



OCHRATOXIN / WINE ASSAY (48-well kit)

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 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. 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 (Assessment of Dietary Intake of Ochratoxin A by the Population of EU Member States: Report of Experts Participating in Task 3.2.7, Jan 2002).

INTENDED USE

The HELICA Ochratoxin A / Wine 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 ($\mu\text{g/L}$). Samples whose visual estimation lies between 1 and 2ppb should be further

evaluated by an alternative method such as HPLC or quantitative ELISA. This should facilitate in process control of wine production and provide a quick and inexpensive check on wines from emerging producers.

ASSAY PRINCIPLE

The HELICA Ochratoxin A / Wine Assay is a solid phase direct enzyme immunoassay. An antibody with high affinity to Ochratoxin A is coated onto polystyrene microwells. 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 horse-radish peroxidase (HRP) is added and binds to the antibody not already occupied by Ochratoxin A present in the standard or sample. After this incubation period, the contents of the wells are decanted, washed and 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 in the sample by comparing to the color of the standards.

MATERIALS SUPPLIED

1 pouch:	Antibody coated microwells	48 wells (6 x 8-well strips) in a microwell holder coated with a mouse anti-ochratoxin A antibody
1 plate:	Mixing wells	48 wells non-coated (6x8-well strips) in a microwell holder
3 vials:	Ochratoxin A Standards *	0.8 mL/vial of ochratoxin A at the following concentrations

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	(see page 2)	0.0, 1.0, and 2.0 ng/mL in 70% methanol (see page 2) *
1 bottle:	Sample diluent	12 mL proprietary sample diluent
1 bottle:	Ochratoxin A HRP-conjugate	6 mL ochratoxin A conjugated to HRP in buffer with preservative
1 bottle:	Substrate Reagent	6 mL stabilized TMB
1 bottle:	Stop Solution	6 mL proprietary stop solution
1 pouch:	Wash buffer (PBS-T)	PBS WITH 0.05% Tween20®, bring to 1 liter with distilled water and store refrigerated

* standard ng/mL	commodity (ppb)
0.0	0.0
0.3	1.0
0.6	2.0

MATERIALS REQUIRED BUT NOT PROVIDED

Pipettor with tips: 100 µl and 200 µl
 Absolute methanol
 10 mL capped tubes.
 Disposable pipettes capable of delivering 3 and 7 mL
 Wash bottle
 Absorbent paper towels
 Timer

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. Never pipette reagents or samples by mouth.
6. Standards are flammable. Caution should be taken in the use and storage of these reagents.
7. 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.
8. Dispose of all materials, containers and devices in the appropriate receptacle after use.

SAMPLE PREPARATION

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.

ASSAY PROCEDURE

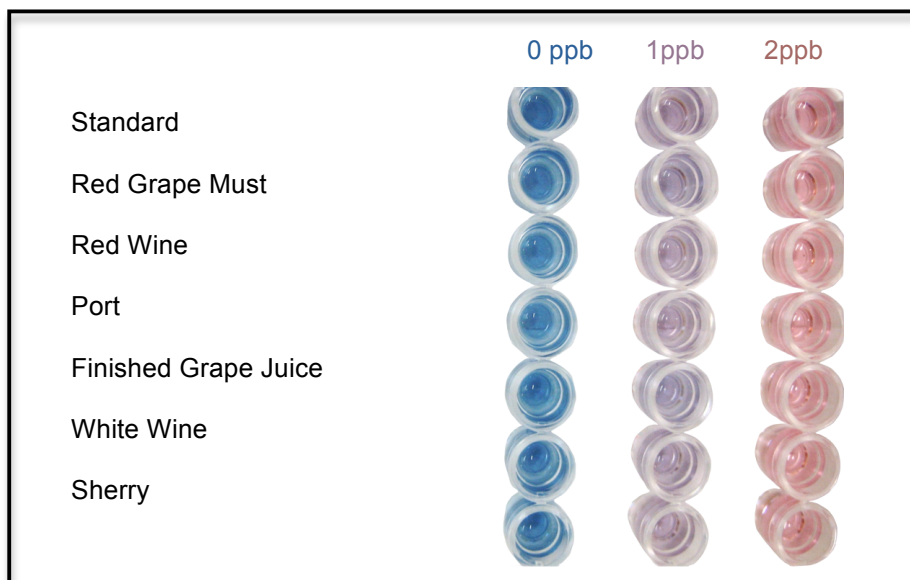
1. Bring all the reagents to room temperature before use. Reconstitute the PBS-Tween packet by washing out the contents with a gentle stream of distilled water into a 1-Liter container. Q.S. to 1 Liter with distilled water and store refrigerated when not in use.
2. Place one mixing well in a microwell holder for each Standard and Sample to be tested. Place an equal number of Antibody Coated Microtiter wells in another microwell holder.

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3. Dispense 200 μl of the sample 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.
Note: 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 Microtiter Well. 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 20 minutes. 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 3 washes.
7. Tap the microwells (face down) on a layer of absorbent towels to remove residual water.
8. Add 100 μl of conjugate to each antibody coated well and incubate at ambient temperature for 10 minutes.
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 ambient temperature for 10 minutes.
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 was added.
12. Compare the color of the sample wells to the zero, 1 ppb and 2 ppb standards 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.

ASSAY CHARACTERISTICS

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



PERFORMANCE PARAMETERS

The HELICA ochratoxin/wine assay is intended primarily as a qualitative/semi-quantitative assay with a visual end-point. The inter-assay and intra-assay variability of zero, 1, 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 (i.e. yellow endpoint). The following data were obtained:

INTRA-ASSAY COMPARISON: 8 replicates of each methanol standard were compared with 8 replicates of spiked commodities

ASSAY 1				
	Standard		GRAPE MUST	
ppb	OD	CV%	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

ASSAY 2			RED WINE	
ppb	Standard			
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

ASSAY 3			WHITE WINE	
ppb	Standard			
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

ASSAY 4			GRAPE JUICE	
ppb	Standard			
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

INTER-ASSAY COMPARISON: Between assay comparisons for 0, 1, and 2 ppb spiked samples were calculated as a percentage of the 0, 1, and 2 ppb methanol standards in 8 assays.

	0 ppb		1 ppb		2 ppb	
	% standard	CV%	% standard	CV%	% 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