Read instructions carefully before starting test

Veratox®

for DON 5/5

Quantitative Test

FGIS-2018-110

REFRIGERATE AT 2–8°C (35–46°F) • DO NOT FREEZE

THE TOXIN

Deoxynivalenol (DON) is most commonly produced by the pink mold Fusarium graminearum. DON, a member of the trichothecene family, is produced by fungi living on cereal commodities such as wheat, corn, barley and ensilages. The toxicological effects attributed to DON include: nausea (vomiting), feed refusal, gastroenteritis, diarrhea, immuno-suppression and blood disorders.

Pigs have been shown to be highly sensitive to DON. They will refuse to eat feeds when DON levels of >1 parts per million (ppm) are present. The toxin and its analogs cause toxic effects in other species as well, with varying degrees of sensitivity. DON has been implicated as causing problems in processed food, including off flavor in ready-to-eat cereals and adverse affects on dough quality. Accurate determination of the presence of the toxin is of major importance to those monitoring the quality of feed and food in which DON may occur. Testing these commodities for the toxin requires careful sampling, extraction, sanitation and quantitative analysis.

The FDA has issued advisory levels for DON as follows:

For	DON Level	Commodities
Humans	1 ppm (flour, bran & germ)	Finished wheat products
Ruminating beef, feedlot cattle, chickens	10 ppm in <50% of diet (5 ppm total diet)	All grains, grain by-products
Swine	5 ppm in <20% of diet (1 ppm total diet)	All grains, grain by-products
All other animals	5 ppm in <40% of diet (2 ppm total diet)	All grains, grain by-products

INTENDED USE
Veratox for DON 5/5 is intended for the quantitative analysis of DON in grain and grain products.

INTENDED USER
The test kit is designed for use by quality control personnel and others familiar with food and feed possibly contaminated by DON. Since technique is very important, operators should be trained by a Neogen representative or someone who has completed the Neogen training.

ASSAY PRINCIPLES
Veratox® for DON 5/5 is a competitive direct enzyme-linked immunosorbent assay (CD-ELISA) which allows the user to obtain exact concentrations in parts per million (ppm). Free DON in the samples and controls is allowed to compete with enzyme-labeled DON (conjugate) for the antibody binding sites. After a wash step, substrate is added, which reacts with the bound conjugate to produce blue color. More blue color means less DON. The test is read in a microwell reader to yield optical densities. The optical densities of the controls form the standard curve, and the sample optical densities are plotted against the curve to calculate the exact concentration of DON.

STORAGE REQUIREMENTS
The kit can be used until the expiration date on the label when stored refrigerated at 2–8°C (35–46°F).

MATERIALS PROVIDED
1. 48 antibody-coated microwells
2. 48 red-marked mixing wells
3. 5 yellow-labeled bottles of 1.5 mL each 0, 0.25, 0.5, 1, and 2 ppm DON controls
4. 1 blue-labeled bottle of DON-HRP conjugate solution
5. 1 green-labeled bottle of K-Blue® Substrate solution
6. 1 red-labeled bottle of Red Stop solution

MATERIALS RECOMMENDED BUT NOT PROVIDED
1. Extraction materials:
    a. Distilled or deionized water
    b. Graduated cylinder
    c. Container with 125 mL capacity or 500 mL for GIPSA method
    d. Whatman #1 filter paper, Neogen filter syringe, or equivalent
    e. Sample collection tubes
2. Agri-Grind grinder, or equivalent (Neogen item #9401)
3. Scale capable of weighing 5–50 grams (Neogen item #9427)
4. Microwell reader with a 650 nm filter (Neogen item #9301/#9302)
5. 12-channel pipettor (Neogen item #9273)
6. 100 µL pipettor (Neogen item #9272)
7. Tips for 100 µL and 12-channel pipettors (Neogen item #9410)
8. Paper towels or equivalent absorbent material
9. Plastic bucket for use as waste receptacle
10. Microwell holder (Neogen item #9402)
11. Timer (Neogen item #9426)
12. Waterproof marker
13. Wash bottle (Neogen item #9400)
14. 2 reagent boats for 12-channel pipettor (Neogen item #9435)
15. Distilled and deionized water

PRECAUTIONS

1. Store test kit between 2–8°C (35–46°F) when not in use.
2. Kits should be brought to room temperature (18–30°C, 64–86°F) before use.
3. Do not use kit components beyond expiration date.
4. Do not mix reagents from one kit serial with reagents from a different kit serial.
5. Do not run more than 24 wells at one time.
6. Follow proper pipetting techniques, including the tip priming.
7. Use of incubation times other than those specified may give inaccurate results.
8. Avoid prolonged storage of kits at ambient temperatures.
9. Do not freeze test kits.
10. Commodity extracts should have a pH of 6–8 before testing. Excessively acidic or alkaline samples should be adjusted. For instructions on adjusting pH contact your Neogen representative or Technical Services.
11. Treat all used liquids, including sample extract, and labware as if contaminated with DON. Gloves and other protective apparel should be worn at all times.
12. To avoid cross-contamination, use clean pipette tips and glassware for each sample, and thoroughly detoxify and wash all glassware between samples.

