

U.S. DEPARTMENT OF AGRICULTURE  
GRAIN INSPECTION, PACKERS AND STOCKYARDS  
ADMINISTRATION  
FEDERAL GRAIN INSPECTION SERVICE  
STOP 3630  
WASHINGTON, D.C. 20250-3630

DON HANDBOOK  
CHAPTER 17  
7-30-07

CHAPTER 17

NEOGEN-AGRISCREEN DON QUALITATIVE TEST KIT (Product # 8310)

<u>Section Number</u>	<u>Section</u>	<u>Page Number</u>
17.1	TESTING AREA .....	17-1
17.2	EXTRACTION PROCEDURES .....	17-1
17.3	TEST PROCEDURES.....	17-2
17.4	CLEANING LABWARE .....	17-4
17.5	WASTE DISPOSAL .....	17-5
17.6	EQUIPMENT AND SUPPLIES.....	17-5
17.7	STORAGE CONDITIONS.....	17-6



## 17.1 TESTING AREA

The extraction solution and other materials used in the Neogen/AgriScreen DON qualitative test kit, product # 8310, does not necessitate the use of 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.

## 17.2 EXTRACTION PROCEDURES

### a. Barley and Wheat.

- (1) Place a sheet of filter paper (Whatman #1 folded or equivalent) into a clean funnel mounted over a test tube or collection container.
- (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-ounce Nasco Whirlpack bag or similar type of sealable plastic bag.
- (5) Add 250 ml of distilled or deionized water and shake (by hand or mechanically) for 3 minutes.
- (6) Let material stand for 2 minutes to enable some of the sample to settle before filtering the extract.
- (7) Filter the extract by pouring at least 15 ml through the filter paper.
- (8) The collected filtrate sample is now ready for official testing.

## 17.3 TEST PROCEDURES

### a. Analysis Procedures.

- (1) Allow reagents, antibody coated wells, mixing wells, and sample extracts to reach room temperature prior to running any test (approximately one hour).

**NOTE: The AgriScreen test kit product # 8310 is supplied with a 1 ppm control only. No other controls standards (2 ppm, 4 ppm etc;) are approved for use by TSD to perform screening test.**

- (2) Remove the red-marked mixing well strip and break off the needed number of wells (one well for each sample and one well for control standard). Return the unused strip to the package.

**NOTE: Do not run more than six wells (five samples plus one control) at a time unless using a multichannel pipettor.**

- (3) Remove the antibody-coated well strip and break off the same number of wells. Return the unused strip to the package and tightly close the package opening.
- (4) Mark one end of the antibody-coated well strip with C for control so that you can identify the wells after washing.
- (5) Mix all reagents by swirling prior to use. Prime pipette tips before dispensing liquids.
- (6) Firmly place a new pipette tip on the pipettor and add 100 microliters ( $\mu$ l) of DON-HRP conjugate (blue labeled bottle) to each mixing well. Discard the tip.
- (7) Firmly place a new pipette tip on the pipettor and add 100 $\mu$ l of the control (yellow labeled bottle) to the first mixing well. Thoroughly mix by depressing the plunger five times. Discard the tip.
- (8) Firmly place a new pipette tip on the pipettor and add 100  $\mu$ l from the sample collection tube to second well of the red-marked mixing strip. Thoroughly mix by depressing the plunger five times. Discard the tip.
- (9) Repeat step (8) for each additional sample.

- (10) Transfer 100  $\mu$ l from each red-marked mixing well to the corresponding antibody-coated well (s). Use a new pipette tip for each well. Discard the red-marked wells.
- (11) Mix by sliding the wells back and forth on a flat surface in a manner to ensure adequate mixing (10 to 20 seconds) without splashing reagents from wells. **Wait 5 minutes** (begin time after mixing).
- (12) The initial reaction is now completed. Shake out the contents of antibody-coated wells.
- (13) Using a wash bottle, fill each antibody-coated well with distilled/deionized water and shake out contents. Repeat five times. Remove all water droplets by turning the wells upside down and vigorously tapping wells on a paper towel.
- (14) Firmly place a new pipette tip on the pipettor and add 100  $\mu$ l of substrate (green labeled bottle) to each antibody-coated well. Discard the tip.
- (15) Mix as instructed in step (11) and **wait 5 minutes** (begin time after mixing).
- (16) Firmly place a new pipette tip on the pipettor and add 100  $\mu$ l of stopsolution (red labeled bottle) to each well. Discard the tip. Mix by tapping gently on the side of the antibody well strip.

b. Visual Interpretation of Results.

- (1) If sample well is **as blue** or **darker blue** than the control well, the sample contains less than 1 ppm of DON.
- (2) If sample well shows less blue color, or more red color, than the control. The Sample contains more than 1 ppm of DON.

c. Reading Results using the Microwell Reader at 650 nm.

- (1) Properly calibrate, wait minimum of 15 minutes before using.
- (2) Remove the sample carriage and hit "Enter."

- (3) Insert the W2 filter (405 nm) and hit "Enter."
- (4) Insert the W1 filter (650 nm) and hit "Enter."
- (5) Hit "Clear" and then "Blank." This will cause the instrument to read air as the blank sample.
- (6) Load the antibody-coated wells into the sample carriage so that the control is in position A1.
- (7) Load the sample carriage into the strip reader so that position A1 is under the reader.
- (8) Hit "Read" and record the value obtained for A1 (the control).
- (9) Slide the carriage to position A2 and hit "Read."
- (10) If the value is **EQUAL TO** or **LARGER THAN** that recorded for A1, the sample is **LESS THAN** or **EQUAL TO** the control. If the value is **SMALLER THAN** that recorded for A1, the sample contains **MORE THAN** the control.
- (11) Slide the carriage to the next sample and hit "Read."
- (12) Repeat step (11) for each of the remaining samples.

d. Reporting and Certifying Test Results.

- (1) Report results on the pan ticket and inspection log as being equal to or less than a threshold (e.g., 1 ppm) or as exceeding the threshold.
- (2) Certify results as being equal to or less than a threshold. (See the Certification Chapter of this handbook for detailed procedures and statements).

## 17.4 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

## 17.5 WASTE DISPOSAL

After the test has been completed, the remaining sample extract and sample solutions may be poured down the drain. Discard solid material in the trash can for routine disposal.

## 17.6 EQUIPMENT AND SUPPLIES

### a. Materials Provided in Test Kits:

- (1) 24 antibody-coated microwells.
- (2) 24 red-marked mixing wells.
- (3) 1 yellow-labeled bottle 1 ppm DON control.
- (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.

### b. Materials Required but not Provided:

- (1) Mixing Bags -18-ounce Nasco Whirlpack bags; Fisher Scientific No. 01-812-6C, or similar type of sealable plastic bag.
- (2) Funnels.
- (3) Cylinders - graduated, 250 ml, 50 ml capacity.
- (4) Filter paper; Whatman No. 1, or equivalent.
- (5) Timmer 5 minute capacity.
- (6) Markers - Sharpie or equal (permanent ink that will not wash off).

- (7) Absorbent material - Kim wipes or paper towels.
- (8) Wash Bottle 250 ml plastic squeeze bottle.
- (9) Pipettor and Pipette Tips (100  $\mu$ l) - Pipetteman, MLA or equivalent.
- (10) Microwell Holder.
- (11) Deionized or distilled water.
- (12) Whirlpack Bag Rack; Fisher Scientific No. 01-812-5E, or equivalent.
- (13) Balance.
- (14) Sample Grinder.

#### **17.7 STORAGE CONDITIONS**

Test kits should be refrigerated at temperatures between 36° F and 46° F.