

# Test Kit Instruction

March 6, 2018

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## NEOGEN VERATOX DON 5/5

### **FORWARD**

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at [Ajit.K.Ghosh@ams.usda.gov](mailto:Ajit.K.Ghosh@ams.usda.gov).

Refer to the Mycotoxin Handbook for information on use of this test kit in the official inspections including sampling, general sample preparation, reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at [Patrick.J.McCluskey@ams.usda.gov](mailto:Patrick.J.McCluskey@ams.usda.gov).

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## 1. GENERAL INFORMATION

VERATOX DON 5/5 is a competitive direct enzyme-linked immunosorbent assay (CD-ELISA) which allows the user to determine the concentration of deoxynivalenol (DON) 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 a blue color. More blue color means less DON. The test is read in a micro well 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.

Approved Test Kit Information	
<b>Test Kit Vendor:</b>	<i>Neogen Corporation 800/234-5333</i>
<b>Test Kit Name:</b>	VERATOX DON 5/5
<b>Product Number:</b>	8331
<b>Effective Date of Instructions:</b>	03/06/2018
<b>Instructions Revision Number:</b>	0
<b>Conformance Range:</b>	0.50 – 30 ppm
<b>Number of Analyses to Cover Conformance Range:</b>	3
<b>Type of Service:</b>	Quantitative
<b>Supplemental Analysis:</b>	Yes
<b>Approved Commodities:</b>	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.
<b>Extraction method:</b>	Shake vigorously by mechanical shaker (250 rpm) or by hand with similar shaking action for three minutes.
<b>Test Format:</b>	Competitive direct enzyme-linked immunosorbent assay
<b>Detection Method:</b>	Stat Fax Reader, Model 4700

## 2. PREPARATION OF TESTING MATERIALS

### a. Stat Fax Reader 4700 set up.

- (1) Turn reader on and wait for the main menu to appear.
- (2) Press "Run Test".
- (3) Select the "DON 00" test from the menu.
- (4) Select "Yes" to accept that test.
- (5) Press "# Wells" and enter how many wells will be read then press "OK".
- (6) Press "Accept" then "Start" when the wells are ready to be read.

### b. Preparation of 1N Sodium Hydroxide (NaOH) Solution.

**NOTE: One can buy premade 1N NaOH from any commercial supplier (e.g. Sigma Aldrich catalog# 72082) or it may be prepared from solid sodium hydroxide pellets (Sigma Aldrich catalog# S8045) as described below:**

- (1) Add slowly 4 grams of NaOH into 100 mL distilled or deionized water with stirring.

**CAUTION! NaOH is corrosive. Addition of solid NaOH pellets into water is an exothermic reaction (produces heat). Stir constantly and add the NaOH slowly.**

- (2) This solution should be used to adjust the pH of any sample extract that shows pH below 7.0
- (3) Label the container stating the name, date of preparation, and initials of technician that prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container under fume hood.

### c. Preparation of 1N Hydrochloric (HCl) Acid Solution

**NOTE: One can buy premade 1N HCl from any commercial supplier (e.g. Sigma Aldrich catalog# 38283) or it may be prepared from concentrated HCl (Sigma Aldrich catalog# 320331) as described below:**

- (1) Add slowly 8.2 mL of 12.1N HCl (concentrated Hydrochloric Acid) into 91.8 mL distilled or deionized water with stirring.

**CAUTION! HCl is corrosive. Addition of acid into water is an exothermic reaction (produces heat). Stir constantly and add the HCl slowly.**

- (2) This solution should be used to adjust pH of any sample extract that shows pH above 8.0
- (3) Label the container stating the name, date of preparation, and initials of technician that prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container under a fume hood.

