins in corn including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta and corn screenings.

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Introduction – Aflatoxins

Aflatoxins are toxic metabolites produced by a variety of molds such as *Aspergillus flavus* and *Aspergillus parasiticus*. They are carcinogenic and can be present in grains, nuts, cottonseeds and other commodities associated with human food or animal feeds. Crops may be contaminated by one or more of the four sub-types of aflatoxin: B1, B2, G1 and G2. Aflatoxin B1 is the most toxic and frequently detected form. The other types present a significant danger if the concentration is high. Aflatoxins have been implicated in human health disorders including hepatocellular carcinoma, aflatoxicosis, Reye's syndrome and chronic hepatitis. Animals are exposed to aflatoxins by consuming feeds that are contaminated by aflatoxin-producing fungal strains during growth, harvest or storage. Symptoms of toxicity in animals range from death to chronic diseases, reproductive interference, immune suppression and decreased milk and egg production. Most controlling government agencies worldwide have regulations regarding the amount of aflatoxins allowable in human and animal foodstuffs. Accurate and rapid determination of the presence of aflatoxin in commodities is of paramount importance.

Intended Use

Hygiena's Helica® Mycotoxin ELISA kits are user-friendly, cost-effective kits for the detection of mycotoxins in a wide range of commodities including animal feeds, grains, corn and animal urine, designed to protect humans and animals from dangerous side effects of mycotoxins.

The Helica Mycotox Total Aflatoxin ELISA assay is a competitive direct enzyme-linked immunosorbent assay intended for the quantitative detection of aflatoxins in corn. It was developed to determine aflatoxins with a wide range of 5 - 300 ppb in grains and certified by the Federal Grain Inspection Service (FGIS) for the quantitative determination of aflatoxins in corn (Certificate No. FGIS 2019-129).

Data obtained from assays should not be used for human diagnostic or human treatment purposes. Assays are not approved by the United States Food and Drug Administration or any other US or non-US regulatory agency for use in human diagnostics or treatment. Assays should not be used as the sole basis for assessing the safety of products for release to consumers. The information generated is only to be used in conjunction with the user's regular quality assurance program.

Not approved for clinical diagnosis. Use for research and development, quality assurance and quality control under the supervision of technically qualified persons.

Principle of the Method

The Helica Mycotox Total Aflatoxin ELISA assay is a competitive direct enzyme-linked immunosorbent assay intended for the quantitative detection of aflatoxins in corn. An aflatoxin-specific antibody is coated to a polystyrene microwell. Aflatoxins are extracted from a ground sample with 70% methanol. The extracted sample and HRP-conjugated aflatoxin are mixed and added to the antibody-coated microwell. Aflatoxin from the extracted sample and HRP-conjugated aflatoxin compete to bind with the antibody coated to the microwell. Microwell contents are decanted and non-specific reactants are removed by washing. An enzyme substrate (TMB) is added and color (blue) develops. The intensity of the color is directly proportional to the amount of bound conjugate and inversely proportional to the concentration of aflatoxin in the sample or standard. Therefore, as the concentration of aflatoxin in the sample or standard increases, the intensity of the blue color will decrease. An acidic stop solution is added, which changes the chromogen color from blue to yellow. The microwells are measured optically by a microplate reader with an absorbance filter of 450 nm (OD450). The

optical densities of the samples are compared to the ODs of the kit standards and a result is determined by interpolation from the standard curve.

