



## OCHRATOXIN A ASSAY FOR GRAINS

CAT. NO. 941OCH01M - 96

### **OCHRATOXIN A**

Ochratoxin A is a toxic metabolite produced by several molds of the *Aspergillus flavus* and *Penicillium* genera, including *Aspergillus ochraceus*. The fungal species has the potential to produce ochratoxin A, a known nephrotoxin and carcinogen. It has been frequently detected in human foods and animal feed, mainly in cereal products, although a range of commodities has been reported to contain the toxin. In humans, exposure to ochratoxin A has been linked with Balken endemic nephropathy (BEN), a chronic kidney disease associated with tumors of the renal system. In animals, impairment of renal function has been reported in swine. In turkeys and chickens symptoms included 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.

### **INTENDED USE**

The Helica Ochratoxin A Assay is a competitive enzyme-linked immunoassay intended for the quantitative detection of Ochratoxin A levels in grains, cereals, coffee, and other commodities including animal feeds.

### **ASSAY PRINCIPLE**

The Helica™ Ochratoxin A Assay is a solid phase direct competitive enzyme immunoassay. An Ochratoxin specific antibody optimized to cross react primarily with Ochratoxin A (see cross-reactivity information) is coated to a polystyrene microwell. Toxins are extracted from a ground sample with 70% methanol. The extracted sample and HRP-conjugated Ochratoxin are mixed and added to the antibody-coated microwell. Ochratoxin from the extracted sample and HRP-conjugated Ochratoxin 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 Ochratoxin in the sample and standards. Therefore, as the concentration of Ochratoxin in the sample or standard increases, the intensity of the blue color will decrease. An acidic stop solution is added which changes the chromagen color from blue to yellow. The microwells are measured optically by a microplate reader with an absorbance filter of 450 nm (OD<sub>450</sub>). The optical densities of the samples are compared to the OD's of the kit standards and an interpretative result is determined.

### **MATERIALS SUPPLIED**

1 pouch: Antibody coated microwells	96 wells (12 eight well strips) in a microwell holder coated with a mouse anti-ochratoxin antibody
1 plate: Dilution wells (green)	96 non-coated wells (12 eight well strips) wells in a microwell holder.
6 vials: Ochratoxin A Standards	1.5 mL/vial of ochratoxin A at the following concentrations: 0.0, 0.4, 1.0, 2.0, 4.0, and 8.0 ng/mL in organic solution
2 bottle: Ochratoxin A HRP-conjugate	2 x 12 mL of ochratoxin A conjugated to peroxidase in buffer with preservative
1 bottle: Substrate Reagent	15 mL stabilized tetramethylbenzidine (TMB)
1 bottle: Stop Solution	15 mL Acidic Solution

## **MATERIALS REQUIRED BUT NOT PROVIDED**

### **Extraction Procedure**

Grinder sufficient to render sample to particle size of fine instant coffee  
Collection Container: Minimum 125 mL capacity  
Balance: 20 g measuring capability  
Graduated cylinder: 100 mL  
Methanol: 70 mL reagent grade per sample  
Distilled or deionized water: 30 mL per sample  
Filter Paper: Whatman #1 or equivalent  
Filter Funnel

### **Assay Procedure**

Pipettor with tips: 100 µl and 200 µl  
Timer  
Wash bottle  
Absorbent paper towels  
Microplate reader with 450 nm filter

## **PRECAUTIONS**

1. Bring all reagents to room temperature (19° - 27°C) before use.
2. Store reagents at 2 to 8°C, and do not use beyond expiration date(s). Never freeze kit components.
3. Do not return unused reagents back into their original bottles. The assay procedure details volumes required.
4. Adhere to all time and temperature conditions stated in the procedure.
5. Samples tested should have a pH of 7.0 (±1.0). Excessive alkaline or acidic conditions may affect the test results.
6. Never pipette reagents or samples by mouth.
7. Standards are flammable. Caution should be taken in the use and storage of these reagents.
8. The Stop Solution contains acid. Do not allow to contact skin or eyes. If exposed, flush with water.
9. Consider all materials, containers and devices that are exposed to sample or standards to be contaminated with toxin. Wear protective gloves and safety glasses when using this kit.
10. Dispose of all materials, containers and devices in the appropriate receptacle after use.

## **EXTRACTION PROCEDURE**

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

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 (50% 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.
2. Place one Dilution 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.
3. Dispense 200  $\mu$ l of the Conjugate into each Dilution Well.
4. Using a new pipette tip for each, add 100  $\mu$ l of each Standard and Sample to appropriate Dilution Well containing Conjugate. 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 100  $\mu$ l of contents from each Dilution Well to a corresponding Antibody Coated Microtiter Well. Incubate at room temperature for 15 minutes.
6. Decant the contents from microwells into a discard basin. Wash the microwells by filling each with distilled or deionized water, then decanting the water 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 water.
8. Measure the required volume of Substrate Reagent (1 mL/strip or 120  $\mu$ l/well) and place in a separate container. Add 100  $\mu$ l to each microwell. Incubate at room temperature for 5 minutes.
9. Measure the required volume of Stop Solution (1 mL/strip or 120  $\mu$ l/well) and place in a separate container. Add 100  $\mu$ l in the same sequence and at the same pace as the Substrate was added.
10. 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.

