



QUANTITATIVE ASSAY FOR OCHRATOXIN A IN ALCOHOLIC BEVERAGES
(96-well kit)
 (CAT NO. 9810CH01ALC)

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 Alcoholic Beverage Assay has been specifically designed for the quantitative estimation of Ochratoxin A in liquid vine products, from

grape must to fortified wines, around the EU limit of 2 ppb ($\mu\text{g/L}$) and in beer from 0.04-0.8 ng/mL.

ASSAY PRINCIPLE

The HELICA Ochratoxin A Alcoholic Beverage 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. The reaction is stopped by the addition of an acid solution which causes the blue color to change to yellow.

MATERIALS SUPPLIED

1 pouch:	Antibody coated microwells	96 wells (12 x 8-well strips) in a microwell holder coated with a mouse anti-ochratoxin A antibody
1 plate:	Mixing wells	96 wells non-coated (12x8-well strips) in a microwell holder. The wells are color coded red.
6 vials:	Ochratoxin A Standards *	1.5 mL/vial of ochratoxin A at the following concentrations 0.0, 0.02, 0.05, 0.1, 0.2, 0.4 ng/mL in 70% methanol (see page 3) *
2 bottles:	Assay diluent	2 x12 mL proprietary assay diluent
1 bottle:	Ochratoxin A HRP-conjugate	12 mL ochratoxin A conjugated to HRP in buffer with preservative
1 bottle:	Substrate Reagent	12 mL stabilized TMB
1 bottle:	Stop Solution	12 mL acidic stop solution
1 pouch:	Wash buffer (PBS-T)	PBS WITH 0.05% Tween20®, bring to 1 liter with distilled water and store refrigerated

MATERIALS REQUIRED BUT NOT PROVIDED

Pipettor with tips: 100 µl and 200 µl
 Absolute methanol
 10 mL capped tubes.
 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

Dilute samples of wine, grape must or juice 1:20 in 70% methanol. Dilute samples of beer 1:2 with absolute methanol.

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.
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.
 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 30 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 30 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. Construct a dose-response standard curve of optical density (OD) against Ochratoxin A content. Sample unknowns are measured by interpolation from the standard curve. If a sample is higher than the highest standard, it should be further diluted in 70% methanol and re-tested. The added dilution factor should be taken into account when expressing the result.

ASSAY CHARACTERISTICS

The values for Ochratoxin A given on the standards refer to the Ochratoxin A content of the vial. As wine, grape must and juice are diluted 1:20 and beer 1:2. this translates to a value in the commodity as follows:

Standard (ng/mL)	Wine, Must, or Juice (ng/mL)	Beer (ng/mL)
0.0	0.0	0.0
0.02	0.4	0.04
0.05	1.0	0.10
0.10	2.0	0.20
0.20	4.0	0.40
0.40	8.0	0.80

PERFORMANCE PARAMETERS

All commodities were grown and processed in California except for the beer which was brewed in Belgium.

Comparing the commodities to the zero standard (70% methanol) in four assays in duplicate gave the following results:

	OD	SD	CV%
Standard Zero	1.918	0.09	4.7
Red Wine (Merlot)	1.922	0.09	4.7
Standard Zero	1.847	0.11	6.0
White Wine (Chardonnay)	1.817	0.11	6.1
Standard Zero	1.926	0.11	5.7
Port	1.909	0.13	6.8
Standard Zero	1.945	0.11	5.7
Sherry	1.955	0.05	2.6
Standard Zero	2.030	0.12	5.9
Red Grape Must	2.045	0.09	4.4
Standard Zero	1.827	0.07	3.8
Red Grape Juice	1.819	0.07	3.8
Standard Zero	1.870	0.09	4.8
Beer	1.841	0.11	6.0

RECOVERY DATA

Having shown that none of the commodities contained Ochratoxin A, each was spiked with Ochratoxin A, at levels of 0.0,0.4,1.0,2.0, 4.0, and 8.0 ng/mL and the standard solvent (70% methanol) was similarly spiked. Beer was spiked at 0.0, 0.04, 0.1, 0.2, 0.4, and 0.8 ng/mL. All samples were diluted 1:20 with 70% methanol except the beer which was diluted 1:2 in absolute methanol and assayed as described above (In the kit as presented the standards are pre-diluted and should be used without further dilution). Recoveries for each commodity with reference to the standards are given below.

Standard	Red Wine%	White Wine%	Port %	Sherry %	Must %	Juice %	Beer %
0.02	93	85	100	105	98	65	120
0.05	104	98	102	104	101	72	112
0.10	100	92	98	105	99	80	115
0.20	103	110	98	93	98	93	120
0.40	110	99	108	101	91	95	113

The results demonstrate that the Helica Biosystems Quantitative Ochratoxin A assay can be used to measure Ochratoxin A in a wide variety of alcoholic and non-alcoholic beverages.

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