Kit Contents

Package/ Number	Component	Description
1X Pouch	Antibody- coated microwell plate	96 wells (12 eight-well strips) in a microwell holder coated with a mouse anti-aflatoxin monoclonal antibody, *Ready-to-Use*.
1X Plate	Dilution plate	96 non-coated wells (12 eight-well strips) in a microwell holder, *Ready-to-Use.*(Mixing wells)
6X Vials	Standards	1.5 mL/vial of Aflatoxin at the following concentrations: 0.0, 0.2, 0.6, 1.8, 5.0 and 15.0 ng/mL in 70% Methanol, *Ready-to-Use*.
2X Bottles	Conjugate	2 x 12 mL of Aflatoxin conjugated to peroxidase in buffer with preservative, *Ready-to-Use*.
1X Bottle	Substrate	12 mL stabilized tetramethylbenzidine (TMB), *Ready-to-Use*.
1X Bottle	Stop solution	12 mL acidic solution, *Ready-to-Use*.
1X Pouch	PBS-T powder	PBS with 0.05% Tween® 20*, bring to 1 Liter with distilled water and store refrigerated. (Wash buffer)

**TWEEN® 20 is a registered trademark of CRODA International Plc.*

Materials Required But Not Provided

- Balance: up to 20 g measuring capability
- Graduated cylinder: 250 and 1000 mL
- Microtube
- Water bath
- Vortex mixer
- Kitchen blender
- Mason jar: 16 fluid oz. (473 mL)
- Methanol ACS grade
- Distilled or deionized water: 30 mL per sample
- Pipettor with tips: 100 µL and 200 µL
- Timer
- Wash bottle
- Absorbent paper towels
- Kimwipe™ or similar lint-free wipe (Kimwipe™ is a trademark of Kimberly Clark)
- Microplate reader with 450 nm filter

Storage and Shelf Life

- Store reagents at 2 to 8 °C. Do not freeze
- Reagents should be used by the expiration date stamped on the individual labels.

- HRP-labeled conjugate and TMB-substrates are photosensitive and are packaged in a light-protective opaque bottle. Store in the dark and return to storage after use.

Precautions and Waste Disposal

General Precautions:

- Bring all reagents to room temperature (19 to 25 °C) before use.
- Do not interchange reagents between kits of different lot numbers.
- Do not use solutions if cloudy or precipitate is present.
- Do not return unused reagents to their original bottles. The assay procedure details volumes required.
- Adhere to all time and temperature conditions stated in the procedure.
- During the sample extraction, avoid cross-contamination.
- Devices, such as a blender, must be cleaned after each sample preparation.
- Samples tested should have a pH of 7.0 (± 1.0). Excessive alkaline or acidic conditions may affect the test results.

Safety Precautions:

Mycotoxins (aflatoxins, trichothecenes and others) are well-known human carcinogens and are considered highly toxic. Because mycotoxins can cause human illness, appropriate safety precautions must be taken and personal protective equipment worn when handling samples, reagents, glassware and other supplies and equipment that potentially could be contaminated with mycotoxins.

- Before using this assay, please review the Safety Data Sheet(s) available at www.hygiena.com.
- Refer to your site practices for safe handling of materials.
- It is strongly advised that protective gloves, a lab coat and safety glasses be worn at all times while handling mycotoxin kits and their respective components. Consider all materials, containers and devices that are exposed to samples or standards to be contaminated with mycotoxins.
- Never pipette reagents or samples by mouth.
- Standards are flammable. Caution should be taken in the use and storage of these reagents.
- The stop solution contains sulfuric acid, which is corrosive. Please refer to the SDS. Do not allow to contact skin or eyes. If exposed, flush with water.

Disposal:

Decontaminate materials and dispose of waste per your site practices and as required by local regulations. Do not dispose of these materials down the drain. Please note that there is a potential for mycotoxin contamination in or on any of the kit components provided.

- Dispose of all materials, containers and devices in the appropriate receptacle after use.
  Before conducting the assay, prepare a waste container to act as a receptacle for your kit waste.
  Eject contaminated pipette tips and discard all other related materials into this container.
- Once the assay is completed, the container should be treated with a sufficient amount of 5 - 6% sodium hypochlorite (NaOCl) to saturate the contents of the container (approximately 1/10th the volume of the container). The NaOCl will denature the mycotoxins and neutralize the waste, making it safe for disposal. Invert the container several times to thoroughly coat all waste.
- In the case of an accidental toxin spill, treat the spill surface with 5 - 6% NaOCl for a minimum of 10 minutes, followed by 5% aqueous acetone. Wipe dry with absorbent paper towels.

