

## Product Manual

### **AgraQuant® Ochratoxin 2/40 ELISA kit** **Article number 10002102/10002103**

#### **Intended use**

The AgraQuant® Ochratoxin 2/40 ELISA kit is an immunoassay designed for the quantitative analysis of ochratoxin (A and B) presence in food and feed components. This product is intended for laboratory use.

#### **Performance characteristics**

**Limit of detection (LOD):** 1.9 ppb \*

**Limit of quantification (LOQ):** 2 ppb \*

**Range of quantification:** 2 -40 ppb

**Plate format:** 96 (10002102) or 48 wells (10002103)

**Assay time:** 15 minutes

\* Determined in red wine. The LOD and LOQ can vary and may depend on the type of matrix being tested. Please contact your Romer Labs representative with any questions.

#### **About ochratoxin**

Ochratoxins (OTA) are a group of mycotoxins mainly produced by the fungal species *Aspergillus ochraceus* and *Penicillium verrucosum*. Ochratoxins can be found in a wide variety of commodities such as grains, raisins, barley, soy products, and coffee. The International Agency for Research on Cancer (IARC) has classified ochratoxin A as a Group 2B potential human carcinogen, as shown by carcinogenicity in animal studies. Moreover, ochratoxins have nephrotoxic, hepatotoxic and teratogenic effects.

## Product information

### About AgraQuant® Ochratoxin 2/40 ELISA kit

The AgraQuant® Ochratoxin 2/40 test kit is a direct competitive enzyme-linked immunosorbent assay (ELISA) that quantitatively determines ochratoxin (A and B) presence. This product is intended for use in grains, coffee, soybean, beer, wine and other commodities.

### Storage information

Upon receipt, immediately transfer the AgraQuant® Ochratoxin 2/40 ELISA kit to refrigerated storage conditions and keep it at 2-8°C (35-46°F) when not in use. Do not freeze. Do not use the kit beyond the expiration date indicated on the label.

### Contents of the kit

The AgraQuant® Ochratoxin 2/40 ELISA kit contains the following items:

#### 10002102

- 96 antibody-coated microwells (12 eight-well strips) in a microwell holder sealed in a foil pouch
- 96 non-coated dilution microwells (12 white eight-well strips)
- 5 vials each with 1.5 mL of ochratoxin standard (0, 2, 5, 20 and 40 ppb)
- 1 green-capped bottle with 25 mL of ochratoxin-conjugate solution
- 1 blue-capped bottle with 15 mL of substrate solution
- 1 red-capped bottle with 15 mL of stop solution

#### 10002103

- 48 antibody-coated microwells (6 eight-well strips) in a microwell holder sealed in a foil pouch
- 48 non-coated dilution microwells (6 white eight-well strips)
- 5 vials each with 0.75 mL of ochratoxin standard (0, 2, 5, 20 and 40 ppb)
- 1 green-capped bottle with 12.5 mL of ochratoxin-conjugate solution
- 1 blue-capped bottle with 7.5 mL of substrate solution
- 1 red-capped bottle with 7.5 mL of stop solution

## Materials required but not included

### Extraction procedure:

- Grinding mill
- Blender or a tightly sealing jar with lid
- Analytical balance with a weighing capacity up to 200 g
- Conic flask with a plug and a minimum capacity of 250 mL
- Graduated cylinder with a minimum capacity of 100 mL
- 100% ACS grade methanol (for certain commodities)
- 70% (70:30 methanol:water) methanol or ACS grade methanol and distilled or deionized water for preparing a 70% methanol solution
- 1 M NaOH (for certain commodities)
- Container for filtrate collection (e.g. flask or falcon tubes)
- Whatman #1 filter paper, or equivalent
- Filter funnel

### Assay procedure:

- Calibrated 8-channel and single-channel pipettes with 100 µL and 200 µL disposable plastic tips
- Timer
- Wash bottle
- Distilled or deionized water
- Absorbent paper towels
- 3 reagent boats for use as reagent containers for an 8-channel pipette
- Microwell reader with 450 nm and 630 nm filters

Visit [www.romerlabs.com](http://www.romerlabs.com) or get in touch with your Romer Labs technical sales representative to find out which of these items are also available from Romer Labs®.

