# AgraQuant® Deoxynivalenol Assay 0.25/5.0

## Order No.: COKAQ4000/COKAQ4048

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# AgraQuant® Deoxynivalenol Assay 0.25/5.0
## Competitive ELISA

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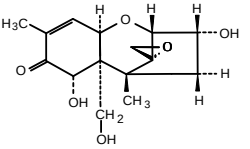
Order #: COKAQ4000/COKAQ4048

Deoxynivalenol (Vomitoxin)

Deoxynivalenol (DON) is a type B trichothecene. DON is produced by fungi of the *Fusarium* genus, particularly *Fusarium graminearum*. This mycotoxin occurs predominantly in grains such as wheat, barley, oats, rye, and maize.




Deoxynivalenol

Short Instruction:

1. Pipette 200 µL conjugate solution into dilution wells
2. Add 100 µL of each standard or sample into the dilution wells.
3. Mix well and transfer 100 µL from dilution wells into antibody coated wells and incubate at RT for 15 minutes
4. Wash 5 times with diluted wash solution
5. Tap dry washed wells
6. Pipette 100 µL substrate solution into the antibody coated wells and incubate at RT for 5 minutes
7. Add 100 µL stop solution into the antibody coated wells
8. Read the strips with ELISA reader using 450nm filter and 630nm differential filter

Performance Characteristics: LOD: 0.2 ppm LOQ: 0.25 ppm
Range: 0.25 - 5.0 ppm

## Sample Preparation / Extraction

1. Obtain a representative sample (*) and grind it using a Romer Series II® Mill so that 95% will pass through a 20-mesh screen, 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 distilled or deionized water and seal jar. Note: Samples should be extracted in a ratio of 1:5 (w:v) of sample to extraction solution respectively.
4. 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 1:4 with deionized or distilled water. For example, add 1 ml of extract to 3 ml of distilled or deionized water.
7. The sample is ready for testing without further preparation.

(*) Please read relevant literatures [1, 2, 3] about sampling procedures in order to obtain a representative sample.

## Wash Solution Preparation

Transfer contents of Wash Solution Concentrate bottle to a 500mL plastic squeeze bottle and add 475mL distilled/deionized water. Swirl to mix.

## Assay Procedure in Detail

Note: All reagents and kit components must be at room temperature 18-30°C (64-86°F) before use. It is recommended that an 8-channel pipette be used to perform the assay. No more than 48 samples and standards total (6 test strips) should be run in one experiment when using an 8-channel pipette. If an 8-channel pipette is not used (i.e. using only single channel pipettes), it is recommended that no more than a total of 16 samples and standards (2 test strips) be run in any one experiment.

1. Place the appropriate number of blue/green-bordered Dilution Strips in a microwell strip holder. One Dilution Well will be required for each standard, (i.e. 0, 0.25, 1.0, 2.0, & 5.0 ppm) or sample.
2. Place an equal number of Antibody Coated Microwell strips in a microwell strip holder. Return unused microwell strips to the zip-lock pouch and reseal.
3. Measure the required amount of Conjugate from the green-capped bottle (~240 µL/well or 2 mL/strip) and place in a separate container (e.g. reagent boat when using the 8-channel pipette). Using an 8-channel pipette, dispense 200 µL of Conjugate into each blue/green-bordered Dilution Well.
4. Using a single channel pipette, add 100 µL of each standard or sample into the appropriate Dilution Well 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 it up and down 3 times and immediately transfer 100 µL of the contents from each Dilution Well into a corresponding Antibody Coated Microwell. Incubate at room temperature for 15 minutes. Note: Do not agitate the plate to mix as it may cause well-to-well contamination.

5. Empty the contents of the microwell strips into a waste container. Wash by filling each microwell with wash solution, and then dumping the wash solution 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 wash procedure.
6. Lay several layers of absorbent paper towels on a flat surface and tap microwell strips on towels to expel as much residual water as possible after the fifth wash. Dry the bottom of the microwells with a dry cloth or towel.
7. Measure the required amount of Substrate from the blue-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 the Substrate into each microwell strip using an 8-channel pipette. Incubate at room temperature for 5 minutes.
8. 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 strip using an 8-channel pipette. The color should change from blue to yellow.
9. Read the strips with a microwell reader using a 450 nm filter with a 630nm differential filter. Record OD readings

for each microwell. Note: Air bubbles should be eliminated prior to reading strips as they may affect analytical results.

