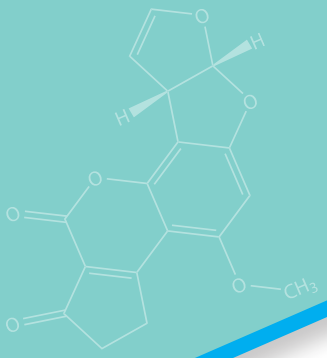

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Helica™ Total Aflatoxin Cannabis ELISA

For the quantitative detection of aflatoxin B_1, B_2, G_1 and G_2 in cannabis flower, gummy, tincture, and vape.

Catalog# 201AFL01C-96

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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: B_1, B_2, G_1 and G_2. The risks also extend to cannabis products, which are rising in popularity with more states approving their recreational use. Aflatoxin B_1 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. Most state government agencies have regulations regarding the amount of aflatoxins allowable in cannabis products. Accurate and rapid determination of the presence of aflatoxin in commodities is of paramount importance.

Cannabis growers, processors, and analytical laboratories can use the Helica™ Total Aflatoxin Cannabis ELISA as a fast, reliable method to screen for total aflatoxins in cannabis flower, gummy, tincture, and vape.

Field of Use

Data obtained from Helica™ 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 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 Cannabis ELISA is a solid phase competitive inhibition enzyme immunoassay. An aflatoxin specific antibody optimized to cross react with all four subtypes of aflatoxin, is coated to a polystyrene microwell. Toxins are extracted from a ground sample and after dilution, added to the appropriate well. If aflatoxin is present it will bind to the coated antibody. Subsequently, aflatoxin bound to horse-radish peroxidase (HRP) is added and binds to the antibody not already occupied by aflatoxin present in the sample or standard. After this incubation period, the contents of the wells are decanted, washed and an HRP substrate is added which develops a blue color in the presence of enzyme. The intensity of the color is directly proportional to the amount of bound conjugate and inversely proportional to the amount of aflatoxin in the standard or sample. 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 450nm (OD_{450}). 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.

Storage and Shelf Life

- Store reagents at 2°C to 8°C, and do not use beyond expiration date(s). Never freeze kit components.

Technical Assistance

For questions or comments, please contact your local distributor. You can call 1-714-578-7830 or email techsupport@hygiena.com. Technical support can also be requested at https://www.hygiena.com/hygiena/technical-support-request.html.

Precautions

- Bring all reagents to room temperature (19° - 25°C) before use.
- 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.
- Never pipette reagents or samples by mouth.
- The Stop Solution contains acid. Do not allow to contact skin or eyes. If exposed, flush with water.
- Consider all materials, containers and devices that are exposed to sample or standards to be contaminated with aflatoxin. Wear protective gloves and safety glasses when using this kit.
- Dispose of all materials, containers and devices in the appropriate receptacle after use.
- HRP-labeled conjugate and TMB-substrates are photosensitive and are packaged in a protective opaque bottle. Store in the dark and return to storage after use.
- For samples highly contaminated with subtypes other than B_1, the assay may underestimate the total aflatoxin level.
- Mycotoxins (aflatoxins, trichothecenes, and others) are well known carcinogens in humans and are thus considered highly toxic. Do not dispose of these materials down the drain.

    1. Before conducting the assay, prepare a waste container to act as a receptacle for your kit waste. Eject contaminated pipette tips and all other related materials into this container.
    2. 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). 5-6% NaOCl will denature the mycotoxins and neutralize the waste, making it safe for disposal. Invert the container several times to thoroughly coat all waste.
    3. 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.

Kit Contents

1X Pouch	Antibody coated microwell plate		96 wells (12 x 8 well strips) in a microwell holder coated with a mouse anti-ochratoxin A antibody, *Ready-to-Use*.
1X Plate	Mixing wells	Green	96 non-coated wells (12 x 8 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, 0.02, 0.05, 0.1, 0.2, and 0.4ng/mL in 50% methanol, *Ready-to-Use*.
1X Bottle	Aflatoxin HRP-conjugate	Green cap	12 mL of aflatoxin conjugated to peroxidase in buffer with preservative, *Ready-to-Use*.
2X Bottles	Assay diluent	Brown cap	2 x12 mL proprietary assay diluent, *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.

