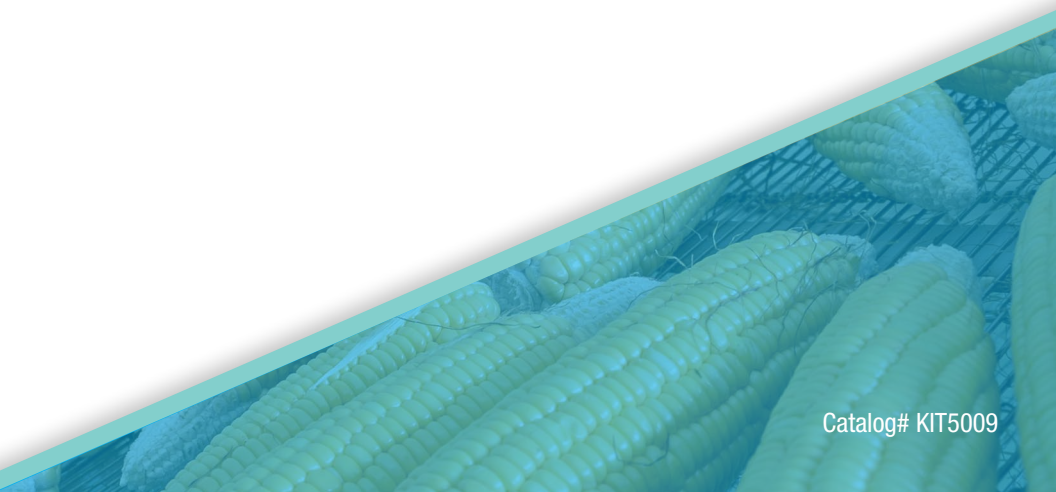
Helica™ Total Aflatoxin Hydro ELISA

For the quantitative detection of total aflatoxins in corn.



Catalog# KIT5009

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This package insert must be read in its entirety before using this product.

Contents

Intended Use

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 following 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 at a high level. Aflatoxins have been implicated in human health disorders including hepatocellular carcinoma, aflatoxicosis, Reye's syndrome and chronic hepatitis. Animals are exposed to aflatoxins by consumption of 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, 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. The Helica™ Total Aflatoxin Hydro ELISA kit was developed to determine aflatoxins with a wide range of 4 - 320 ppb in corn using aqueous extraction procedure.

Field of Use

Data obtained from Helica™ assays should not be used for human diagnostic or human treatment purpose. Assays are not approved by the United States Food and Drug Administration or any other U.S. or non-U.S. 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 supervision of technically qualified persons.

Principle of the Method

The Helica™ Total Aflatoxin Hydro 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. 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 an interpretative result is determined.

Storage and Shelf Life

- Bring all reagents to room temperature (19 - 25°C) before use.
- Store reagents at 2 to 8°C, and do not use beyond expiration date(s). Never freeze kit components.
- Do not return unused reagents back into their original bottles. The assay procedure details volumes required.
- Adhere to all time and temperature conditions stated in the procedure.

Precautions

Read this manual carefully before starting the test. The test must be performed by specialized and trained staff.

- Never pipette reagents or samples by mouth.
- Handle the test kit in accordance with good laboratory practices (GLP).
- Do not interchange reagents between kits of different lot numbers.
- Do not use reagents beyond the expiration date of the kit.
  The alteration of a reagent can cause inaccurate results.
- Do not exchange the vial caps.
- Use sterile pipette tips.
- Do not use solutions if they become cloudy or precipitate.
- Substrate solution is light sensitive. Avoid exposure to direct light.
- Do not allow wells to dry completely.
- Handle any solution with gloves.
- During the sample extraction, avoid cross-contamination.
- Devices such as a blender must be cleaned after each sample preparation.
- Substrate solution contains TMB, which is highly toxic if inhaled, ingested, or comes in contact with the skin. Please refer to the SDS.
- If you get in contact with toxic or irritating substances, rinse the affected skin area with plenty of water. Please refer to the SDS.
- Stop Solution contains sulfuric acid, which is corrosive. Please refer to the SDS.
- Avoid incubating on cold work benches.

Kit Contents

1X Pouch	Antibody coated microwell plate		96 wells (12 eight-well holder) coated with a mouse anti-aflatoxin monoclonal antibody, *Ready-to-Use*.
1X Plate	Mixing wells	Green	96 non-coated wells (12 eight-well strips) in a microwell holder, *Ready-to-Use.*
6X Vials	Aflatoxin standards	Black cap	1.5 mL/vial of aflatoxin at the following concentrations: 0.0, 0.1, 0.25, 0.8, 2.5, 8.0 ng/mL in aqueous solution, *Ready-to-Use*.
2X Bottles	Aflatoxin-HRP conjugate	Green cap	2 x 12 mL of aflatoxin conjugated peroxidase in buffer with preservative, *Ready-to-Use*.
1X Bottle	Substrate reagent	Blue cap	12 mL stabilized tetramethylbenzidine (TMB), *Ready-to-Use*.
1X Bottle	Stop solution	Red cap	12 mL acidic solution, *Ready-to-Use*.
1X Pouch	Washing buffer		PBS WITH 0.05% Tween20®, bring to 1 liter with distilled water and store refrigerated.
90X Capsules	Hydro extraction buffer*		Buffer powder for extraction, use two capsules for each sample (5 g).