PROCEDURAL NOTES

1. K-Blue Substrate is ready for use. The substrate should be clear to light blue — discard if it has turned dark blue. Only pour the needed volume of substrate into a reagent boat. Do not return unused substrate to the bottle. Cover the reagent boat to keep the substrate protected from light until it is needed.
2. Antibody wells: Keep wells sealed in the foil pouch until needed. Remove wells from the foil pouch only after the samples are extracted, and the test procedure is set to begin.

SAMPLE PREPARATION AND EXTRACTION

The sample to be tested should be collected according to accepted sampling techniques. The sample should be ground and thoroughly mixed prior to proceeding with the extraction. Store samples at 2–8°C (35–46°F) until analyzed.

1. Obtain a representative sample. Grind the entire sample so that at least 95% of the ground material passes through a 20 mesh sieve, the particle size of a fine instant coffee.
2. Using hand or mechanical means, vigorously shake 10 grams of ground sample in 100 mL of distilled or deionized water for 3 minutes.
3. Let extract sit for at least 3 minutes to allow for some of the particles to settle.
4. Filter a minimum of 5 mL of the extract through a Neogen syringe filter, collecting a minimum of 3 mL into a sample collection tube.
5. The sample is ready for testing.

FGIS METHOD:

1. Obtain a representative sample. Grind the entire sample so that at least 95% of the ground material passes through a 20 mesh sieve, the particle size of espresso.
2. Using hand or mechanical means, vigorously shake 50 grams of ground sample in 250 mL of distilled or deionized water for 3 minutes.
3. Let material set for 2–3 minutes to enable some of the sample to settle before filtering extract.
4. Filter the extract by pouring at least 5 mL through a Whatman #1 filter (or Neogen filter syringe) and collecting the filtrate as a sample.
5. Dilute the sample extract 1:2 (1+1) with distilled or deionized water. For example, add 1 mL of extract to 1 mL of distilled or deionized water.
6. The sample is ready for testing.

Note: See FGIS Test Kit instructions for additional dilution protocols.

TEST PROCEDURE

Allow all reagents to warm to room temperature (18–30°C, 64–86°F) before use.

1. Remove 1 red-marked mixing well for each sample to be tested plus 5 red-marked wells for controls, and place in the well holder.
2. Remove an equal number of antibody-coated wells. Return antibody wells which will not be used immediately to the foil pack with desiccant and reseal the foil pack to protect the antibody. Mark one end of strip with a "1", and place strip in the well holder with the marked end on the left. Do not mark the inside or bottom of the wells.
3. Mix each reagent by swirling the reagent bottle prior to use.
4. Place 100 µL of conjugate from the blue-labeled bottle in each red-marked mixing well.
5. Using a new pipette tip for each, transfer 100 µL of controls and samples to the red-marked mixing wells as described below.

0	0.25	0.5	1	2	S1	S2	S3	S4	S5	S6	S7	Strip 1
S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	Strip 2

6. Using a 12-channel pipettor, mix the liquid in the wells by pipetting it up and down 3 times. Transfer 100 µL to the antibody-coated wells. Mix by sliding the microwell holder back and forth on a flat surface for 10–20 seconds without splashing reagents from the wells. Incubate for 5 minutes at room temperature (18–30°C, 64–86°F). Discard red-marked mixing wells.
7. Shake out the contents of the antibody wells. Fill the wells with distilled or deionized water and dump them out. Repeat this step 5 times, then turn the wells upside-down and tap out on a paper towel until the remaining water has been removed.
8. Pour the needed volume of substrate from the green-labeled bottle into the green-labeled reagent boat.
9. With new tips on the 12-channel pipettor, prime and pipette 100 µL of substrate into the wells and mix by sliding back and forth on a flat surface for 10–20 seconds.
10. Incubate 5 minutes. Discard remaining substrate and rinse the reagent boat with water.
11. Pour Red Stop solution from the red-labeled bottle (same volume as the substrate) into the red-labeled reagent boat.
12. Eject the excess substrate from the 12-channel pipettor, prime the tips, and pipette 100 µL of Red Stop to each well. Mix by sliding back and forth on a flat surface. Discard the tips.
13. Wipe the bottom of the microwells with a dry cloth or towel and read in a microwell reader using a 650 nm filter. Air bubbles should be eliminated, as they could affect analytical results. Results should be read within 20 minutes after the addition of Red Stop.
14. Read and calculate results using Neogen's StatFax microwell reader. If using an EL301 reader or other strip/plate reader, calculate results using Neogen's Veratox for Windows software.

