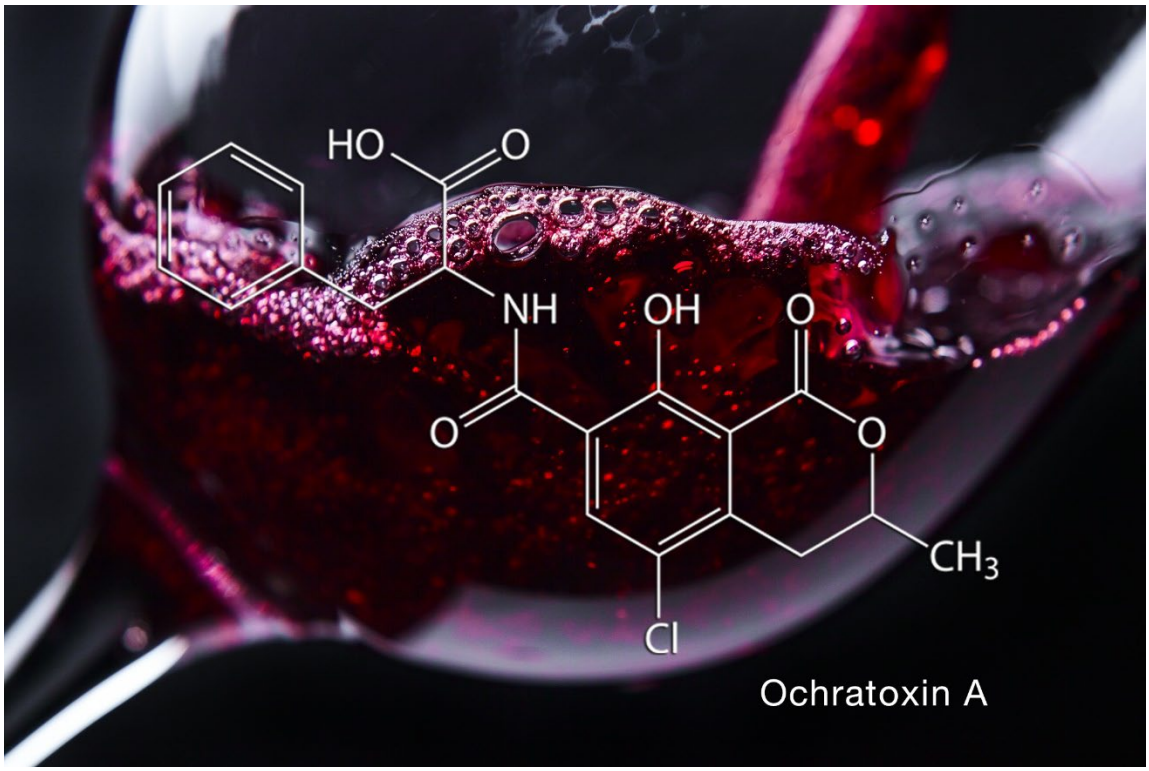
Helica® Ochratoxin A Wine ELISA

Product Number – KIT5016 (981OCH01W – 96)



Helica® Ochratoxin A Wine ELISA

For the qualitative or semi-quantitative estimation of ochratoxin A in liquid vine products from grape must to fortified wines, around the EU limit of 2 ppb (µg/L).

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Introduction – Ochratoxin A

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 Balkan endemic nephropathy (BEN), a chronic kidney disease associated with tumors of the renal system. Impairment of renal system has also been reported in swine. 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. These include green and roasted coffee, cocoa, spices and grape derivatives such as raisins, grape juice and wines.

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 Ochratoxin A Wine ELISA assay has been specifically designed for the qualitative or semi-quantitative estimation of ochratoxin A in liquid vine products from grape must to fortified wines, around the EU limit of 2 ppb (µg/L). Samples whose visual analysis lies between 1 ppb and 2 ppb should be further evaluated by an alternative method such as HPLC or quantitative ELISA. This should facilitate the in-process control of wine production and provide a quick and inexpensive check on wines from emerging producers.

