

**Product Instructions** 

# **Reveal Q+ for Zearalenone**

**Quantitative Test** 

# The Toxin

Zearalenone primarily is produced by the mold *Fusarium graminearum*, which also commonly produces deoxynivalenol (DON). Hence, there is evidence if zearalenone is detected, there is a high probability other fusarial mycotoxins may be present. Zearalenone is classified as an estrogenic mycotoxin because it frequently causes estrogenic responses in animals.

When zearalenone-contaminated feed or grain is eaten by livestock, it can cause a wide variety of reproductive problems. In swine, it causes vulvovaginitis, low birth weights, fetal reabsorption, aborted pregnancies, reduced litter sizes, abnormal estrus, and feminization of immature males. Zearalenone can delay the breeding process and cost the producer significant economic and physical losses.

Livestock producers increasingly are becoming aware of zearalenone problems, and have looked for ways to reduce risks related to contaminated feed.

The best protection against mycotoxins is monitoring for their presence in feeds and foods, which means testing all along the pathway from initial harvest of grains to the finished product.

# **Intended Use/User**

Reveal<sup>®</sup> Q+ for Zearalenone is intended for the quantitative analysis of corn and wheat products for zearalenone. The test kit is designed for use by quality control personnel and others familiar with commodities possibly contaminated by zearalenone.

# **User Responsibility**

- Users are responsible for familiarizing themselves with product instructions and information. Visit our website at neogen.com, or contact your local Neogen® representative or authorized distributor for more information.
- When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, laboratory technique and the sample itself may influence results.
- It is the user's responsibility in selecting any test method or product to evaluate a sufficient number of samples with the appropriate matrices and challenges to satisfy the user that the chosen test method meets the user's criteria.
- It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.
- As with any test method, results obtained from use of any Neogen Food Safety product do not constitute a guarantee of the quality of the matrices or processes tested.

# **Assay Principles**

Reveal Q+ for Zearalenone is a single-step lateral flow immunochromatographic assay based on a competitive immunoassay format. The extract is wicked through a reagent zone, which contains antibodies specific for zearalenone conjugated to colloidal gold particles. If zearalenone is present, it will be captured by the particle-antibody complex. The zearalenone-antibody-particle complex then is wicked onto a membrane, which contains a zone of zearalenone conjugated to a protein carrier. This zone captures any uncomplexed zearalenone antibody, allowing the particles to concentrate and form a visible line. As the level of zearalenone in a sample increases, free zearalenone will complex with the antibody-gold particles. This allows less antibody-gold to be captured in the test zone. Therefore, as the concentration of zearalenone in the sample increases, the test line density decreases. Algorithms programmed into the readers convert these line densities into a quantitative result displayed in parts per billion (ppb). The membrane also contains a control zone where an immune complex present in the reagent zone is captured by an antibody, forming a visible line. The control line always will form regardless of the presence of zearalenone, ensuring the strip is functioning properly.

# **Storage Requirements**

Store kit components at room temperature (18–30°C, 64–86°F) for full shelf life. Test strips should remain capped in their original tubes until used (for optimal performance).

# **Materials Provided**

- 1. 25 Reveal Q+ for Zearalenone test strips
- 2. 25 red sample dilution cups
- 3. 25 clear sample cups
- 4. 1 bottle of sample diluent

# Materials Recommended, Not Provided

- 1. Sample collection cups with lids (700004011 | 9428, 700004012 | 9428B)
- 2. 65% ethanol solution (700002492 | 8073, 700002493 | 8074)
- 3. Agri-Grind grinder or equivalent (100001350 | 9401, 700004021 | 9453)
- 4. Microcentrifuge tubes (700003932 | 9172)
- 5. Mini centrifuge (700003963 | 9330)
- 6. Filter syringes (700002724 | 9420)
- 7. Sample collection tubes with caps (700002726 | 9421, 700002727 | 9421B)
- 8. Whatman #4 filter paper or equivalent (700004035 | 9519, 700006493 | 9429)
- 9. Pipettor, 200 µL (700004020 | 9488)
- 10. Pipettor, 100  $\mu L$  (100001422 | 9860, 100001330 | 9272)
- 11. Pipette tips, 1–200  $\mu L$  (100001352 | 9407 or 100001353 | 9410, 700004099 | 9417)
- 12. Scale capable of weighing 5–50 g  $\pm 0.1$  g (700004010  $\mid$  9427)
- 13. Timer (100001358 | 9426)
- 14. Reveal sample cup rack (700002734 | 9475)
- 15. Dispensing pump or graduated cylinder (700004020 | 9448, 100001367 | 9447)

