

**NEOGEN**  
**AGRI-SCREEN FOR DON TEST**

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## GENERAL INFORMATION

AGRI-SCREEN for DON is a competitive direct enzyme-linked immunosorbent assay (CD-ELISA) which allows the user to determine how the concentration of deoxynivalenol (DON) in a sample compares to the concentration of DON in a supplied control of 1 part per million (ppm). Water is used to extract any existing DON from a ground sample. Extracted DON in the filtrate is mixed with enzyme-labeled DON (conjugate). The mixed solution is transferred to antibody-coated wells, where free DON and conjugate compete for antibody binding sites. After a wash step, substrate is added. Color develops as a result of the presence of bound conjugate. The stopping reagent is added, and the color of the resulting solution is observed. Blue color indicates negative samples. Red indicates strong positives.

The instructions presented in this document cover only the procedure for performing the analytical test. 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@usda.gov](mailto:Ajit.K.Ghosh@usda.gov).

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division for information on use of this test kit in official FGIS inspections including sampling, general sample preparation (e.g., grinding and dividing), 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@usda.gov](mailto:Patrick.J.McCluskey@usda.gov).

### Approved Test Kit Information

<b>Test Kit Vendor:</b>	<i>Neogen Corporation 800/234-5333</i>
<b>Test Kit Name:</b>	AGRI-SCREEN for DON
<b>Product Number:</b>	8310
<b>Effective Date of Instructions:</b>	02/18/2015
<b>Instructions Revision Number</b>	2
<b>Detection Threshold:</b>	1 ppm
<b>Number of Analyses to Cover Conformance Limit:</b>	1
<b>Type of Service:</b>	Qualitative
<b>Supplemental Analysis:</b>	No
<b>Approved Commodities:</b>	Wheat, corn, and barley
<b>Extraction method:</b>	Shake 50 gram sample with 250 mL of deionized or distilled water for 3 minutes using mechanical shaker at 250 rpm (or shake vigorously by hand with similar shaking action).
<b>Test Format:</b>	Competitive direct enzyme-linked immunosorbent assay
<b>Detection Method:</b>	Visual

## **SAMPLE PREPERATION AND EXTRACTION PROCEDURES**

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for information regarding sampling and general sample preparation (grinding, dividing, etc.).

### **Extraction Procedure (wheat, corn, and barley):**

- (1) Weigh  $50 \pm 0.1$  grams ground samples into a suitable container or whirl pak 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) Allow the extraction mixture to sit for 2-3 minutes.
- (5) Filter about 2 mL of the extract through a Neogen syringe filter. This filtrate is now ready for testing.

## **TEST PROCEDURES**

### **a. Analysis Procedure.**

- (1) Take out reagents and antibody wells from refrigerator and allow (about 30 to 40 minutes) reaching the room temperature (68 - 77° F) prior to use for running the test.
- (2) Remove 1 red-marked mixing well from the foil pack for each sample to be tested, and one for the control, and place in the well holder.

**NOTE: Do not run more than 6 wells at a time unless you are using a multichannel pipettor. Contact Neogen for more information.**

- (3) Remove an equal number of antibody-coated wells. Return well which will not be used immediately back to the foil pack and reseal to protect the antibody. Mark one end of the strip with a "1", and place in the well holder with the marked end on the left.
- (4) Mix each reagent by swirling the reagent bottle prior to use.
- (5) Using a new pipette tip, add 100  $\mu$ L of conjugate from the blue-labeled bottle to each red-marked mixing well. Discard the tip.
- (6) Using a new tip, add 100  $\mu$ L of the control (1 ppm of DON solution provided by the manufacturer) to the first red-marked well. Mix by inserting the tip in the liquid and pipetting up and down 5 times. Discard the tip.

