Helica® Aflatoxin B1 Rapid ELISA

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For the quantitative detection of Aflatoxin B1 in grains, nuts, cottonseeds, cereals and other commodities including animal feeds.

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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, cottonseed 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 at high concentrations.

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 Aflatoxin B1 Rapid ELISA assay is a competitive enzyme-linked immunoassay intended for the quantitative detection of aflatoxin B1 in grains, nuts, cottonseeds, cereals and other commodities including animal feeds.

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 Aflatoxin B1 Rapid ELISA assay is a solid phase direct competitive enzyme immunoassay (ELISA). An aflatoxin specific antibody optimized to react with B1 is coated to a polystyrene microwell. Toxins are extracted from a ground sample with 70% methanol. The extracted sample and HRP-conjugated Aflatoxin B1 are mixed and added to the antibody-coated microwell. Aflatoxin from the extracted sample and HRP-conjugated Aflatoxin B1 compete to bind with the antibody coated to the microwell. After this incubation period, the contents of the wells are decanted, washed and an HRP substrate (TMB) is added which develops a blue color in the presence of an enzyme. 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 450nm (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 B1 at the following concentrations: 0.0, 0.2, 0.5, 1.0, 2.0 and 4.0 ng/mL in organic 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)

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Materials Required but Not Provided

- A grinder sufficient to render sample to the particle size of fine instant coffee
- Collection container with minimum 125 mL capacity
- Balance with 20 g measuring capability
- Graduated cylinder: 100 mL
- Methanol, reagent grade: 70 mL per sample
- Distilled or deionized water: 30 mL per sample
- Filter Paper: Whatman #1 or equivalent
- Filter Funnel
- Glass tubes
- Centrifuge
- Pipettor with tips: 100 µL and 200 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.

Extraction Procedure

1. Prepare the extraction solution (70% Methanol) by adding 30 mL of distilled or deionized water to 70 mL of methanol (reagent grade) for each sample to be tested.
2. Grind a representative sample to the particle size of fine instant coffee (95% passes through a 20-mesh screen).
3. Weigh out a 20 g ground portion of the sample and add 100 mL of the extraction solvent (70% methanol).
   Note: The ratio of sample to extraction solvent is 1:5 (w/v).
4. Mix by shaking in a sealed container or in a blender for a minimum of 2 minutes.
5. Allow the particulate matter to settle, then filter 5 – 10 mL of the extract through a Whatman #1 filter paper (or equivalent) and collect the filtrate to be tested. The sample is now ready for testing.

Assay Procedure

Note: It is recommended that a multi-channel pipettor be utilized to perform the assay. If a single channel pipettor is used, it is recommended that no more than a total of 16 samples and standards (2 test strips) are run.

1. Bring all the reagents to room temperature before use. 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 in a microwell holder for each standard and sample to be tested. Place an equal number of antibody-coated microtiter wells in another microwell holder. 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 the conjugate into each Dilution Well.
5. Using a fresh pipette tip for each, dispense 100 µL aliquots of each standard and sample to appropriate dilution well containing conjugate. Mix by priming pipettor at least 3 times.
   Note: The operator must record the location of each standard and sample throughout the test.
6. Using a new pipette tip for each, transfer 100 µL from each dilution well to a corresponding antibody-coated microtiter well. Incubate at room temperature for 15 minutes.
   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 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 wash for a total of 5 washes.
8. Tap the microwells (face down) on a layer of absorbent towels to remove residual buffer.
9. Measure the required volume of substrate reagent (1mL/strip or 120 µL/ well) and place in a separate container. Add 100 µL to each microwell. Incubate at room temperature for 5 minutes. Cover to avoid direct light.
10. Measure the required volume of stop solution (1mL/strip or 120µL well) and place in a separate container. Add 100 µL in the same sequence and at the same pace as the substrate was added.
11. Read the optical density (OD) of each microwell with a microtiter plate reader using a 450nm filter (using an air blank or a differential filter of 630 nm). Record the optical density (OD) of each microwell.

12. Setting the zero standard as 100% binding (B_o), calculate % binding (%B) for each standard and sample as a percentage of the zero binding (%B/B_o).