### 3. SAMPLE PREPARATION AND EXTRACTION PROCEDURES

- a. The sample to be tested should be collected and prepared according to accepted sampling techniques (see Mycotoxin Handbook).
- b. **Standard Extraction Procedure.**
  - (1) Weigh  $50 \pm 0.2$  grams ground samples into a suitable container or bag.
  - (2) Add 250 mL of distilled or deionized water and close the bag securely to prevent spillage.
  - (3) Shake vigorously by mechanical shaker (250 rpm) or by hand with similar shaking action for three minutes.
  - (4) Let the extract sit for at least 3 minutes to allow for some of the particles to settle.
  - (5) Filter about 3 to 5 mL of the extract through a Neogen syringe filter. This is the **filtered extract** to be used in the Extract Dilution Procedure below (3.c)
  - (6) For Corn Germ Meal and Malted Barley, check the pH of the filtered extract. For all other commodities, proceed to Extract Dilution Procedures (Section 3.c, below).
    - (a) If the pH is not between 6.0 -8.0 it needs to be adjusted.
    - (b) Using a disposable polyethylene transfer pipette, add one drop of 1N NaOH (sodium hydroxide) to the sample extract, vortex to mix, and check the pH.
    - (c) If pH is still below 7.0, add another drop of 1N NaOH, mix, and check pH again. Continue this process until the pH falls between 6.0 and 8.0, and then proceed to Extract Dilution Procedures (Section c, below).

c. **Extract dilution Procedure.**

Three different dilution procedures are needed to cover the full range (0.50 to 30 ppm) of this test kit, one for each separate quantitation range.

(1) **Diluted Filtered Extract (for the 0.50 – 5.0 ppm quantitation range)**

Dilute the filtered extract (from 3.b, above) twofold with distilled or deionized water. Using a 1000 µL pipette, add 1.0 mL of filtered extract to 1.0 mL of distilled or deionized water. Vortex for 10 seconds.

(2) **Diluted Extract A (for the 5.0 – 10 ppm quantitation range)**

Dilute the **Diluted Filtered Extract** two-fold with distilled or deionized water to prepare **Diluted Extract A**. Using a 1000 µL pipette, add 1.0 mL of **diluted filtered extract** (from Step 3.c.1, above) to 1.0 mL of distilled or deionized water. This is **Diluted Extract A**. Vortex for a few seconds prior to the analysis.

(3) **Diluted Extract B (for the 10 – 30 ppm quantitation range)**

Dilute the **Diluted Filtered Extract** (from Step 3.c.1, above) ten-fold with distilled or deionized water to prepare **Diluted Extract B**:

Using a 100 µL pipette, add 100 µL of diluted filtered extract to 900 µL (measured using 100 -1000 µL variable volume pipet) of distilled or deionized water. This is **Diluted Extract B**. Vortex for a few seconds prior to the analysis.

**4. TEST PROCEDURES**

**NOTE: For all unknown samples, standard analysis using the Diluted Filtered Extract (3.c. (1). above) should be performed first. If the results are above 5.0 ppm, Diluted Extract A and B (from Sections 3.c. (2) and 3.c. (3), above) should be prepared and analyzed simultaneously on a single assay.**

a. **Analysis Procedure.**

- (1) Allow reagents and antibody wells to reach room temperature (68 - 77°F) prior to running the test.
- (2) Mix each reagent by swirling the reagent bottle prior to use.
- (3) 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.

**Note: Remember to use two wells per sample if you are doing supplemental testing (additional dilutions) for higher ranges.**

- (4) 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 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.
- (5) Using a 100 µL pipette, place 100 µL of conjugate from the blue-labeled bottle in each red-marked mixing well.
- (6) Using a new pipette tip for each, transfer 100 µL of controls and test samples to the red-marked mixing wells.

**Test Samples:** For 0.50 to 5 ppm quantitation range, use 100 µL of the **Diluted Filtered Extract**. For 5.0 to 10 ppm quantitation range, use 100 µL of **Diluted Extract A**, and for 10 to 30 ppm quantitation range, use 100 µL of **Diluted Extract B**.

- (7) Using a 12-channel pipette, mix the liquid in the wells by pipetting it up and down 3 times. Transfer 100 µL into the antibody-coated wells.
- (8) Mix by sliding the micro well holder back and forth on a flat surface for about 20 seconds without splashing the reagents from the wells. Incubate for 5 minutes at room temperature (68 – 77°F). Discard red-marked mixing wells.
- (9) 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.
- (10) Pour the needed volume of substrate from the green-labeled bottle into the green- labeled reagent boat.
- (11) With new tips on the 12-channel pipette, 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.
- (12) Incubate for 5 minutes. Discard remaining substrate and rinse the reagent boat with water.
- (13) Pour Red Stop solution from the red-labeled bottle (same volume as the substrate) into the red-labeled reagent boat.
- (14) Using 12-channel pipette, 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.
- (15) Wipe the bottom of the micro wells with a lint free tissue and read on the Stat Fax 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. In order to accept the result, the R-value should be  $\geq 0.98$ .

b. **Reporting of Results.**

- (1) For Diluted Filtered Extract, results are valid in the range of **0.50 to 5 ppm**. If the result falls outside this range, retest with **Diluted Extract A and Diluted Extract B**.
- (2) For **Diluted Extract A**, multiply the printed result by 2 to obtain the final concentration. If the result falls above this range, use the result from **Diluted Extract B**.