- (7) Using a new tip, add 100  $\mu$ L of the first sample to the second red-marked well. Mix by inserting the tip in the liquid and pipetting up and down 5 times. Discard the tip.
- (8) Repeat the process for each additional sample in a following red-marked well.
- (9) Using a new tip for each, transfer 100  $\mu$ L from each red-marked well to the corresponding antibody-coated well. Discard the red-marked wells.
- (10) Mix by sliding the wells back and forth on a flat surface in a manner to ensure adequate mixing for 10-20 seconds, without splashing reagents. Incubate for 5 minutes at room temperature.
- (11) Dump the content of the well and turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.
- (12) Using a wash bottle, fill each well with deionized water. Empty the wells again and remove all remaining liquid. Repeat this step 4 times (total of 5 washes). Remove all water droplets by turning wells upside down and vigorously tapping on an absorbent paper.
- (13) Using a new tip, add 100  $\mu$ L of substrate from the green-labeled bottle to each well. Discard the tip.
- (14) Mix thoroughly by sliding wells back and forth on a flat surface for 10-20 seconds,
- (15) Incubate for 5 minutes at room temperature.
- (16) Using a new tip, add 100  $\mu$ L of red stop solution from the red-labeled bottle to each well. Discard the tip.
- (17) Visually check that each well is one homogeneous color and no striations in color are present. Mix gently if needed to ensure thorough mixing within each well.

**b. Interpretation of the Results**

- (1) If a sample well is as blue as or darker than the control well, the sample contains less than 1 ppm of DON.
- (2) If a sample well shows less blue color, or more red color, than the control, the sample contains more than 1 ppm of DON.
- (3) For optimum observation of color differences, place the wells on a white surface and read looking down through the solution.

**REPORTING AND CERTIFYING TEST RESULTS**

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or [Patrick.J.McCluskey@udsa.gov](mailto:Patrick.J.McCluskey@udsa.gov)).

## 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 any components beyond expiration date.
- (3) Do not mix reagents from one kit lot with reagents from a different kit lot.
- (4) Do not run more than 6 wells per batch, unless using multichannel pipettor.
- (5) Follow proper pipetting techniques, including priming pipette tips by filling and dispensing solution once before use.
- (6) Use of incubation times other than those specified may give inaccurate results.
- (7) Bring kits to room temperature (18-30°C, 64-86°F) prior to use.
- (8) Avoid prolonged storage of kits at ambient temperatures.
- (9) Treat all used liquids, including sample extract, and labware as if contaminated with DON. Use precaution when handling
- (10) To avoid cross-contamination, use new pipette tips for each measurement.
- (11) Do not use substrate that has turned blue prior to use.

## EQUIPMENT AND SUPPLIES

### a. Materials Provided in Test Kits (24 well kit).

- (1) 24 antibody-coated microwells
- (2) 24 red-marked mixing wells
- (3) 1 yellow-labeled bottle of 1 ppm DON control
- (4) 1 blue-labeled bottle of DON-HRP conjugate solution
- (5) 1 green-labeled bottle of Substrate solution

- (6) 1 red-labeled bottle of Red Stop solution

**b. Materials required but not provided.**

- (1) Extraction materials (items i through iii available in kit form from Neogen item #8052)
  - i. Distilled or deionized water
  - ii. Neogen filter syringe, (Neogen item #9420)
  - iii. Sample collection tubes (Neogen item #9421)
- (2) 50 mL graduated cylinder (Neogen item #9447)
- (3) Agri-Grind grinder or equivalent (Neogen item #9401)
- (4) Scale capable of weighing 5-25 grams (Neogen item #9427)
- (5) 100 µL pipettor (Neogen item #9272/#9278)
- (6) Tips for 100 µL pipettor (Neogen item #9410/#9407)
- (7) Paper towels or equivalent absorbent material
- (8) Plastic bucket for use as a waste receptacle
- (9) Microwell holder (Neogen item #9402)
- (10) Timer (Neogen item #9426)
- (11) Waterproof marker
- (12) Wash bottle (Neogen item #9400)
- (13) Distilled or deionized water

## **REVISION HISTORY**

### **Revision 2 (02/18/2015)**

- The test kit name was changed from “AGRI-SCREEN DON” to “AGRI-SCREEN for DON”.

### **Revision 1 (01/07/2015)**

- Correct Acronym of Policies, Procedures, and Market Analysis Branch (PPMAB) has been used.
- Phone number of Patrick McCluskey (816-659-8403) has been corrected.