Helica® Total Aflatoxin Hydro ELISA

Product Number – KIT5009 (941AFL01AQ-96)

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For the quantitative detection of total aflatoxins in corn.

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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 allowed in human and animal foodstuffs. Accurate and rapid determination of the presence of aflatoxin in commodities is critical.

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 Total Aflatoxin Hydro ELISA assay was developed to determine the level of aflatoxins at a wide range of 4 - 320 ppb in corn using an aqueous extraction procedure.

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. Helica 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 Total Aflatoxin Hydro 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 deionized water and extraction buffer. 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. A chromogenic 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, 0.1, 0.25, 0.8, 2.5 and 8.0 ng/mL in aqueous solution, *Ready-to-Use*.
2X Bottles	Conjugate	2 x 12 mL of Aflatoxin B1 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)
90X Capsules	Hydro extraction buffer**	Buffer powder for extraction; use two capsules for each sample (5 g).

* *TWEEN® 20 is a registered trademark of CRODA International Plc.*
** Additional capsules (Product No. 928XB001) can be purchased separately.

Materials Required But Not Provided

- Microcentrifuge and tubes
- Analytical balance
- Extraction cup
- Graduated cylinders: 25 mL and 1000 mL
- Vortex mixer
- Distilled water (or deionized) water
- Water bath
- Pipettor with tips: 100 µL, 200 µL and 1,000 µL
- Timer
- Wash bottle
- Absorbent paper towels
- 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

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

1. Place a bottle containing deionized or distilled in a water bath set at 40 °C.
2. Let it pre-warm for 1 hour before use.
3. Weigh 5.0 ± 0.2 grams of ground sample into an extraction cup.
4. Add two capsules of Hydro extraction buffer into the cup. (Cat# 928XB001)
5. Add 25 mL of warm deionized or distilled water, wait 5 minutes to soften capsules.
6. Shake vigorously for 2 - 3 minutes.
7. Transfer 1 mL into a microcentrifuge tube and centrifuge for 1 minute.
8. Using a new pipette tip, dispense 700 µL of water into a clean tube and add 100 µL of the supernatant. Vortex for a few seconds to mix prior to analysis.
9. The sample is now ready for testing.
10. The final dilution for use in calculation is **1:40**.

Assay Procedure

Note: It is recommended to use a multi-channel (8-channel) pipettor for this assay.

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-Tween 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. Remove one mixing well for each sample and another six (6) mixing wells for the six (6) standards. Remove double the 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 each green-marked 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 pipettor at least three (3) times.
6. Using a new pipette tip for each, transfer 100 µL of contents from each mixing well to a corresponding antibody-coated microtiter well. 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 microwells into a discard basin. Wash the microwells by filling each with PBS-T wash buffer, then decanting the wash into a discard basin. Repeat this step 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 antibody-coated well and 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.

Interpretation of Results

Construct a dose-response curve using either the unmodified OD values or 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 1:40 ratio. Therefore, the level of aflatoxin shown by the standard must be multiplied by 40 in order to indicate the ng of aflatoxin per gram of commodity (ppb) as follows.

Standard (ng/mL)	Commodity (ppb)
0.0	0.0
0.1	4.0
0.25	10.0
0.8	32.0
2.5	100.0
8.0	320.0

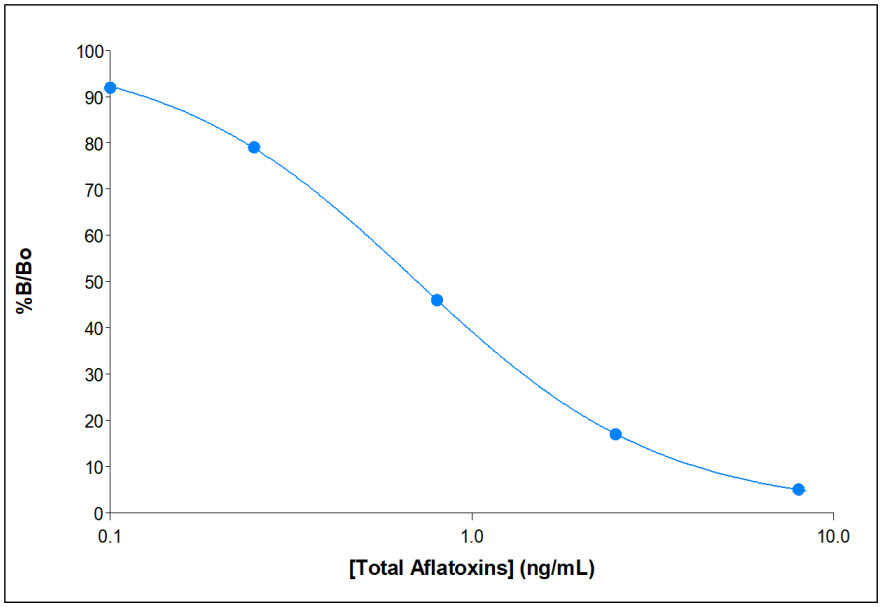
The sample dilution results in a standard curve from 4 ppb to 320 ppb. If a sample contains aflatoxin at a greater concentration than the highest standard, it should be diluted appropriately with water and retested.
The extra dilution step should be taken into consideration when expressing the final result.

Assay Characteristics

Data from the average of seven consecutive standard curves gave the following results

Aflatoxin (ng/mL)	%B/B_0	CV (%)
0.0	1.835	1.9
1.0	1.626	1.5
2.5	1.283	1.0
5.0	0.720	2.4
10.0	0.338	1.0
20.0	0.147	1.0

The graph below represents the data in the table above.



Recovery

Average recovery of corn samples naturally contaminated with aflatoxins. (n = 5 per each contamination level)

Aflatoxin Contamination in Corn (ppb)	Average (ppb)	Recovery (%)	CV (%)
4.8	5.23	109	12.23
18.3	16.64	91	3.09
87.9	81.49	93	5.40
300	246.54	82	2.61

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.