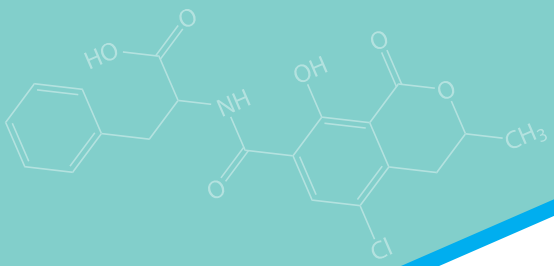

hygiena®

Helica® Ochratoxin A Cannabis ELISA

For the quantitative detection of ochratoxin A in cannabis flower, gummy, tincture and distillate.

Catalog# KIT5024

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

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Intended Use

Ochratoxin A is a toxic secondary metabolite produced by several molds of the *Aspergillus* and *Penicillium* genera, including *Aspergillus ochraceus*. Ochratoxin A is a nephrotoxin and carcinogen. In humans, exposure to ochratoxin A has been linked to Balken endemic nephropathy (BEN), a chronic kidney disease associated with tumors of the renal system. Impairment of renal system has also been reported in swine. In turkeys and chickens, symptoms include retarded growth, decreased feed conversion, nephropathy and mortality. Feed refusal has also been observed in turkeys. A decrease in egg production and shell quality was reported in both turkeys and chickens. Ochratoxin A has been frequently detected in human foods and animal feed with the main human bioburden deriving from cereals and grain products, although a wide range of commodities has been found to contain the toxin, including cannabis products.

Cannabis growers, processors, and analytical laboratories can use the Helica Ochratoxin A Cannabis ELISA as a quick, reliable method to screen for ochratoxin A in cannabis flower, gummy, tincture, and distillate.

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 Ochratoxin A Cannabis ELISA is a solid phase competitive inhibition enzyme immunoassay. An ochratoxin A specific antibody is coated to a polystyrene microwell. Toxins are extracted from a ground sample and after dilution, added to the appropriate antibody coated well. If ochratoxin A is present it will bind to the coated antibody. Subsequently, ochratoxin A bound to horse-radish peroxidase (HRP) is added and binds to the antibody not already occupied by ochratoxin A 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 ochratoxin A in the standard or sample. Therefore, as the concentration of ochratoxin A 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 (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 helica-cs@hygiena.com

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 ochratoxin A. 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.
- 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 eight-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 eight-well strips) in a microwell holder, *Ready-to-Use*.
6X Vials	Ochratoxin A standards	Black cap	1.5 mL/vial of ochratoxin A at the following concentrations: 0.0, 0.05, 0.1, 0.2, 0.4 and 0.8 ng/mL in 70% methanol, *Ready-to-Use*.
1X Bottle	Ochratoxin A HRP-conjugate	Green cap	12 mL of ochratoxin A conjugated to peroxidase in buffer with preservative, *Ready-to-Use*.
2X Bottles	Assay diluent	Brown cap	2 x 12 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*.
2X Pouches	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 50 mL capacity
- Balance: 20 g measuring capability
- Methanol reagent grade
- Distilled or deionized water
- Microcentrifuge tubes (1.5 mL)
- Centrifuge
- Pipettor with tips: 50 µL, 100 µL, 200 µL and 1,000 µL
- Timer
- Wash bottle
- Absorbent paper towels
- Microplate reader with 450 nm filter
- Water bath that reaches 55 °C

Preparation of Cannabis Samples

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

Flower, tincture and distillate

1. Prepare a warm water bath at 55 °C.
2. For flower, grind in a blender.
3. Weigh 1.0 g of sample into a clean tube.
4. Add 80% methanol at 20-times the sample amount (i.e., 20 mL for 1 g). Place sample into the warm water bath and incubate for 15 minutes. Vortex for 2 minutes.
5. Transfer 1 mL to a microcentrifuge tube. Let cool for 10 minutes, and centrifuge at 4,000 rpm for 3 minutes.
6. Remove clear mid-layer and dilute 1:10 in 70% methanol. For example, add 100 µL of sample to 900 µL of 70% methanol. Vortex briefly.
7. The sample is now ready for testing.
8. Final dilution for use in calculation is 1:200.

Preparation of Cannabis Samples, con't.