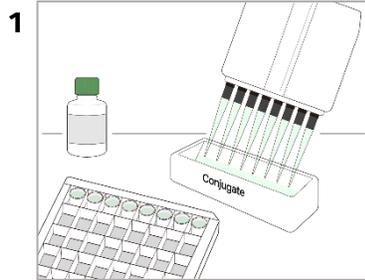
## AgraQuant® Ochratoxin 2/40 ELISA kit – assay principle

The AgraQuant® Ochratoxin 2/40 test kit is a direct competitive enzyme-linked immunosorbent assay (ELISA). Ochratoxins (A and B) are extracted from a ground sample with 70% methanol. The extracted sample and enzyme-conjugated ochratoxin are then mixed and added to the antibody-coated microwell. Ochratoxins in the samples or standards are allowed to compete with enzyme-conjugated ochratoxin for the antibody binding sites. After a washing step, the enzyme substrate is added, which results in color development. The intensity of the color is inversely proportional to the concentration of ochratoxins in the sample or standard. A stop solution is then added, which changes the color from blue to yellow. The absorbance of each well is then measured at 450 nm and with a differential filter at 630 nm. The measurement must take place within 10 minutes after adding the stop solution.

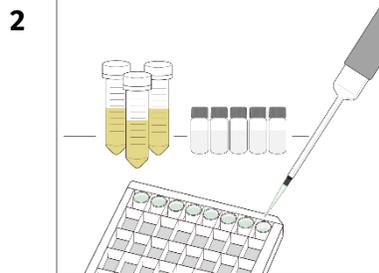
To analyze the results, please refer to the “Results analysis” section at the end of this product manual.

## Protocol at a glance

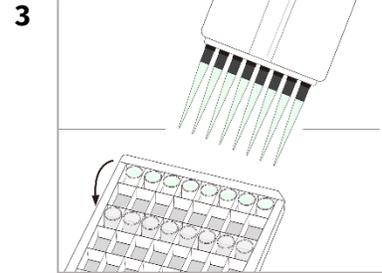
This section offers a brief overview of the AgraQuant® ELISA procedure. It is essential to read the complete product manual thoroughly before initiating the assay.



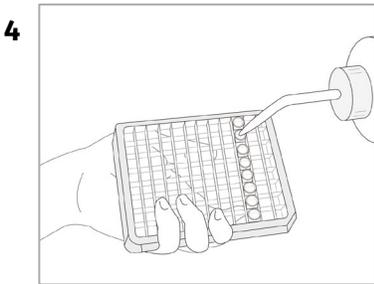
Pipette **200 µL of conjugate solution** into the dilution wells.



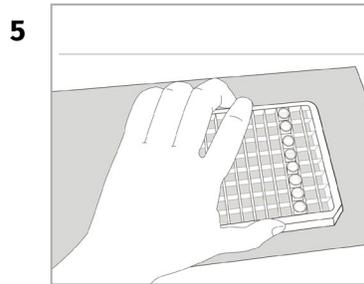
Add **100 µL of each standard or sample extract** into the dilution wells.



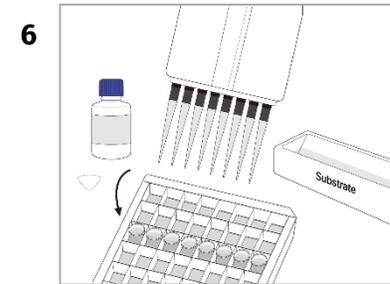
Mix well and **transfer 100 µL** from the dilution wells into the antibody-coated wells. **Incubate at RT for 10 minutes.**



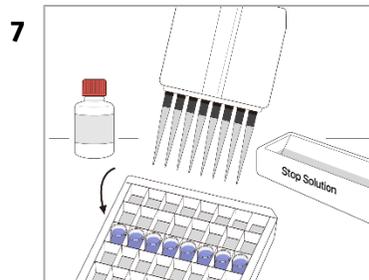
**Wash 5 times** with distilled or deionized water.



