AgraQuant® Zearalenone Plus 25/1000 ELISA kit

Package insert

Article number 10002111/10002112

Intended use

The AgraQuant® Zearalenone 25/1000 ELISA test kit is an immunoassay designed for the quantitative analysis of the presence of zearalenone in food and feed components.
This product is intended for laboratory use.

Performance characteristics

Limit of detection (LOD): 20 ppb *

Limit of quantification (LOQ): 25 ppb *

Range of quantification: 25-1000 ppb

Plate format: 96 (10002111) or 48 wells (10002112)

Assay time: 15 minutes

About Zearalenone

Zearalenone is mainly produced by the fungus *Fusarium graminearum*. This compound is mostly found in corn, however it can be present also in other important commodities such as wheat, barley, sorghum and rye. Grain infected by *Fusarium graminearum* often has a pink color, because of a pigment that is produced with zearalenone. Zearalenone appears to bind to estrogen receptors and is therefore linked to hormonal changes and alterations in the development of the urogenital system. Swine are the animals most significantly affected by the toxic effects of zearalenone.

* Determined for corn.

Product information

About the ELISA test kit

The AgraQuant® Zearalenone Assay is a direct competitive enzyme-linked immunosorbent assay (ELISA) that quantitatively determines zearalenone presence. This product is intended for use in grains, cereals and other commodities.

Storage information

Upon receipt, immediately transfer the AgraQuant® Zearalenone 25/1000 to refrigerated storage 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 package.

Contents of the kit

The AgraQuant® Zearalenone 25/1000 ELISA test kit contains the following items:

10002111
- 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 of 1.5 mL each of zearalenone standard (0, 25, 100, 300 and 1000 ppb)
- 1 green-capped bottle of 25 mL of zearalenone-conjugate solution
- 1 blue-capped bottle of 15 mL of substrate solution
- 1 red-capped bottle of 15 mL of stop solution

10002112
- 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 of 0.75 mL each of zearalenone standard (0, 25, 100, 300 and 1000 ppb)
- 1 green-capped bottle of 12.5 mL of zearalenone-conjugate solution
- 1 blue-capped bottle of 7.5 mL of substrate solution
- 1 red-capped bottle of 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
- Graduated cylinder with a minimum capacity of 100 mL
- 70% (70:30 methanol:water) methanol or ACS grade methanol and distilled or deionized water for preparing a 70% methanol solution
- Container with a minimum capacity of 125 mL
- 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** or get in touch with your technical sales representative to find out which of these items are also available from Romer Labs®.

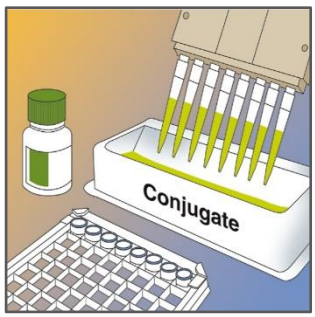
ELISA kit – Assay principle

The AgraQuant® Zearalenone Assay is a direct competitive enzyme-linked immunosorbent assay (ELISA). Zearalenone is extracted from a ground sample with 70% methanol. The extracted sample and enzyme-conjugated zearalenone are then mixed and added to the antibody-coated microwell. Zearalenone in the samples or standards is allowed to compete with enzyme-conjugated zearalenone 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 zearalenone 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 *Results analysis* at the end of this package insert.

Protocol at a glance

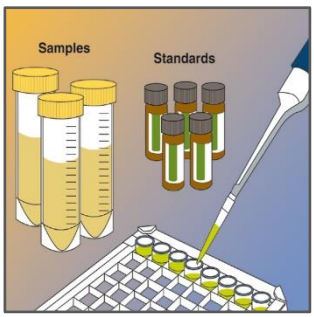
The following section gives only an overview of the ELISA procedure. Before performing the assay, please read this package insert carefully.

1



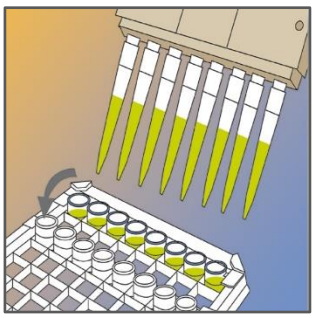
Pipette **200 µL of conjugate solution** into the dilution wells.

2



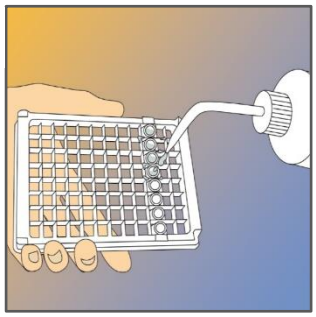
Add **100 µL of each standard or sample extract** into the dilution wells.

3



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

4

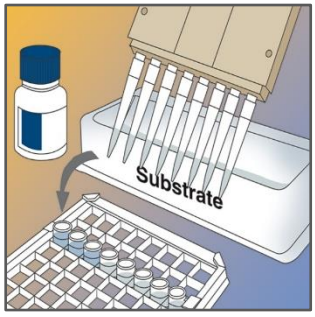


Wash 5 times with distilled or deionized water.

5

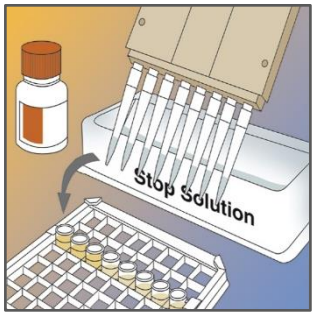
Tap dry the washed wells.

6



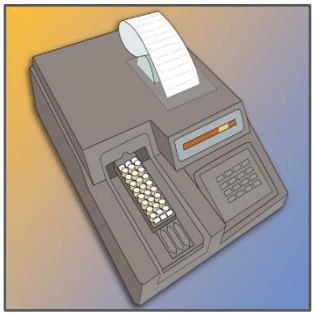
Pipette **100 µL of substrate** solution into the antibody-coated wells. **Incubate at RT for 5 minutes**.

7



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

8



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 (extraction ratio of 1:5 of sample to extraction solution).
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.

   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. Dilute the sample extract 1:5 with 70% methanol. For example, add 1 mL of extract to 4 mL of 70% methanol.
7. Samples are ready for testing. Please read the *ELISA procedure* section and carefully follow the protocol.

➲ **Did you know?** AgraQuant® Zearalenone 25/1000 ELISA test kits share 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 is not valid for AgraQuant® Deoxynivalenol and all three AgraQuant® Aflatoxin M1.

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 the technical sales representative in your region to know more.

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 allowed to reach 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 recommend 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 into 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, 25, 100, 300 and 1000 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?** The 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 must remain at 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.

5. 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 µL 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.

6. Empty the content of the microwell strips into a waste container. **Wash** by filling each microwell with distilled or deionized water, and then dump 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.

7. 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.

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

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

10. 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 and -1.000. The zearalenone 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 package insert, a dilution factor of 25 is applied during sample extraction. This dilution factor is already taken into account, thus the zearalenone concentration can be read directly from the standard curve obtained.

Note: Soybean meal has been validated in the quantitation range of 40 – 1000 ppb. To detect zearalenone in soybean meal within a quantitation range of 25 – 1000 ppb, please contact technical support for sample clean-up method.

Note: If a sample contains zearalenone levels higher than the highest standard (>1000 ppb), the filtered extract should be further diluted with 70% methanol such that the diluted sample results in a range of 25-1000 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.

Visit **www.romerlabs.com** to find worldwide contact information.
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