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 Ochratoxin A Wine ELISA assay is a solid phase competitive inhibition enzyme immunoassay. An antibody with high affinity to ochratoxin A is coated to a polystyrene microwell. Standard or sample is added to the appropriate well and if ochratoxin A is present it will bind to the coated antibody. Subsequently, ochratoxin A bound to horseradish peroxidase (HRP) is added and binds to the antibody not already occupied by the ochratoxin A present in the standard or sample. After this incubation period, the contents of the wells are decanted, washed and a 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. A solution containing a red dye is added to stop the enzyme reaction and simultaneously causes a color change leaving the bluest wells deep blue/purple and the lightest wells a mauve/pink, thus facilitating a visual qualitative estimate of ochratoxin A in the sample by comparing to the color of the standards.

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-ochratoxin A 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)
3X Vials	Standards	1.5 mL/vial of ochratoxin A at the following concentrations: 0.0, 0.3 and 0.6 ng/mL in 70% methanol, *Ready-To-Use*.
1X Bottle	Conjugate	12 mL of ochratoxin A conjugated to peroxidase in buffer with preservative, *Ready-to-Use*.
2X Bottles	Assay Diluent	2 x 12 mL proprietary sample diluent, 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)

** TWEEN® 20 is a registered trademark of CRODA International Plc.*

Materials Required but Not Provided

- Collection container with a minimum 100 mL capacity
- Balance with 20 g measuring capability
- Graduated cylinder: 100 mL
- Methanol or acetonitrile: 7 or 80 mL, reagent grade per sample
- Distilled or deionized water: 3 or 20 mL per sample
- Centrifuge
- Pipettor with tips: 100 µL and 200 µL
- Timer
- Wash bottle
- Absorbent paper towels
- Microplate reader with 650 nm filter (optional)

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
Wine, Port, Sherry, Grape Must and Grape Juice

1. Pipette 3 mL of sample into a disposable 10 mL tube.
2. Add 7 mL of absolute methanol, cap the tube and shake vigorously to mix. Note: The ratio of sample to solvent is 1:3.33 (v/v).
3. The sample is now ready for testing.
4. The final dilution for use in interpretation = **1:3.33**

Licorice

1. Prepare extraction solvent (80% acetonitrile) by adding 20 mL of distilled water to 80 mL of acetonitrile for each sample to be tested.
2. Transfer 100 mL of 80% acetonitrile to a container and add 10 g of sample.
   Note: The ratio of sample to solvent is 1:10 (w/v).
3. Mix by shaking in a sealed container for a minimum of 5 minutes.
4. Allow the acetonitrile and licorice layers to separate. Alternatively, centrifuge a portion of the sample at 3,500 rpm for 5 minutes to speed the separation. Collect the upper layer containing the ochratoxin to be tested.
5. Dilute an aliquot of the extract 1:10 with 70% methanol.
6. The sample is now ready for testing.
7. The final dilution for use in interpretation = **1:100**

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 to run no more than a total of 16 samples and standards (2 test strips.

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 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 sample diluent into each mixing well.
5. 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 three (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 of contents from each mixing well to a corresponding antibody-coated microtiter well. It is recommended that a multichannel pipettor be used for this step in order to minimize beginning-to-end variation. Incubate at room temperature for 20 minutes. The mixing wells contain enough solution to run each standard and/or sample in duplicate if desired.
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 3 washes.
8. Tap the microwells (face down) on a layer of absorbent towels to remove residual wash buffer.

9. Add 100 µL of ochratoxin A-HRP conjugate to each antibody-coated well and incubate at room temperature for 10 minutes. Cover to avoid direct light.
10. Repeat steps 6 and 7.
11. Measure the required volume of substrate reagent (1 mL/strip or 120 µL/well) and place into a separate container. Add 100 µL to each microwell. Incubate at room temperature for 10 minutes. Cover to avoid direct light.
12. Measure the required volume of Stop Solution (1 mL/strip or 120 µL/well) and place into a separate container. Add 100 µL in the same sequence and at the same pace as the substrate reagent was added.
13. Compare the color of the sample wells to the standards with the appropriate final dilution factor to determine compliance with local and internationally accepted limits for ochratoxin A. Alternatively, a permanent record can be made by reading the OD of the wells at 650 nm.