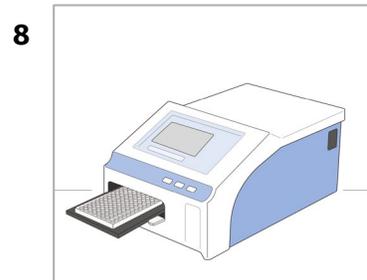
**Tap dry** the washed wells.



Pipette **100 µL of substrate solution** into the antibody-coated wells. **Incubate at RT for 5 minutes.**



Pipette **100 µL of stop solution** into the antibody-coated wells.



Read the absorbance of each well at **450 nm** with a differential filter at **630 nm**.

## Reagent and sample preparation

### Sample preparation

1. Obtain a representative sample of the specimen you want to analyze and grind it so that 95% will pass through a 20-mesh (sieve opening 0.84 mm), then thoroughly mix the subsample portion.
2. Weigh out 20 g of ground sample into a clean jar that can be tightly sealed.
3. Add 100 mL of 70% methanol extraction solution and seal the jar (1 part of the respective sample and 5 parts extraction solution in a total of 6 parts)
4. Vigorously shake or blend for 3 minutes.
5. Allow sample to settle, then filter the top layer of extract through a Whatman #1 filter and collect the filtrate. Alternatively, centrifuge the extract 4 minutes at 3000 x g.  
**Note:** Commodity extracts should have a pH of 6-8. Excessive alkaline or acidic conditions may affect the test results and should be adjusted before testing.
6. Samples are ready for testing. Please read the ELISA procedure section and carefully follow the protocol.

**For beer:** measure 3 mL of beer sample into a test tube and add 7 mL of 100% methanol. Vortex or mix for 30 seconds: the sample is now ready for testing. The result of ochratoxin in beer is calculated by multiplying a factor of (2/3) to the ELISA result.

**For wine:** pipette 3 mL of a wine sample into a test tube; pipette 5.7 mL of 100% methanol into the same tube. Vortex and mix for 30 seconds. Adjust pH value in the range of 6.5 - 7.5 using 1M NaOH. The sample is now ready for testing. The result of ochratoxin in wine is calculated by multiplying a factor of 0.58 to the ELISA result.

- ➡ **Did you know?** AgraQuant® Ochratoxin 2/40 ELISA test kit shares the same extraction solution with most of our AgraQuant® ELISA test kits. This allows you to use the same sample extract for several mycotoxin tests. This does not apply to AgraQuant® Deoxynivalenol, AgraQuant® Aflatoxin M1 Sensitive & M1 High Sensitive and AgraQuant® T-2/HT-2.

## ELISA procedure

### Before starting

#### Procedural guidelines:

- Make sure you have everything you need ready before starting the assay.
- All reagents and kit components must be equilibrated to room temperature, i.e. 18-30°C (64-86°F), before use.
- Run a standard curve with each assay.
- Adhere to the incubation times stated in the procedure. Use of incubation times other than those specified may give inaccurate results.
- We strongly recommended to perform the assay with an 8-channel pipette.
- Do not run more than 6 eight-well strips in one experiment when using an 8-channel pipette. If an 8-channel pipette is not used (i.e. using only single channel pipettes), we recommended that you run no more than a total of 16 samples and standards (2 test strips) in any one experiment.
- Do not return unused reagents in their original bottles.

#### Precautions:

- Store reagents at 2-8°C (35-46°F) when not in use, and do not use beyond the expiration date.
- Methanol is flammable. Caution must be taken in its use and storage.
- The stop solution contains acid. Avoid contact with skin or eyes. If exposed, flush with water.
- Treat all materials, containers and devices that are exposed to the sample or standards as if they were contaminated with toxin.
- Wear protective gloves and safety glasses when using the kit.
- Dispose of all single-use materials, containers and devices appropriately after use.