Preparation of Samples

Preparation of Extraction Solvent

1. Using a 1,000 mL graduated cylinder, measure 700 mL of methanol and pour it into a glass bottle.
2. Using a 500 mL graduated cylinder, add 300 mL of distilled or deionized water to the methanol and shake until completely mixed.
3. Label the glass bottle stating 70% methanol/30% water, date of preparation and initials of technician who prepared it.
4. To prepare smaller or larger amounts of the extraction solvent, use the ratio of 7 parts methanol to 3 parts distilled or deionized water.
5. Place the extraction solvent bottle in a water bath with a temperature set to 40 °C and let it sit at least 1 hour before use. Use a thermometer to check the water bath temperature.

Note: The sample must be collected according to the appropriate established sampling techniques.

Extraction Procedure

1. Weigh 50 ± 0.2 grams ground sample into a 16 fluid oz. mason jar.
2. Using a 250 mL graduated cylinder, add 250 mL of warmed extraction solvent (70% methanol). Return the solvent extraction bottle to the warm water bath between samples.
3. Blend for 3 minutes at high speed. Extraction should be performed immediately so that the extraction solvent temperature is close to 40 °C.
4. Using a 1,000 µL pipette, transfer 1,000 µL into a microcentrifuge tube and centrifuge for 10 seconds.
5. Using a 1,000 µL pipette and a new pipette tip, dispense 300 µL of 70% methanol in a microtube.
6. Using a 100 µL pipette, add 100 µL of the supernatant into the microtube. Vortex for a few seconds to mix prior to analysis.
7. The final dilution for use in calculation is **1:20**.

Assay Procedure

1. Bring all reagents and samples to room temperature before use and perform the sample preparation at room temperature. Prepare wash buffer by reconstituting the PBS-T powder packet by washing out the contents with a gentle stream of distilled or deionized water into a 1-Liter container. Fill to 1 Liter with distilled or deionized water and store refrigerated when not in use.
2. Place one mixing well for each sample and another six (6) mixing wells for six (6) standards. Remove an equal number of antibody-coated wells. Return unused wells to the foil pouch with desiccant and reseal.
3. Mix each reagent by swirling the reagent bottle prior to use.
4. Dispense 200 µL of conjugate into the appropriate mixing well.
5. Using a new pipette tip for each, add 100 µL of each standard and sample to the appropriate mixing wells. Mix by priming the pipettor at least three (3) times.
6. Using a new pipette tip for each, transfer 100 µL into the antibody-coated wells. Incubate for 15 minutes at room temperature.
   Note: The mixing wells contain enough solution to run each standard and/or sample in duplicate (recommended). If running each standard or sample in singlets or if more replicates are needed, the volumes of assay diluent and sample/standard should be scaled accordingly.
7. Decant the contents from the wells into a discard basin. Wash the microwells by filling each with PBS-T wash buffer, then decanting the wash into a discard basin. Repeat wash for a total of five (5) times.
8. Tap the wells (face down) on a layer of absorbent towels to remove residual buffer.

9. Measure the required volume of substrate solution (1 mL/strip or 120 µL/well) and place in a separate container. Add 100 µL of substrate reagent to each well. Incubate at room temperature for 5 minutes. Cover to avoid direct light.
10. Measure the required volume of stop solution (1 mL/strip or 120 µL/well) and place in a separate container. Add 100 µL of stop solution in the same sequence and at the same pace as the substrate reagent was added. Mix gently by sliding the plate back and forth on a flat surface for 10 – 15 seconds.
11. Wipe the bottom of the wells with a lint-free Kimwipe and remove air bubbles.
12. Read the optical density (OD) at 450 nm. Read within 10 minutes after addition of stop solution.

Note: It is the nature of immunoassay curves that they become flat at the extreme low and high values. Extrapolation to values beyond the lowest and highest point on the standard curve will lead to imprecise and inaccurate results.