# Precautions

- 1. The test strips must remain inside the stay-dry tube before use.
- 2. Ethanol is highly flammable. Keep container tightly closed, and away from heat, sparks, open flame, and those who are smoking. It is toxic if swallowed, or if vapor is inhaled. Avoid contact with skin.
- 3. Store test kit at room temperature (18–30°C, 64–86°F) when not in use. Do not freeze.
- 4. Do not use kit components beyond expiration date.
- 5. Treat all used liquids, including sample extract, and labware as if contaminated with zearalenone. Gloves and other protective apparel should be worn at all times.
- 6. To avoid cross-contamination, use clean glassware for each sample, and thoroughly wash all glassware between samples.
- 7. Ensure the device lot number and the curve details match the lot ID number selected on the reader. Failure to update the lotspecifc QR code within the AccuScan<sup>®</sup> Pro and AccuScan Gold will cause inaccurate results.
- 8. Commodities tested should have a pH of 6–8. Excessively acidic or alkaline samples should be adjusted. For instructions on adjusting pH, contact Neogen Technical Services.
- 9. Please refer to the SDS information available at neogen.com for complete safety information on the assay and components.

## Accuscan Reader Set Up

- 1. Enter the lot-specific and the commodity-specific QR code by selecting Scan QR from the main screen. Place the specific QR code into the white cartridge adapter labeled Cal/QR and place the cartridge into the reader.
- The valid code will be scanned by the reader and provide information on the lot number and expiry date. Verify this information is correct and then add the lot ID to the reader by pressing Add Lot ID.
  Note: The lot ID for the current lot will now be stored with the test ID (e.g., Q+ Zearalenone Corn or Q+ Zearalenone Wheat) and can be selected when running a test.
- 3. Return to the main screen and select the Mycotoxin Q+ category then select the Q+ Zear test type.
- 4. The reader will prompt the user to select the lot ID of the kit being tested. A sample ID can be added at this time.

# Raptor<sup>°</sup> Solo Reader Set Up

The Solo reader must be in standard mode to read the Reveal Q+ for Zearalenone test strips.

- 1. From the main menu, select the  $\{\mathcal{O}\}$  in the upper left hand corner
- 2. Select track replacement
- 3. Return to the home screen by pressing the 🔟 in the lower left hand corner
- 4. Insert the track replacement tool with the arrow facing towards you into the track in the unit
- 5. Gently pull up to remove the track
- 6. Insert the standard Endpoint testing track into the unit (track with the blue dot on the right hand side)



### **Sample Preparation**

The sample to be tested should be collected according to accepted sampling techniques (see FGIS sampling protocol or contact your Neogen representative). Obtain a representative sample (minimum 100 g). Grind the sample so at least 95% of the ground material passes through a 20-mesh sieve (about the particle size of fine espresso).

If not using Neogen's prepared solution, prepare a 65% ethanol solution by mixing 6.5 parts ethanol with 3.5 parts distilled or deionized water for each sample.

### **Protocol for Corn Curve Set**

#### **Sample Extraction**

- 1. Extract at a ratio of 1 part sample to 3 parts 65% ethanol. For example, combine 10 g of ground sample with 30 mL of 65% ethanol.
- 2. Vigorously shake, using hand or mechanical means (250 rpm) for 3 minutes, or blend for 1 minute.
- 3. Allow the sample to settle, then filter at least 4 mL with a filter syringe, or Whatman No. 1 filter paper. Alternatively, pipette sample into a 2.0 mL microcentrifuge tube and centrifuge for 30 seconds.
- 4. The sample is now ready for testing.