**INTERPRETATION OF RESULTS**

Construct a dose-response curve using either the unmodified OD values or the OD values expressed as a percentage 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 sample dilution results in a standard curve from 2 ppb to 40 ppb. If a sample contains Ochratoxin A 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.

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, and so the level of ochratoxin shown by the standard must be multiplied by 5 in order to indicate the ng of ochratoxin per gram of commodity (ppb) as follows:

standard (ng/mL)	commodity (ppb)
0.0	0.0
0.4	2.0
1.0	5.0
2.0	10.0
4.0	20.0
8.0	40.0

**APPENDIX****PERFORMANCE DATA****WITHIN ASSAY VARIATION**

A typical example of the Helica Ochratoxin A assay run in duplicate yielded the following standard curve and within assay variation.

<b>ppb in sample</b>	<b>mean OD</b>	<b>CV%</b>
0.0	1.726	1.0
2.0	1.385	1.8
5.0	0.965	1.1
10.0	0.610	3.8
20.0	0.343	1.0
40.0	0.157	1.0

**BETWEEN ASSAY VARIATION**

Between assay variation is expressed as percentage of Bo for each standard.  
n= 6 assays.

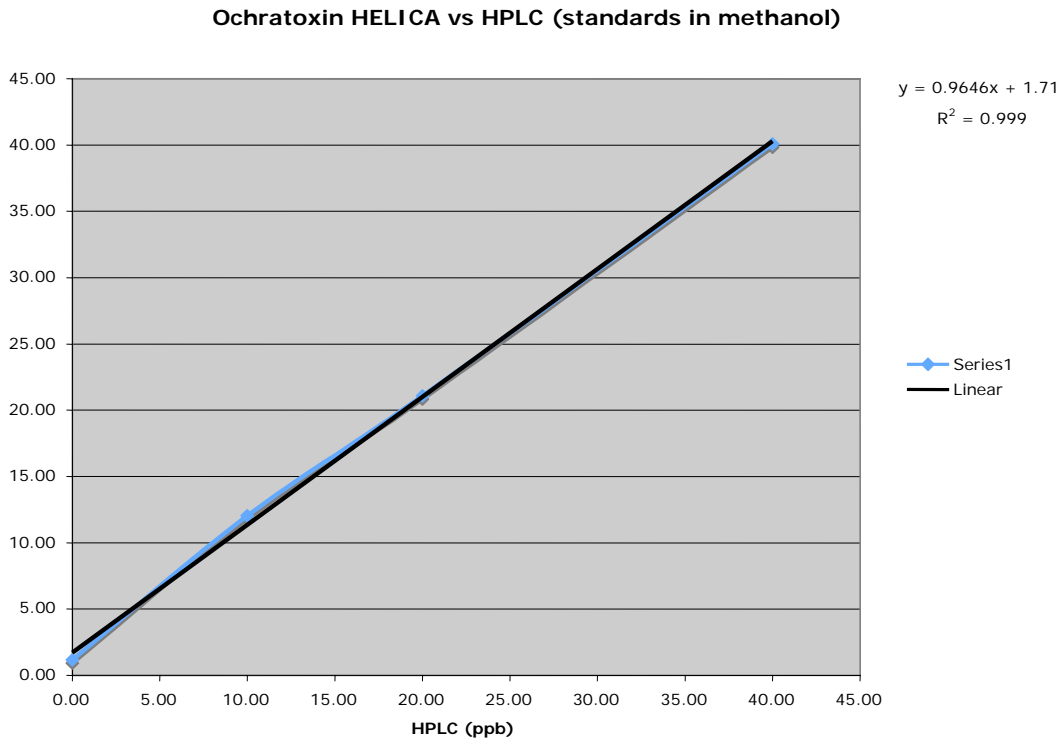
<b>ppb in sample</b>	<b>B/Bo%</b>	<b>CV%</b>
2.0	77.7	4.0
5.0	54.1	5.5
10.0	35.2	7.1
20.0	19.7	4.3
40.0	9.6	4.7

Limit of detection (LOD) is defined as the mean plus two standard deviations of multiple determinations of an ochratoxin-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 Ochratoxin A assay:

LOD for corn is 2.0 ppb n= 10

LOD for green coffee is 2.0 ppb n= 10



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

#### **CROSS-REACTIONS**

The assay will cross-react with Ochratoxin A analogues as follows:

Ochratoxin A-100%

Ochratoxin B- 9.3%

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