Interpretation of Results

Construct a dose-response curve using the OD values expressed as a percentage (%B/B_0) of the OD of the zero (0.0) standard against the aflatoxin content of the standard. Unknowns are measured by interpolation from the standard curve.

The information contained on the label of each standard vial refers to the contents of that vial. However, the sample has been diluted at a 1:20 ratio with 70% methanol. Therefore, the level of aflatoxin shown by the standard must be multiplied by 20 in order to indicate the ng of aflatoxin per gram of commodity (ppb) as follows.

Standard (ng/mL)	Commodity (ng/mL)
0.0	0.0
0.2	4.0
0.6	12.0
1.8	36.0
5.0	100.0
15.0	300.0

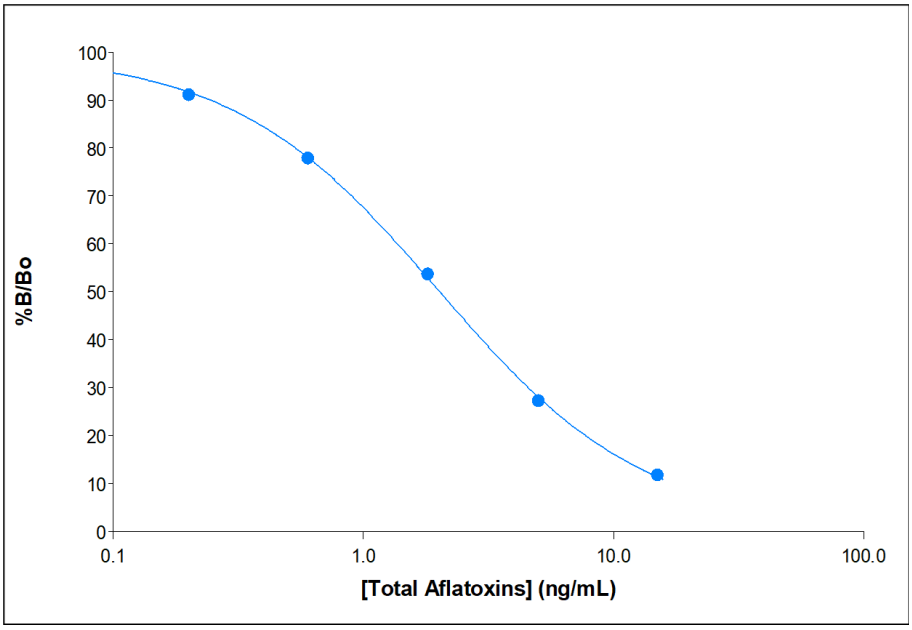
The sample dilution results in a standard curve from 4 ppb to 300 ppb. If a sample contains aflatoxin at greater concentration than the highest standard, it should be diluted appropriately in 70% methanol and retested. The extra dilution step should be taken into consideration when expressing the final result.

Assay Characteristics

A typical example of the Helica Total Aflatoxin assay run in duplicate yielded the following standard curve and within assay variation (average shown).

Aflatoxin (ng/mL)	%B/B_0	CV (%)
0.0	100	-
0.2	86	2.3
0.6	67	2.9
1.8	39	1.8
5.0	18	3.6
15.0	7	10.1

The graph below represents the data in the table above.



Average accuracy on corn samples naturally contaminated with aflatoxins (n = 21 per each concentration)

Aflatoxin in corn (ng/mL)	Average (ng/mL)	Standard Deviation	Acceptable Range (ng/mL)
5.2	5.27	0.60	2.6 - 7.8
18.3	18.13	1.26	11.0 - 25.6
87.9	80.88	6.51	59.8 - 116
300	243.46	7.48	204 - 396

Technical Assistance

For questions or comments, please contact your local distributor. You can also email techsupport@hygiena.com, visit our Contact Us page for regional phone numbers or request technical support at https://www.hygiena.com/hygiena/technical-support-request.html.