RETESTING

Sample results >2.0 should be diluted and retested. If positives occur in commodities not previously tested, confirm with an additional approved method prior to taking action. The test kit will not differentiate between DON and 3 acetyl DON.

PERFORMANCE CHARACTERISTICS

Limit of detection: 0.1 ppm (Determined by the mean average of 10 DON free samples plus 2 standard deviations.)

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

Range of quantitation: 0.25–2 ppm (For quantitating samples above 2 ppm contact Neogen Technical Services for dilution instructions.)

Validated commodities: barley flour, barley lightly pearled, barley silage, barley, beet pulp*, canola meal, corn, corn bran, corn cob, cornmeal, corn germ meal*, corn gluten feed*, corn gluten meal*, corn grits, corn oil, corn screenings, corn silage**, corn/soy blend, corn starch, corn steep, DDGS*, DDDGs backset/recycled water*, DDGs syrup*, DDGs wet cake*, flaxseed, hay**, haylage**, kamut, malted barley*, malted barley flour, millet, milo, oats, oat hulls*, oat fiber, oat flour, pea fiber, petfood*, popcorn, potato (white), potato with skins, powder* quinoa, raw flour, rice, rice gluten, rice hulls, rough rice, rye, rye flour, soy meal, soy flour, soy hydrolysate, sunflower meal, tapioca, TMR**, wheat, wheat bran, wheat bran aleurone, wheat flour, wheat flour 2nd clear, wheat germ, wheat middlings, wheat midds and wheat, waxy

FGIS-validated commodities: Corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), wheat (including whole grain wheat flour, wheat middlings, wheat red dog, wheat 2nd clear, and wheat screenings), corn germ meal, malted barley (including malted barley flour), oats (whole oats with hull), rye, wheat bran (wheat bran aleurone), sorghum, and corn/soy blend

**Generally requires a pH adjustment.*
***Contact Neogen for special procedure.*
NOTE: Neogen continues to validate new commodities. Please contact a representative for the latest validated commodity list.

CUSTOMER SERVICE

Customer assistance and Technical Services for Veratox for DON 5/5 can be reached by calling 800-234-5333 (USA/Canada) or 517-372-9200, 8 a.m. to 7 p.m. Eastern Time. Assistance is available on a 24-hour basis by calling 800-867-0308. Training on this product and all Neogen test kits is available.

SDS INFORMATION AVAILABLE

Safety data sheets (SDS) are available for this test kit, and all of Neogen's Food Safety test kits, at foodsafety.neogen.com, or by calling Neogen at 800-234-5333 or 517-372-9200.

WARRANTY

Neogen Corporation 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 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.

NOTES:

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TESTING KITS AVAILABLE FROM NEOGEN

Natural toxins
- Aflatoxin, DON, ochratoxin, zearalenone, T-2/HT-2 toxins, fumonisin, histamine

Foodborne bacteria
- *E. coli* O157:H7, *Salmonella*, *Listeria*, *Listeria monocytogenes, Campylobacter, Staphylococcus aureus, Salmonella enteritidis*

Sanitation
- ATP, yeast and mold, total plate count, generic *E. coli* and total coliforms, protein residues

Food allergens
- Almonds, coconut, crustaceans, eggs, gliadin, hazelnut, milk, mustard, peanuts, sesame, soy, walnuts, multi-treenut

Genetic modification
- CP4 (Roundup Ready®)

Ruminant by-products
- Meat and bone meal, feed

Species Identification
- Raw and cooked meat samples

North America
Neogen Headquarters
800/234-5333 (USA/Canada)
foodsafety@neogen.com
foodsafety.neogen.com/en

Europe, Middle East and Africa
Neogen Europe
+ 44 (0) 1292 525 600
info_uk@neogeneurope.com
foodsafety.neogen.com/uk

Mexico
Neogen Latinoamerica
+52 (55) 5254-8235
informacion@neogenlac.com
foodsafety.neogen.com/sp

Brazil
Neogen do Brasil
+55 19 3935.3727
info@neogendobrasil.com.br
foodsafety.neogen.com/pt

China
Neogen Bio-Scientific Technology
+86 21 6271 7013
info@neogenchina.com.cn
www.neogenchina.com.cn

India
Neogen Food and Animal Security
+91 484 2306598, 2301582
info@neogenindia.com
www.neogenindia.com

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