A final result, from Diluted Extract A, of less than 3.5 ppm is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Repeat the analysis using 0.50 – 5 ppm quantitation range (using Diluted Filtered Extract), and only perform the analysis using Diluted Extract A again if the value is still greater than 5 ppm.

- (3) For **Diluted Extract B**, multiply the printed result by 11 to obtain the final concentration. Results following this protocol are valid in the range of **10 to 30 ppm**.

A final result, from Diluted Extract B, of less than 7 ppm is indicative of a problem, and troubleshooting is needed. Verify that the procedure is being followed properly. Repeat the analysis using the 10 to 30 ppm quantitation range (Diluted Extract A), and only perform the analysis using Diluted Extract B again if the value is still greater than 10 ppm.

## 5. **REPORTING AND CERTIFYING TEST RESULTS**

Refer to the Mycotoxin Handbook for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or [Patrick.J.McCluskey@ams.uds.gov](mailto:Patrick.J.McCluskey@ams.uds.gov)).



## 6. STORAGE CONDITIONS AND PRECAUTIONS

### a. Storage Conditions.

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

### b. Precautions.

- (1) Store test kit between 2-8°C (35-46°F) when not in use. Do not freeze.
- (2) Do not use kit components beyond expiration date.
- (3) Do not use substrate that has turned blue prior to use.
- (4) Do not mix reagents from one kit lot with reagents from a different kit lot.
- (5) Do not run more than 24 wells per test.
- (6) Follow proper pipetting techniques, including priming pipette tips by filling and dispensing solution once before use.
- (7) Use of incubation times other than those specified may give inaccurate results.
- (8) Bring kits to room temperature (68-77°F) prior to use.
- (9) Avoid prolonged storage of kits at ambient temperatures.
- (10) Treat all used liquids, including sample extract, and lab ware as if contaminated with DON. Use precaution when handling.
- (11) To avoid cross-contamination, use new pipette tips for each sample.
- (12) Commodities tested should have a pH of 6-8. Excessively acidic or alkaline samples should be adjusted. For instructions on adjusting pH contact your Neogen representative or Technical Services.

## 7. EQUIPMENT AND SUPPLIES

### a. Materials Provided in Test Kits.

- (1) 48 antibody-coated micro wells
- (2) 48 red-marked mixing wells
- (3) 5 yellow-labeled bottles of 0, 0.5, 1, 2, and 6 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
- (7) Directions for use

### b. Materials required but not provided.

- (1) Extraction materials.
  - (a) Distilled or deionized water
  - (b) Sealable Container with 500 mL capacity for FGIS method.
  - (c) Neogen filter syringe (Neogen item #9420/#9430)
  - (d) Sample collection tubes (Neogen item #9421)
- (2) 250 mL graduated cylinder (Neogen item #9447)
- (3) Agri-Grind grinder or equivalent (Neogen item #9401)
- (4) Scale capable of weighing 5-50 grams (Neogen item #9427)
- (5) 100 µL pipette (Neogen item #9272/#9278)
- (6) 12-channel pipette (Neogen item #9273)
- (7) Tips for 12-channel and 100 µL pipettes (Neogen item #9410/#9407)
- (8) Paper towels or equivalent absorbent material
- (9) Plastic bucket for use as a waste receptacle
- (10) Micro well 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 pipette (Neogen item #9435)
- (15) Distilled or deionized water
- (16) 1N NaOH (Sigma Aldrich #72082), or NaOH pellets (Sigma Aldrich #S8045)
- (17) 1N HCl (Sigma Aldrich #38283), or concentrated HCl (Sigma Aldrich #320331)

## 8. REVISION HISTORY

### Revision 0 (03/06/2018)