Materials Required But Not Provided

- Grinder sufficient to render sample to particle size of fine instant coffee
- Collection tube: Minimum 50mL capacity
- Balance: 20g measuring capability
- Distilled or deionized water: 3-6mL per sample
- Filter Paper: Whatman #1 or equivalent
- Filter Funnel
- Centrifuge
- Pipettor with tips: 100µL and 200µL
- Timer
- Wash bottle
- Dilution tubes
- Absorbent paper towels
- Microplate reader with 450nm filter
- Water bath that reaches 50°C

Preparation of Cannabis Samples

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

Flower

1. Grind flower in a blender.
2. Weigh 1.0g of sample into a clean tube.
3. Prepare 70% ethanol (i.e. 3mL of dH2O with 7mL of ethanol). Add 70% ethanol at 10-fold over the sample (i.e. 10mL). Vortex continuously for 2 minutes.
4. Pass the sample through a Whatman No. 1 filter to remove particulate. Save the filtrate in a clean tube.
5. Dilute the filtrate 1:20 in PBS-T wash buffer. For example, add 50µL of sample to 950µL of PBS-T wash buffer. Vortex briefly.
6. The sample is now ready for testing.
7. Final dilution for use in calculation is 1:200.

Preparation of Cannabis Samples, con't.

Gummy

1. Prepare a warm water bath at 50°C.
2. Using a clean razor, chop up gummies to smaller pieces (~0.5cm in diameter) to increase surface area that will be exposed to solvent.
3. Weigh 1.0g of diced gummies and transfer to a clean tube.
4. Prepare 70% ethanol (i.e. 6mL of dH2O with 14mL of ethanol). Add 70% ethanol at 20-fold over the sample (i.e. 20mL). Place sample into the warm water bath and incubate for 15 minutes or until the gummy has melted. This will vary depending on the type and concentration of gelling agent employed to make the gummy. Occasionally, remove the sample from water bath to mix and return to water bath.
5. After gummy has fully melted, mix continuously for 2 minutes.
6. Dilute the sample 1:10 in 70% ethanol. For example, add 100µL of sample to 900µL of 70% ethanol. Vortex briefly.
7. The sample is now ready for testing.
8. Final dilution for use in calculation is 1:200.

Tincture oil

1. Transfer 1.0mL of tincture oil to a clean tube.
2. Prepare 70% ethanol (i.e. 1.5mL of dH2O with 3.5mL of ethanol). Add 70% ethanol extraction solution at 5-fold over the sample (i.e. 5mL).
3. Vortex continuously for 2 minutes. Centrifuge the sample for 5 minutes at 3,500 rpm. Use upper ethanol layer for dilution.
4. Dilute sample to 70% ethanol at 1:40 (v/v). For example, add 15µL of sample to 585µL of 70% ethanol. **Note:** some precipitation/cloudiness may be observed. Vortex briefly.
5. The sample is now ready for testing.
6. Final dilution for use in calculation is 1:200.

Preparation of Cannabis Samples, con't.

Vape

1. Transfer 0.5mL of vape to a clean tube.
2. Prepare 70% methanol (i.e. 3mL of dH2O with 7mL of methanol). Add 70% methanol extraction solution at 20-fold over the sample (i.e. 10mL).
3. Vortex continuously for 3 minutes. Let sample settle for 5 minutes.
4. Dilute sample into PBS-T buffer at 1:10 (v/v). For example, add 50µL of sample to 450µL of PBS-T. **Note:** some precipitation/cloudiness may be observed. Vortex briefly.
5. The sample is now ready for testing.
6. Final dilution for use in calculation is 1:200.