## FGIS Method Sample Extraction

- 1. Combine 50 g of ground sample with 150 mL of 65% ethanol.
  - a. For soybeans, rough rice, distiller dried grains (DDG), and corn gluten meal, combine 50 g of ground sample with 300 mL of 65% ethanol.
- 2. Vigorously shake, using hand or mechanical means (250 rpm) for 3 minutes.
- 3. Allow the sample to settle for 1 minute, then filter at least 3–5 mL with a filter syringe.
- 4. The sample is now ready for testing.

**Note:** When the test is complete, for soybeans, rough rice, corn gluten meal, and DDGs, multiply the results by 2.

### **Test Procedure**

- 1. Place the appropriate number of red sample dilution cups and clear sample cups into a sample cup rack. Label cups if necessary.
- 2. Add 100  $\mu$ L of sample extract to the red sample cup.
- 3. Add 200 µL of sample diluent to the red dilution cup with the sample extract. Mix by pipetting up and down 5 times.
- 4. Transfer 100  $\mu$ L of diluted sample extract into a new clear sample cup.
- 5. Place a new Reveal Q+ for Zearalenone test strip with the sample end down into the sample cup and set timer for 6 minutes. Ensure the test strip comes into contact with liquid and begins to wick.
- 6. Remove the strip from the sample cup after it has developed for 6 minutes and read immediately (within 30 seconds).
- 7. Final result will be displayed on the unit. Samples greater than 1200 ppb must be diluted and re-tested.

# **Dilution Procedure**

### Samples greater than 1200 ppb must be diluted and retested.

- 1. Add 100  $\mu$ L sample filtrate to a sample collection tube.
- 2. Add 200  $\mu L$  65 % ethanol solution to the sample collection tube. Mix well.
- 3. Place the appropriate number of red sample dilution cups and clear sample cups into a sample cup rack. Label cups if necessary.
- 4. Add 100 μL of diluted sample extract (from step 2) to the red sample cup.
- 5. Add 200 µL of sample diluent to the red dilution cup with the sample extract. Mix by pipetting up and down 5 times.
- 6. Transfer 100  $\mu$ L of diluted sample extract into a new clear sample cup.
- 7. Place a new Reveal Q+ for Zearalenone test strip with the sample end down into the sample cup and set timer for 6 minutes. Ensure the test strip comes into contact with liquid and begins to wick.
- 8. Remove the strip from the sample cup after it has developed for 6 minutes and read immediately (within 30 seconds). Final result displayed will need to be multiplied by 3.

# **Protocol for Wheat Curve Set**

#### **Sample Extraction**

- 1. Extract at a ratio of 1 part sample to 5 parts 65% ethanol. For example, combine 10 g of ground sample with 50 mL of 65% ethanol.
- 2. Vigorously shake, using hand or mechanical means (250 rpm) for 3 minutes, or blend for 1 minute.
- 3. Allow the sample to settle, then filter at least 4 mL with a filter syringe, or Whatman No. 1 filter paper. Alternatively, pipette sample into a 2.0 mL microcentrifuge tube and centrifuge for 30 seconds.
- 4. The sample is now ready for testing.

## **FGIS Method Sample Extraction**

- 1. Combine 50 g of ground sample with 250 mL of 65% ethanol.
- 2. Vigorously shake, using hand or mechanical means (250 rpm) for 3 minutes.
- 3. Allow the sample to settle for 1 minute, then filter at least 3–5 mL with a filter syringe.
- The sample is now ready for testing.
  Note: When the test is complete, multiply the results by 1.66 for wheat.