Gummy

1. Prepare a warm water bath at 55 °C.
2. Using a clean razor, chop up gummies to smaller pieces (~0.5 cm in diameter) to increase surface area that will be exposed to solvent.
3. Weigh 1.0 g of diced gummies and place into a clean tube.
4. Add washing buffer at 10-times the sample amount (i.e., 10 mL for 1 g). Place sample into the warm water bath and incubate for 15 minutes. Vortex for 2 minutes.
5. Transfer 1 mL to a microcentrifuge tube. Cool for 10 minutes, and centrifuge at 4,000 rpm for 3 minutes.
6. Transfer clear mid-layer to a new tube and dilute 1:20 in 70% methanol. For example, add 50 µL of sample to 950 µL of 70% methanol. Vortex briefly.
7. The sample is now ready for testing.
8. 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 or deionized water into a 1-Liter container. Q.S. 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 twice the number of antibody-coated microwells in another microwell holder to run in duplicate. If running a single well per standard and sample, please scale down the reagent volumes accordingly.
3. Dispense 200 µL of the Assay Diluent into each mixing well.
4. Using a new pipette tip for each, add 100 µL of each standard and prepared sample to appropriate mixing well containing diluent. Mix by priming pipettor at least 3 times. Incubate at room temperature for 20 minutes. **Note:** Some precipitation/cloudiness may be observed from sample mixture. Operator must record the location of each Standard and Sample throughout test.
5. Using a new pipette tip for each, transfer 100 µL of contents from each mixing well to a corresponding antibody-coated microwell. It is recommended that a multi-channel pipettor be used for this step in order to minimize beginning to end variation. Incubate at room temperature for 30 minutes.
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.
7. Tap the microwells (face down) on a layer of absorbent towels to remove residual wash buffer.
8. Add 100 µL of ochratoxin A-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 (1 mL/strip or 120 µL/well) and place in a separate container. Add 100 µL to each microwell. Incubate at room temperature for 10 minutes. Cover to avoid direct light.

Assay Procedure, con't.

11. Measure the required volume of Stop Solution (1 mL/strip or 120 µL/well) and place in a separate container. Add 100 µL 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 450 nm 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 ochratoxin A 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 and also in 70% methanol as instructed in the Preparation of Cannabis Samples, so the level of ochratoxin shown by the standard must be multiplied by the total dilution factor of 200 in order to indicate the ng per gram (ppb) of the commodity according to the table.

Standard (ng/mL)	Sample (ppb) 1:200
0.0	0
0.05	10
0.1	20
0.2	40
0.4	80
0.8	160

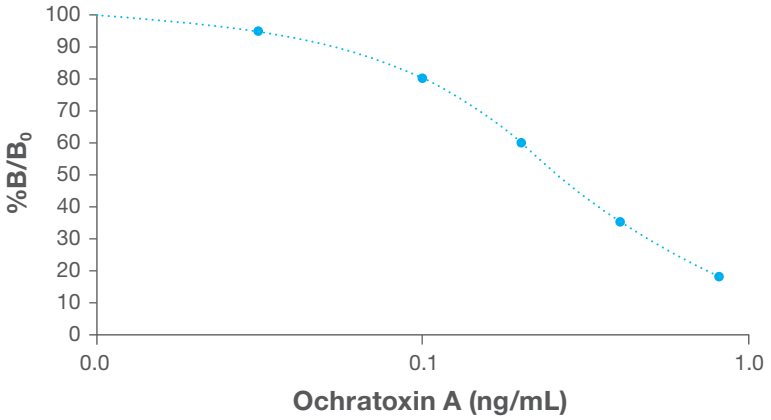
The sample dilution results in a range of detection of 8 - 160 ppb. If a sample contains ochratoxin A at a greater concentration than the highest standard, it should be diluted appropriately in 70% methanol and retested. The extra dilution step should be taken into consideration when expressing the final result.

Assay Characteristics

Data from 21 consecutive standard curves gave the following results:

Standard (ng/mL)	%B/B$_0$	CV (%)
0	100	-
0.05	91	4.0
0.1	80	5.7
0.2	60	7.6
0.4	35	9.7
0.8	18	17.8

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



Recovery

Samples were purchased from a dispensary and spiked with 20 µg/kg of ochratoxin. After drying overnight, the samples were extracted and tested on the Ochratoxin A Cannabis Assay with following results:

Samples	Spike level (µg/kg)	Mean % recovery	CV (%)	n
Flower	20	75	8.4	9
Gummy	20	82	10.6	7
Tincture cannabis oil	20	89	8.7	11
Distillate	20	78	11.1	7

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