Assay Procedure

1. Bring all the reagents to room temperature before use. Reconstitute the PBS-Tween packet by washing out the contents with a gentle stream of distilled water into a 1-Liter container. Q.S. to 1 Liter with distilled 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 twice the number of Antibody Coated Microtiter Wells in another microwell holder to run in duplicate.
3. Dispense 200µL of the Assay Diluent into each mixing well.
4. Using a new pipette tip for each, add 100µL of each standard and prepared sample to appropriate mixing well containing diluent. Mix by priming pipettor at least 3 times. **Note:** Operator must record the location of each Standard and Sample throughout test.
5. Using a new pipette tip for each, transfer 100µL of contents from each mixing well to a corresponding Antibody Coated Microtiter Well. Incubate at room temperature for 30 minutes. It is recommended that a multi-channel pipettor be used for this step in order to minimize beginning to end variation. The mixing wells contain enough solution to run each standard and/or sample in duplicate if so desired.
6. Decant the contents from microwells into a discard basin. Wash the microwells by filling each with PBS-Tween wash buffer, then decanting the wash into a discard basin. Repeat wash for a total of 5 washes.

Assay Procedure, con't.

7. Tap the microwells (face down) on a layer of absorbent towels to remove residual wash buffer.
8. Add 100µL of aflatoxin HRP-conjugate to each antibody coated well and incubate at room temperature for 30 minutes. Cover to avoid direct light.
9. Repeat steps 6 and 7.
10. Measure the required volume of Substrate Reagent (1mL/strip or 120µL/well) and place in a separate container. Add 100µL to each microwell. Incubate at room temperature for 10 minutes. Cover to avoid direct light.
11. Measure the required volume of Stop Solution (1mL/strip or 120µL/well) and place in a separate container. Add 100µL in the same sequence and at the same pace as the Substrate Reagent was added.
12. Read the optical density (OD) of each microwell with a microtiter plate reader using a 450nm filter. Record the optical density (OD) of each microwell.
13. 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).

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 either the unmodified OD values or the OD values expressed as a percentage (%B/B_o) 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 by extraction solvent as instructed in the EXTRACTION PROCEDURE and also in wash buffer or 70% ethanol and so the level of aflatoxin shown by the standard must be multiplied by 200 in order to indicate the ng per gram (ppb) of the commodity as follows:

Standard (ng/mL)	Flower, Gummy, Tincture, and Vape Commodity (ppb)1:200
0	0
0.02	4
0.05	10
0.1	20
0.2	40
0.4	80

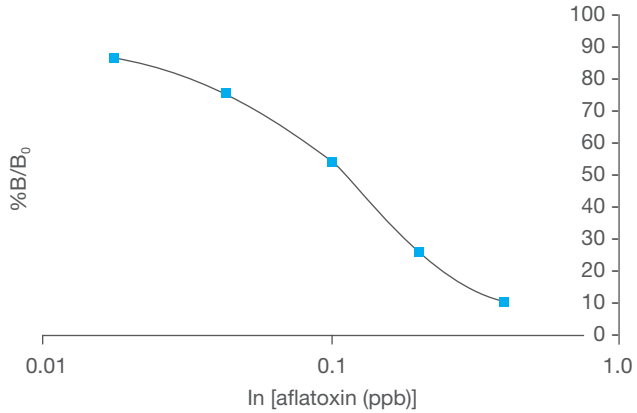
The sample dilution results in a range of detection of 4-80ppb. If a sample contains aflatoxin at a greater concentration than the highest standard, it should be diluted appropriately in extraction solvent and retested. The extra dilution step should be taken into consideration when expressing the final result.

Assay Characteristics

Data from 10 consecutive standard curves gave the following results:

Standard (ng/mL)	%B/B$_0$	CV (%)
0	100	-
0.02	88	2.6
0.05	76	3.2
0.1	53	4.5
0.2	26	8.4
0.4	10	10.8

The below figure is a representative standard curve for aflatoxin based on the above data table.



Cross-Reactivity

The assay will cross-react with aflatoxin analogues as follows:
B_1 - 100%, B_2 – 14.6%, G_1 – 4.8%, G_2 - <0.32%

Recovery

Samples were purchased from a dispensary and spiked with 20 µg/kg of aflatoxin. After drying overnight, the samples were extracted and tested on the Helica™ Total Aflatoxin Cannabis Assay with following results:

Samples	Spike level (µg/kg)	Mean % recovery	CV (%)	n
Flower	20	90	9	9
Gummy	20	106	16	6
Tincture cannabis oil	20	103	10	3
Vape	20	102	4	3

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