#### **Test Procedure**

- 1. Place the appropriate number of red sample dilution cups and clear sample cups into a sample cup rack. Label cups if necessary.
- 2. Add 100  $\mu$ L of sample extract to the red sample cup.
- 3. Add 200 µL of sample diluent to the red dilution cup with the sample extract. Mix by pipetting up and down 5 times.
- 4. Transfer 100  $\mu$ L of diluted sample extract into a new clear sample cup.
- 5. Place a new Reveal Q+ for Zearalenone test strip with the sample end down into the sample cup and set timer for 6 minutes. Ensure the test strip comes into contact with liquid and begins to wick.
- 6. Remove the strip from the sample cup after it has developed for 6 minutes and read immediately (within 30 seconds).
- 7. Final result will be displayed on the unit. Samples greater than 1200 ppb must be diluted and re-tested.

### **Dilution Procedure**

#### Samples greater than 1200 ppb must be diluted and retested.

- 1. Add 100  $\mu$ L sample filtrate to a sample collection tube.
- 2. Add 200  $\mu$ L 65 % ethanol solution to the sample collection tube. Mix well.
- 3. Place the appropriate number of red sample dilution cups and clear sample cups into a sample cup rack. Label cups if necessary.
- 4. Add 100  $\mu$ L of diluted sample extract (from step 2) to the red sample cup.
- 5. Add 200 µL of sample diluent to the red dilution cup with the sample extract. Mix by pipetting up and down 5 times.
- 6. Transfer 100  $\mu$ L of diluted sample extract into a new clear sample cup.
- 7. Place a new reveal Q+ for Zearalenone test strip with the sample end down into the sample cup and set timer for 6 minutes. Ensure the test strip comes into contact with liquid and begins to wick.
- 8. Remove the strip from the sample cup after it has developed for 6 minutes and read immediately (within 30 seconds).
- 9. Final result will be displayed on the unit. Samples greater than 1200 ppb must be diluted and re-tested. Final result displayed will need to be multiplied by 3.

### **Reading Test Results**

**Note:** Test strips should be read within 30 seconds of completion of the 6 minute incubation. Refer to reader set up for test selection and set up information.

Select the assay type from the menu and ensure the device lot number matches the lot ID number selected on the reader.

Note: Failure to update the lot-specific QR code will cause inaccurate results.

Fully insert the Reveal Q+ test strip into the black R-labeled cartridge adapter with the sample end first and results facing out.

Insert the cartridge with test strip upside-down into the AccuScan Gold reader (the test lines will face downward into the reader), test-strip side up for the AccuScan Pro, or test strip facing outwards for Raptor Solo. The reader will automatically begin analyzing the cartridge.

Caution: Removing cartridge prior to completion can result in invalid readings.

The reader will analyze the test strip and results will be displayed and stored in the reader.

### Notes

- 1. Ensure device is fully inserted into cartridge.
- 2. Readings should be made within 30 seconds of the 6 minute incubation time. Readings after 6.5 minutes may be inaccurate due to over-development of the device.
- 3. The strips must be read using AccuScan Pro, AccuScan Gold or Raptor Solo in Standard mode.

# **Performance Characteristics**

### **Corn Curve Commodities**

Range of detection: 50–1200 ppb

#### **Wheat Curve Commodities**

Range of detection: 25–1200 ppb Samples greater than 1200 ppb must be diluted and retested following the dilution protocols.

# **Validated Matrices**

Note: Neogen continues to validate new commodities. Please contact a representative for the latest validated commodity list.

# **FGIS Approved Matrices**

Corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), DDG with solubles, corn gluten meal, rough rice, soybean (including whole soybean and full-fat soy flour), and wheat (including whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings).

# **Customer Service**

Neogen Customer and Technical Services can be contacted through <u>neogen.com</u> and product training is available by request.

# **SDS Information Available**

Safety data sheets are available for all test kits at <u>neogen.com</u> or by calling 800.234.5333 or 517.372.9200.

# **Terms and Conditions**

Neogen's full terms and conditions are available <u>online</u>.

# Warranty

Neogen makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. If any materials are defective, Neogen will provide a replacement of the product. Buyer assumes all risk and liability resulting from the use of this product. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. Neogen shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.