### Assay protocol

1. Place the appropriate number of **white dilution wells** in a microwell strip holder. One dilution well will be required for each standard (i.e. 0, 2, 5, 20 and 40 ppb) or sample.
2. Place an equal number of **antibody-coated microwells** in a microwell strip holder. Return unused microwells to the foil pouch with the desiccant packet and reseal pouch with tape.
3. Measure the required amount of conjugate solution from the green-capped bottle (~240 µL/well or 2 mL/strip) and place it in a separate container (e.g. reagent boat when using the 8-channel pipette). Using an 8-channel pipette, dispense **200 µL of conjugate solution** into each dilution well.  
➡ **Did you know?** Ratio of conjugate to standard/sample should remain at 2:1, but the volumes of conjugate and standards/samples can be reduced, e.g. using 100 µL and 50 µL, respectively. The content to be transferred from dilution wells to antibody-coated wells has to remain 100 µL
4. Using a single channel pipette, add **100 µL of each standard or sample** into the dilution wells containing 200 µL of conjugate. Use a fresh pipette tip for each standard or sample.

**Note:** Make sure the pipette tip has been completely emptied.

- Using an 8-channel pipette with fresh tips for each 8-well strip, mix each well by carefully pipetting up and down 3 times and immediately **transfer 100  $\mu$ L of the content** of each dilution well into a corresponding antibody-coated microwell. Incubate at room temperature for **10 minutes**.

**Note:** Do not attempt to mix the content of the microwells by shaking the plate as this may cause well-to-well contamination.

- Empty the content of the microwell strips into a waste container. **Wash** by filling each microwell with distilled or deionized water, and then dumping the water from the microwell strips. Repeat this step 4 times for a total of 5 washes.

**Note:** Take care not to dislodge the strips from the holder during the washing steps.

- Lay several layers of absorbent paper towels on a flat surface and tap the microwell strips on towels to remove as much residual water as possible after the fifth wash. Dry the bottom of the microwells with a dry cloth or towel.

**Note:** Never insert absorbent paper directly into the wells.

- Measure the required amount of substrate solution from the blue-capped bottle (~120  $\mu$ L/well or 1 mL/strip) and dispense it into a separate container (e.g. reagent boat for an 8-channel pipette). **Pipette 100  $\mu$ L of the substrate solution** into each microwell using an 8-channel pipette. Incubate at room temperature for **5 minutes**.

- Measure the required amount of stop solution from the red-capped bottle (~120  $\mu$ L/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipette). **Pipette 100  $\mu$ L of stop solution** into each microwell using an 8-channel pipette. The color should change from blue to yellow.

- Read the absorbance of each well within 10 minutes after the addition of the stop solution at **450 nm** (reference wavelength 630 nm) with a microwell reader.

**Note:** Carefully remove any air bubbles prior to reading the absorbance as they may affect the result.

**Note:** Do not return unused reagents to their original bottles. Carefully note which rows/strips contain standards or samples during the assay.

## Results analysis

Results can be easily calculated using the **Romer Labs® spreadsheet** that is provided free of charge upon request. With the Romer Labs® spreadsheet you only need to insert the obtained OD values for standards and samples. The spreadsheet applies the Log/Logit regression model to construct a calibration curve. The correlation coefficient (R) of the calibration curve must be between -0.990 to -1.000. The ochratoxin concentration in your samples is calculated automatically by interpolation with the calibration curve. Alternatively, construct a dose-response curve using either the unmodified OD values of the standards or the OD values expressed as a percentage of the OD of the zero (0) standard. If the Log/Logit regression model is used for results interpretation, the correlation coefficient (R) of the calibration curve must be between -0.990 and -1.000.

When working according to the sample preparation section described in this product manual, a dilution factor of 5 is applied during sample extraction. This dilution factor is already taken into account, thus the ochratoxin concentration can be read directly from the standard curve obtained.

**Note:** If a sample contains ochratoxin levels higher than the highest standard (>40 ppb), the filtered extract should be further diluted with 70% methanol such that the diluted sample results in a range of 5 - 40 ppb. The diluted sample should be reanalyzed to obtain accurate results. The applied dilution factor must be included in the calculation of the results.

**Note:** An OD value of less than 0.5 absorbance units for the 0 ppb standard may indicate the deterioration of reagents.

## Technical support

Not sure if the test works with your specific samples or matrices? Let our longstanding experience in mycotoxin testing work for you. Contact your Romer Labs technical sales representative for more information.

Visit [www.romerlabs.com](http://www.romerlabs.com) to find worldwide contact information.  
For further information please contact us at:

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