Interpretation of Results

Construct a dose-response curve using the OD values expressed as a percentage of the OD of the zero standard (0.0 ng/mL) 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 5:1 ratio with 70% methanol, so the level of aflatoxin shown by the standard must be multiplied by 5 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	1.0
0.5	2.5
1.0	5.0
2.0	10.0
4.0	20.0

The sample dilution results in a standard curve from 1 ppb to 20 ppb. If a sample contains aflatoxin at greater 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.

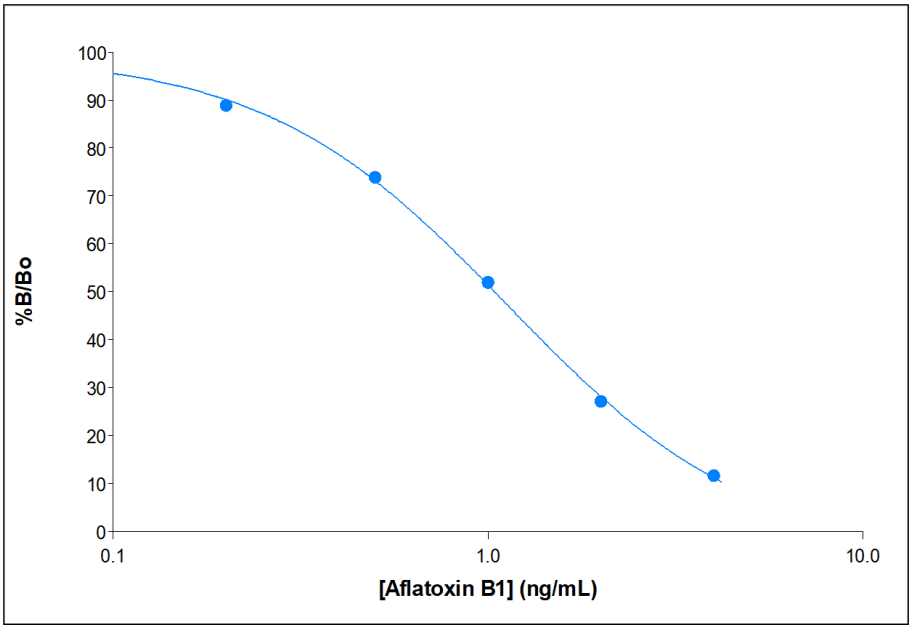
Assay Characteristics

Performance Data - Precision

Intra-assay Variation A typical example of the Helica Aflatoxin B1 assay run in duplicate yielded the following standard curve and within assay variation.			
Sample (ng/mL)	**Mean OD**	**%B/B_0**	**CV (%)**
0.0	1.835	100	1.9
1.0	1.626	88.6	1.5
2.5	1.283	69.9	1.0
5.0	0.720	39.2	2.4
10.0	0.338	18.4	1.0
20.0	0.147	8.0	1.0

Inter-assay Variation Between assay variation is expressed as percentage of B_0 for each standard. n= 6 assays		
Sample (ng/mL)	**%B/B_0**	**CV (%)**
1.0	88.5	4.2
2.5	75.0	3.3
5.0	53.9	4.8
10.0	28.1	8.7
20.0	12.0	11.9

The below figure is a representative standard curve for aflatoxin B1 based on the above data table (inter-assay). Data from seven (7) separate standard curves was used to generate this graph.



The limit of detection (LOD) is defined as the mean plus two standard deviations of multiple determinations of an Aflatoxin-free commodity extract. As different commodities generate somewhat different zeros due to 'matrix inhibition' effects, it follows that the LOD is commodity specific and should be measured empirically for each different commodity.

Using the Helica Aflatoxin B1 assay:

LOD for corn in <1.0 ppb, n=10
LOD for peanuts is <1.0 ppb, n= 10

The Helica Aflatoxin B1 ELISA has been tested at 1mg/mL (1 million ppb) without evidence of anomalous binding behavior (high-dose hook effect). Therefore, it may be used to assess gross environmental contamination.

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.