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# Product Information

**Ochratoxin A ELISA Kit**
**for alcoholic beverages**

Catalog Number **SE120011**
Storage Temperature 2–8 °C

## TECHNICAL BULLETIN

### Product Description

The Ochratoxin A ELISA (Enzyme-Linked Immunosorbent Assay) Kit for alcoholic beverages 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 horseradish 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.

The Ochratoxin A ELISA Kit for alcoholic beverages has been specifically designed for the quantitative determination of Ochratoxin A in liquid wine products, from grape must to fortified wines, around the EU limit of 2 ppb (μg/L) and in beer from 0.04–0.8 ng/mL.

### Components

1. Ochratoxin Low Matrix Microplate - 981OCH01ALC: 96 wells (12 x 8 well strips) in a microwell holder coated with a mouse anti-ochratoxin A antibody.
2. Ochratoxin Low Matrix Standard – 6 vials, 983S1OCH01ALC: 1.5 mL/vial of ochratoxin A at the following concentrations 0.0, 0.02, 0.05, 0.1, 0.2, and 0.4 ng/mL in 70% methanol.
3. Ochratoxin Low Matrix HRP Conjugate - 984ALCOCH01: 12 mL of ochratoxin A conjugated to HRP in buffer with preservative.
4. Assay Diluent - 937AD001: 12 mL of proprietary assay diluent.
5. TMB Substrate - 916T001: 12 mL of stabilized 3,3′,5,5′-tetramethylbenzidine (TMB).
6. Stop Solution - 946P001: 12 mL of Acidic Solution.
7. PBST Wash Buffer Powder - 915X001: 1 packet of PBST. Bring to 1 liter with distilled water and store refrigerated.
8. Dilution Wells: 96 wells non-coated (12 x 8 well strips) in a microwell holder. The wells are color coded red.

### Reagents and Equipment Required but Not Provided.

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 100 μL to 200 μL volumes.
- Absolute Methanol.
- Absorbent paper towels.
- Graph paper, or computer and software for ELISA data analysis.

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

**Preparation Instructions**

Sample Preparation

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

**Storage/Stability**

Store reagents at 2–8 °C, and do not use beyond expiration date(s). Never freeze kit components.

HRP-labelled conjugate and TMB Substrate are photosensitive and are packaged in a protective opaque bottle. Store in the dark and return to storage.

**Procedure**

1. Bring all the reagents to room temperature (19-27 °C) 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 microwells in another microwell holder.
3. Dispense 200 µL of the assay diluent into each dilution well.
4. Using a new pipette tip for each, add 100 µL of each standard and prepared sample to appropriate dilution 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 dilution well to a corresponding Antibody Coated well. It is recommended a multichannel pipettor be used for this step in order to minimize beginning to end variation. Incubate at room temperature for 30 minutes. The dilution wells contain enough solution to run each standard and/or sample in duplicate, if so desired.
6. Decant the contents from microwells. Wash the microwells by filling each with PBST wash buffer, then decanting the wash. Repeat wash for a total of 3 washes.
7. Tap the microwells (face down) on a layer of absorbent towels to remove residual PBST.
8. Add 100 µL of HRP-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 TMB Substrate (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. The blue color will change to yellow.
12. Read the optical density (OD) of each microwell with a microplate reader at 450 nm using an air blank or a differential filter of 630 nm.

**Results**

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.

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.

The values for Ochratoxin A on the standards refer to the contents 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 (ppb) | Beer (ppb) |
|---|---|---|
| 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 |

Recovery Data

Commodities were 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. Recoveries for each commodity with reference to the standards are given below.

| | Recovery % | | | | | | |
|---|---|---|---|---|---|---|---|
| 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 |

Sensitivity

This ELISA kit has been tested to have a sensitivity of 2 ppb (µg/L).

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