Additional Notes: Ratio of Conjugate to Standard/Sample should remain at 2:1, but volumes of Conjugate and Standards/Samples can be reduced, e.g. using 100µL and 50µL, respectively. The content to be transferred from dilution well to antibody coated well remains the same as 100 µL. Do not return unused reagents to their original bottles. Carefully keep track of the position of Samples and Standards during the assay. Do not mix the assay microwells by shaking at any time during test.

## Interpretation of Results

Using either the unmodified OD values or the OD values expressed as a percentage of the OD of the zero (0) standard, construct a dose-response curve using the five standards. Since the amount of DON in each standard is known, the unknowns can be measured by interpolation from this standard curve. Results can also be easily calculated using the Romer® Log/Logit spreadsheet that is provided (free of charge) upon request. If the Log/Logit regression model is used for results interpretation, the linearity coefficient (r^2) of the calibration curve must be between -0.990 to -1.000. An OD value of less than 0.5 absorbance units for 0ppm standard may indicate deterioration of reagents.
If a sample contains DON levels higher than the highest standard (> 5.0ppm), the filtered extract should be further diluted in distilled or deionized water such that the diluted sample results are in a range of 0.25 – 5.0ppm and reanalyzed to obtain accurate results. The dilution factor must be included when the final result is calculated.

For soybean, a dedicated calculation spreadsheet is needed for results calculation. Please contact technical service to obtain the spreadsheet.

## Performance Characteristics in Detail

Limit of detection: 0.2 ppm (Determined by the average values of 10 DON-free wheat samples plus 2 standard deviation).

Limit of quantitation: 0.25 ppm[1] (Described as the lowest concentration point on the calibration curve that this test can reliably detect DON).

Range of quantitation: 0.25 – 5.0 ppm (For quantitation of samples above 5.0ppm samples should be diluted such that the diluted sample results are in a range of quantitation).

[1] Referring to AOAC RI approved method, the LOQ is 0.4ppm (determined by the average values of 10 DON-free wheat samples plus 10 standard deviation, the LOQ for oats is 0.6ppm)

## Materials supplied

Order #: COKAQ4000

- 96 antibody coated microwells (12 eight-well strips) in a microwell holder (sealed in a zip-lock pouch)
- 96 non-coated dilution microwells (12 eight-well strips marked with blue/green at base)
- 5 vials of 1.5mL of each DON standard (0, 0.25, 1.0, 2.0 and 5.0 ppm)
- 1 bottle of 25mL of DON conjugate (green-capped bottle)
- 1 bottle of 15mL of substrate solution (blue-capped bottle)
- 1 bottle of 15mL of stop solution (red-capped bottle)

- 1 bottle of 25mL of 20X Wash Solution Concentrate (blue-capped bottle)

Order #: COKAQ4048

- 48 antibody coated microwells (6 eight-well strips) in a microwell holder (sealed in a zip-lock pouch)
- 48 non-coated dilution microwells (6 eight-well strips marked with blue/green at base)
- 5 vials of 0.75mL of each DON standard (0, 0.25, 1.0, 2.0 and 5.0 ppm)
- 1 bottle of 12.5mL of DON conjugate (green-capped bottle)
- 1 bottle of 7.5mL of substrate solution (blue-capped bottle)
- 1 bottle of 7.5mL of stop solution (red-capped bottle)
- 1 bottle of 25mL of 20X Wash Solution Concentrate (blue-capped bottle)

## Materials required but not supplied

Extraction Procedure

- *EQMMS2010: Romer Series II® Mill or equivalent
- *EQOLE1025: Blender or a tightly sealing jar with lid
- *EQOLE1010: Balance, 400 g
- *EQOLE1050: Graduated cylinder: 100mL
- Container with a minimum 125mL capacity
- *Whatman#1 filter paper, or equivalent
- *Filter funnel

Assay Procedure

- *8-channel and single channel pipettes capable of pipetting 100µL and 200µL with tips
- *EQOLE1300: Timer
- *COKAD1150: Wash bottle
- Distilled or de-ionized water

- Absorbent paper towels
- *3 reagent boats for use as reagent containers for an 8-channel pipette
- *Microwell reader with a 450nm filter and a 630nm differential filter

*Items available from Romer Labs.