Interpretation of Results

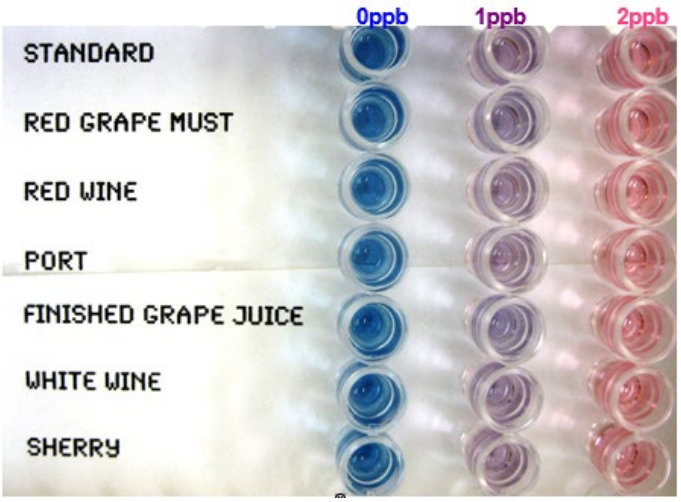
The information contained on the label of each standard vial refers to the contents of that vial. However, the wine samples have been diluted at a 3.33:1 ratio with absolute methanol in the extraction procedure. Therefore, the level of ochratoxin shown by the standard must be multiplied by 3.33 in order to indicate the ng per mL (ppb) of the commodity. Additionally, the licorice sample has been diluted at a 10:1 ratio by extraction solvent as instructed in the EXTRACTION PROCEDURE and also 10:1 in 70% methanol and so the level of ochratoxin shown by the standard must be multiplied by 100 in order to indicate the ng per gram (ppb) of the commodity as follows:

Standard (ng/mL)	Wine, Port, Sherry, Grape Must & Grape Juice Commodity (ppb) 1:3.33	Licorice Commodity (ppb) 1:100
0	0	0
0.3	1	30
0.6	2	60

Compare the color of the unknown sample to the color of the standards to determine if the unknown sample is above or below the desired cut-off value.

Assay Characteristics

Typical assay results are shown for vine products ranging from grape must to fortified wines spiked with 1 ppb or 2 ppb ochratoxin A. There is a clear distinction between 0 ppb, 1 ppb and 2 ppb.



The Ochratoxin A in Wine Assay is intended primarily as a qualitative/semi-quantitative assay with a visual endpoint. The inter-assay and intra-assay variability of 0 ppb, 1 ppb, and 2 ppb ochratoxin A that were spiked into the commodities with respect to the methanol standards was assessed using a standard acid stop method with subsequent OD measurements of 450 nm (i.e., yellow endpoint). The following data were obtained:

Intra-assay Comparison

Data from the averages of eight consecutive standard curves performed alongside the averages of eight replicates of spiked commodities gave the following results:

	Standard		Grape Must	
Ochratoxin A (ppb)	Mean OD	CV (%)	Mean OD	CV (%)
0	1.854	4.2	1.850	4.4
1	0.874	5.7	0.959	3.9
2	0.371	8.9	0.413	5.3

	Standard		White Wine	
Ochratoxin A (ppb)	Mean OD	CV (%)	Mean OD	CV (%)
0	1.515	2.8	1.487	3.8
1	0.702	2.8	0.756	4.1
2	0.278	6.5	0.322	6.5

	Standard		Red Wine	
Ochratoxin A (ppb)	Mean OD	CV (%)	Mean OD	CV (%)
0	1.881	4.0	1.870	4.7
1	1.011	3.1	1.109	3.3
2	0.427	4.9	0.451	3.1

Inter-assay Comparison

Data from the average of eight independent assays performed alongside spiked commodities gave the following results. Spiked samples were calculated as a percentage of the 0 ppb, 1 ppb and 2 ppb methanol standards.

	0 ppb		1 ppb		2 ppb	
Commodity	% Standard	CV (%)	% Standard	Commodity	% Standard	CV (%)
Grape Must	98.5	3.2	116.2	4.8	102.6	8.5
Red Wine	97.2	2.8	99.5	3.0	103.7	8.5
White Wine	97.2	2.3	100.3	7.0	98.5	5.8
Grape Juice	95.5	2.2	107.5	5.1	85.8	5.9

	Standard		Grape Juice	
Ochratoxin A (ppb)	Mean OD	CV (%)	Mean OD	CV (%)
0	2.035	1.2	2.009	5.5
1	1.001	3.0	1.044	3.4
2	0.447	6.3	0.366	7.1

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.