*Additional capsules (Cat# 928XB001) can be purchased separately.

Materials Required But Not Provided

- Single or multi-channel pipettor with 100, 200, and 1000 µL tips
- Timer
- Wash bottle
- Absorbent paper towels
- Microcentrifuge and tubes
- Analytical balance
- Extraction cup
- Graduated cylinders (25 mL and 1000 mL)
- Vortex mixer
- Distilled water (or deionized) water
- Water bath
- Microplate reader equipped with a 450 nm filter

Preparation of Sample

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

1. Place a bottle containing deionized or distilled in water bath set at 40 °C.
2. Let it pre-warm for 1 hour before use.
3. Weigh 5.0 ± 0.2 grams 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. Final dilution for use in calculation is 1:40.

Assay Procedure

1. Bring all reagents and samples to room temperature before use and perform the sample preparation at room temperature. Reconstitute the PBS-Tween packet by washing out the contents with a gentle stream of distilled water into a 1-Liter container.
2. Remove 1 green-marked mixing well for each sample and another 6 green-marked mixing wells for 6 standards.
3. Remove double the number of antibody-coated wells and return unused wells to the foil pack with desiccant.
4. Mix each reagent by swirling the reagent bottle prior to use.
5. Dispense 200 µL of conjugate (green-capped bottle) into each green-marked mixing well.
6. Using a 100 µL pipettor with a new pipette tip for each, add 100 µL of standards and samples to the green-marked mixing wells.
7. Using an 8-channel pipettor, mix the liquid in the wells by pipetting it up and down 3 times. Transfer 100 µL into the antibody-coated wells. The mixing wells contain enough solution to run each standard or sample in duplicate.
8. Incubate for 15 minutes at room temperature.
9. Discard the contents from the wells into a discard basin. Using a wash bottle, fill the wells with PBS-Tween wash buffer, then dump the buffer out of the wells into a discard basin. Repeat this step four more times.
10. Tap the wells (face down) on a layer of absorbent towels to remove residual buffer.
11. Using an 8-channel pipettor, add 100 µL of substrate reagent (blue-capped bottle) to each well. Incubate at room temperature for 5 minutes. Cover to avoid direct light.
12. Using an 8-channel pipettor, add 100 µL of stop solution (red-capped bottle) in the same sequence and at the same pace as the substrate reagent was added.
13. Read the optical density (OD) of each microwell with a microplate reader using a 450 nm filter. Read within 10 minutes after addition of stop solution.

Interpretation of Results

Construct a dose-response curve using either the unmodified OD values or the OD values expressed as a percentage (%B/Bo) 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 a standard vial refers to the contents of that vial. However, the sample has been diluted at a 1:40 ratio, and so 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

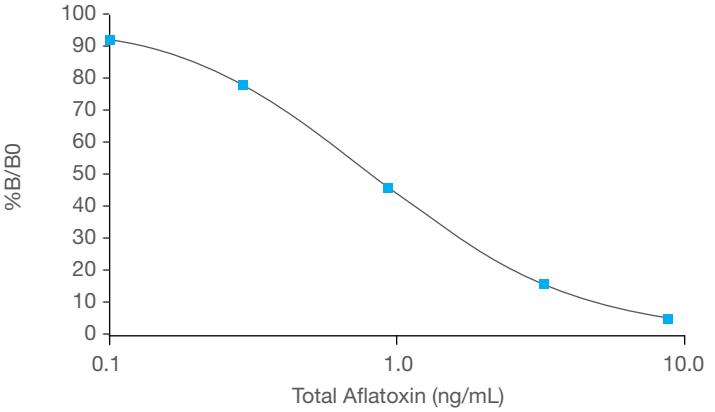
The sample dilution results in a standard curve from 4 ppb to 320 ppb. If a sample contains aflatoxin at greater concentration than the highest standard, it should be diluted appropriately with water and retested. The extra dilution step should be taken into consideration when expressing the final result.

Assay Characteristics

Data from seven consecutive standard curves gave the following results:

Aflatoxin (ng/mL)	%B/B0	CV (%)
0.0	100	-
0.1	92	3.14
0.25	79	4.75
0.8	46	7.12
2.5	17	12.54
8.0	5	12.22

The graph below represents the data in the table above.



Recovery

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

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