## Technical and Background Information

The AgraQuant® Deoxynivalenol (DON) Assay is a direct competitive enzyme-linked immunosorbent assay (ELISA) that determines a quantitative level for the presence of DON and is intended for use in grains, cereals, nuts, and other commodities.
The AgraQuant® DON Assay has been validated for barley[2], malt, malted barley[2], corn[2], corn bran, corn meal[2], milo/sorghum[2], oats[2], popcorn[2], rice, soybean, wheat[2], wheat flour, and wheat midds.

[2] AOAC RI approved method

### Deoxynivalenol (Vomitoxin)

Deoxynivalenol (DON) is a type B trichothecene. DON is produced by fungi of the *Fusarium* genus, particularly *Fusarium graminearum*. This mycotoxin occurs predominantly in grains such as wheat, barley, oats, rye, and maize. DON is highly toxic, levels above 1ppm are considered potentially harmful to swine. Pet foods prepared with wheat contaminated with DON have been involved in acute toxicities. DON is a known immunosuppressant and may cause kidney problems. Humans are thought to exhibit a similar vomition syndrome when consuming DON-contaminated grain.
The US Food and Drug Association advisory levels for DON are as follows: (1) 1ppm for finished wheat products for human consumption; (2) 5ppm for grain and grain byproducts destined for

swine and other animals; and not to exceed 1ppm in the diets for swine and 2ppm in the diets of other animals; (3) 10ppm for grain and grain byproducts for ruminating beef and feedlot cattle older than 4 months and for chickens; and not to exceed 5ppm in the diet.

## Assay Principles

The AgraQuant® DON Assay is a direct competitive enzyme-linked immunosorbent assay (ELISA). DON is extracted from a ground sample with distilled or deionized water. The extracted sample and enzyme-conjugated DON are mixed and added to the antibody-coated microwell. DON in samples and control standards are allowed to compete with enzyme-conjugated DON for the antibody binding sites. After a washing step, an enzyme substrate is added and blue color develops. The intensity of the color is inversely proportional to the concentration of DON in the sample or standard. A stop solution is then added which changes the color from blue to yellow. The microwells are measured optically using a microwell reader with an absorbance filter of 450nm ($OD_{450}$) and a differential filter of 630nm. The optical densities of the samples are compared to the OD's of the standards and an interpretative result is determined.

## Precautions

1. Store reagents at 2-8°C (35-46°F) when not in use, and do not use beyond the expiration date.
2. Adhere to incubation times stated in the procedure. Use of incubation times other than those specified may give inaccurate results.
3. The Stop Solution contains acid. Avoid contact with skin or eyes. If exposed, flush with water.
4. Consider all materials, containers and devices that are exposed to the sample or standards to be contaminated

with toxin. Wear protective gloves and safety glasses when using the kit.

5. Dispose of all materials, containers and devices appropriately after use.
6. The conjugate solution is colored green in order to help customers to distinguish whether conjugate was already added to microwells or not. The greenness of conjugate solution may vary among production batches, nevertheless, this does not affect the conjugate quality.

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## Warranty

The user assumes all risk in using Romer Labs products and services. Romer Labs will warrant that its products and services meet all quality control standards set by Romer Labs and Romer Labs will, at its option, repair or replace any product, components, or repeat services which prove to be defective in workmanship or material within product specific warranty periods or expiration dates and which our examination shall disclose to our satisfaction to be defective as such. This warranty is expressly in lieu of all other warranties, expressed or implied, as to description, quality, merchantability, fitness for any particular purpose, productiveness, or any other matter. Romer Labs shall be in no way responsible for the proper use of its products. Romer Labs hereby disclaims all other remedies, warranties, guarantees or liabilities, expressed or implied, arising by law or otherwise, and it shall have no liability for any lost profits or damage, direct, indirect or otherwise, to person or property, in

connection with the use of any of its products or services. This warranty shall not be extended, altered or varied except by a written instrument signed by an authorized representative of Romer Labs.

References:

1. J. Richard. Sampling and sample preparation for mycotoxin analysis. Romer® Labs' Guide to Mycotoxins, Vol. 2, July 2000.
2. T.B. Whitaker. Standardisation of mycotoxin sampling procedures: an urgent necessity. Food Control. 14, 233-237, 2003.
3. Commission Regulation (EC) No 401/2006 of 23 February 2006. Laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs.

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