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DON HANDBOOK  
CHAPTER 19  
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CHAPTER 19

NEOGEN VERATOX DON DST TEST KITS

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## 19.1 GENERAL INFORMATION

The extraction solution and other materials used in the Neogen Veratox DON DST test kits (part number 8333) do not necessitate the use of a separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this handbook to ensure a safe and efficient work environment.

## 19.2 EXTRACTION PROCEDURES

- a. Barley, Malted Barley, Corn, Oats, Wheat, and Rice.
  - (1) Place a sheet of filter paper (Whatman #1 folded or S&S 24-cm pleated or equivalent) into a clean funnel mounted over a 25 x 200 mm (diameter x length) test tube or collection beaker.
  - (2) Label the collection container with the sample identification.
  - (3) Thoroughly mix the ground sample and weigh a 50-gram portion.
  - (4) Place the ground 50-gram portion into an 18 oz sealable plastic bag or extraction jar.
  - (5) Add 250 ml of distilled or deionized water and shake (by hand or mechanically) for 3 minutes.
  - (6) Let material stand for 2 - 3 minutes to enable some of the sample residue to settle before filtering the extract.
  - (7) Filter the extract by pouring at least 15 ml through a Whatman # 1 filter (or Neogen filter syringe) and collect the filtrate as a sample.
  - (8) The sample is ready for testing.

### 19.3 PREPARATION OF SOLUTIONS

a. Control Standard, Conjugate, Substrate, and Red Stop.

All reagents are packaged ready to use, no pre-mixing necessary. Substrate should be stored in the dark, and 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.

### 19.4 TEST PROCEDURES

a. Test Procedures.

- (1) Allow all reagents to reach room temperature (64 – 86 ° F) prior to use.
- (2) Remove 3 red-marked mixing wells for each sample to be tested from the foil pack. Do not run more than 24 wells or 8 samples when using a repeating pipettor.
- (3) 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.
- (4) Mark one end of both sets of wells (red-marked, & antibody coated) with a (C) for the control standard. Place the marked end in the well holder with the marked end on the left. If testing more than 1 sample mark each control standard well with a (C) for identity. Do not mark the inside or bottom of the wells.

Well#	1	2	3	4	5	6	7	8	9	10	11	12
	C	S1	S1	C	S2	S2	C	S3	S3	C	S4	S4

- (5) Mix each reagent by swirling the reagent bottle prior to use.
- (6) Place 100 µl of conjugate from the blue labeled bottle into each mixing well. Discard the tip.
- (7) Using a new pipette tip, transfer 100 µl of control standard to the designated control mixing well(s). Discard the tip and replace the cap on the control standard bottle.

- (8) Using a new pipette tip for each sample, transfer 100  $\mu$ l of sample extract into each designated sample mixing well(s).
- (9) Using a 12-channel pipettor with new tips, mix the wells by pipetting the liquid up and down in the tips three times. Transfer 100  $\mu$ l to the antibody wells.
- (10) Mix by sliding the well holder back and forth on flat surface in a manner to ensure mixing (10-20 seconds) without splashing reagents from wells. **Wait 5 minutes** (begin time after mixing). Discard the red-marked wells.
- (11) After 5 minute incubation the initial reaction is now completed. Shake out the contents of antibody-coated wells.
- (12) Using a wash bottle, fill each antibody-coated well with distilled/deionize water and shake out. Repeat five times. Remove all water droplets by turning the wells upside down and vigorously tapping wells on a paper towel after the last wash..
- (13) Pour the need volume of substrate from green labeled bottle into a reagent boat. Using new tips on the 12-channel pipettor, pipette 100  $\mu$ l of substrate into the antibody-coated wells and mix as instructed in step (10). **Wait 5 minutes** (begin time after mixing).
- (14) Discard the remaining substrate and rinse the reagent boat with water. If additional test service requested cover the substrate boat to protect from light.
- (15) Pour the need volume of stop solution into the reagent boat. Using new tips, or the same pipette tips used to dispense substrate, add 100  $\mu$ l red stop to each well and mix thoroughly as instructed in step (10). Discard the tips.
- (16) Wipe the bottom of the microwells with a dry cloth or towel. Air bubbles should be eliminated, as they could affect analytical results. Results should be read and calculated within 20 minutes of completion of testing using the Neogen Stat Fax Model 321 Plus Microwell Reader.

b. Reading the Results with the Stat-Fax Model 321 PLUS Microwell Reader.

To begin from the "Ready" prompt, press Menu, key in test number 99, and then press Enter. The preprogrammed test menu will print. Use the test number listed for the DON DST method. For example, if the DON DST method is listed as menu number 5, press Menu, key in the test number 5, and then press Enter. Verify the calibration constants by running a strip. The constants should read:

C 1	0.0
C 2	0.5
C 3	1.0
C 4	2.0
C 5	6.0

If no test for the DON DST is present or the calibrators are not correct, contact Neogen Corporation.

- (1) The screen will read, "Set carrier to A, press enter." Place the wells all the way to the right in the carrier. Push the carrier all the way to the left to line up the notch with the wells, then press enter. The carrier will advance into the reader, and it should start to print.
- (2) When the reader is finished reading the strip, the screen will read, "Plot Curve Y/N?"  
  
Press "Yes" (1/A) to print the graph,  
  
Press "No" (0) to skip this feature.
- (3) The screen will read, "Accept Curve Y/N ?"  
  
Press "Yes" (1/A) to accept the curve and proceed to read another strip. When finished reading the second strip, press "Clear" twice and the results strip will print, "Test Ended."
- (4) Press "No" (0) to end the test. If a diluted sample extract (see Standard Extraction Procedure) is being analyzed, the reader value for the extract will need to be modified to adjust for the dilution of the extract.

If the original extract was diluted 1+1 with water (this is an actual 1:2 dilution), the sample results are multiplied by 2. If the original extract was diluted 1+3 with water (this is an actual 1:4 dilution), the sample results are multiplied by 4.

**NOTE: If the correlation coefficient is less than 0.98 or if the slope exceeds  $-2.0 (\pm 0.5)$ , the reader will print, "Invalid Calibration" and no results will be reported. If the slope value consistently reads outside these tolerances, contact Neogen as soon as possible to report these findings.**

## 19.5 REPORTING AND CERTIFYING TEST RESULTS

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

## 19.6 SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.



- (4) 1 blue-labeled bottle 7 ml DON-HRP conjugate solution.
- (5) 1 green labeled bottle 32 ml K-Blue substrate solution.
- (6) 1 red-labeled bottle 32 ml Red Stop solution.

b. Materials Required but not Provided.

- (1) Mixing Bags -18-ounce Whirlpack bags or equivalent.
- (2) Nalgene funnels - 80 mm top I.D., stem 30 mm, stem O.D. 18 mm; American Scientific Products No. F7465-2.
- (3) Plastic beakers - 250 ml plastic.
- (4) Cylinders - Polypropylene, graduated, 250 ml capacity.
- (5) Filter paper - 24 cm diameter; Whatman No. 1, or equivalent.
- (6) Timer - 10-minute capacity.
- (7) Reagents Boats.
- (8) Absorbent material - Kim wipes or paper towels.
- (9) Wash Bottle (plastic squeeze bottle).
- (10) Stat Fax Model 321 Plus Microwell Reader.
- (11) Multichannel Pipettor - TiterTek 12 channel or equivalent.
- (12) Pipettor and Pipette Tips (100  $\mu$ l) - Pipetteman, MLA or equivalent.
- (13) Pipettor and Pipette Tips (1ml) - Pipetteman, MLA or equivalent.
- (14) Microwell Holder.

(15) Deionized or distilled water.

(16) Scale Balance.

(17) Sample Grinder.

#### **19.10 STORAGE CONDITIONS AND PRECAUTIONS**

a. Storage Conditions.

Store test kits in a dedicated refrigerator between 2-8° C (35-46° F) when not in use.

b. Precautions.

(1) Do not use test kit components beyond expiration date.

(2) Do not mix reagents from one kit lot number with reagents from a different kit lot number.

(3) Do not run more than 12 wells (4 samples) per test.

(4) Kits should be brought to room temperature (18-30° C, 64-86° F) prior to use.

(5) Avoid prolonged storage of test kits at ambient temperatures.

(6) Do not freeze test kits.