

CHAPTER 13

ROSA® DON P/N TEST KIT

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13.1 GENERAL INFORMATION

The ROSA® DON P/N test kit uses lateral flow test strip technology that provides qualitative (equal to or less than a specified threshold) results in wheat and barley. Screening levels of 0.5 ppm (wheat only) and 1.0 ppm (wheat or barley) both available in this kit based on the extract volume used in test.

13.2 PREPARATION OF TESTING MATERIALS

NOTE: A Negative and Positive Control should be run periodically using the Performance Monitoring Mode (see section 13.4 e.) to verify performance of equipment and test strips (daily, weekly, bi-weekly, or monthly, based on internal quality assurance standards).

a. Negative Control.

Use the DON Dilution Buffer for the Negative Control.

b. Positive Control.

Prepare the 0.5 ppm DON Positive Control by adding 6.0 ml of DON Dilution Buffer and mix for 30 seconds. Allow to stand for 10 minutes at room temperature. Mix again before using. Use 300 µl as your diluted extract and test following Test Procedures section 13.4 (c). **A valid positive test result must be received before official testing.**

NOTE: Store at 32-45 °F for up to one week, or freeze at -4 °F for 2 months.

c. Equipment Preparation.

(1) Incubator must be at 45±1°C (temperature indicator is green).

(2) Incubator must be clean and level.

d. DON Dilution Buffer.

(1) Predispense 1.0 ml of DON Dilution Buffer into a micro-centrifuge tube for each sample to be tested.

(2) Use this solution at room temperature.

(3) Store DON Dilution bottle and any unused predispensed tubes at 32-45 °F.

e. Test Strips.

- (1) Remove ROSA® DON P/N moisture resistant container from the refrigerator and allow it to reach room temperature to limit condensation.
- (2) Remove only the number of strips to be used and return container to 32-45 °F storage. Strips are stable at room temperature for at least 12 hours.

NOTE: If blue desiccant packets turn white or pink, performance test the strips with Negative and Positive Controls before continued use.

13.3 EXTRACTION PROCEDURES

- a. Transfer 50 grams of ground sample into a clean extraction container.
- b. Add 250 ml of the deionized or distilled water.
- c. Blend or shake for a minimum of 60 seconds. Allow sample to settle for 1 minute to obtain sample extract.

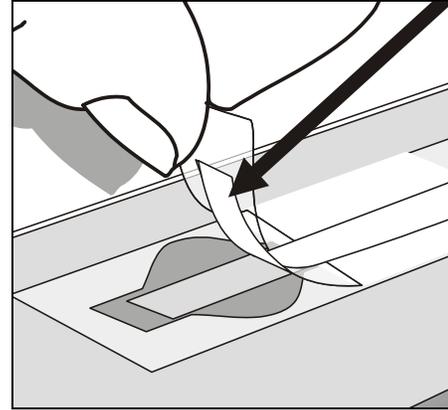
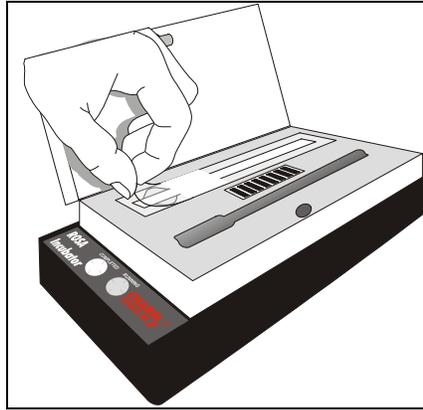
NOTE: If particles are present after settling, filter or centrifuge to clarify sample extract. **To Filter:** pour the extract into Whatman 2V (or equivalent) filter paper and filter into a labeled collection container. **To Centrifuge:** transfer 1.0-1.5 ml of sample extract to a labeled micro-centrifuge tube and centrifuge for 10 seconds. Clarified extract is now ready for testing.

13.4 TEST PROCEDURES

a. Sample Preparation for 0.5 ppm Screening Level

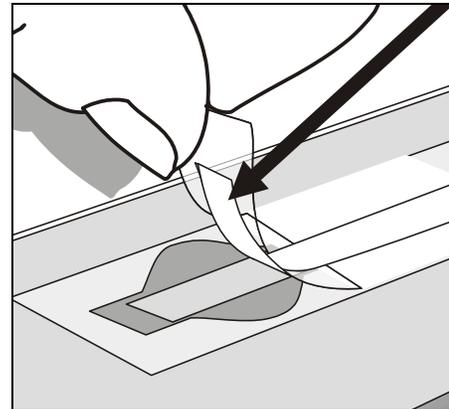
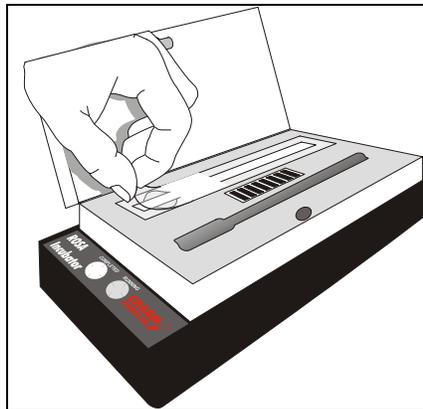
- (1) Pipet 100 µl of clarified extract to a predispensed (1.0 ml DON Dilution Buffer), labeled micro-centrifuge tube, cap, and mix. This is the diluted extract.
- (2) Label the test strip to identify sample.
- (3) Open the incubator lid and place test strip in the ROSA-M Incubator with the flat side facing upward.

- (4) While holding the strip flat on the incubator, use tab to peel tape back to the indicated line exposing the sample pad. Avoid bending back the white wick and sponge under the tape.



b. Sample Preparation for 1.0 ppm Screening Level

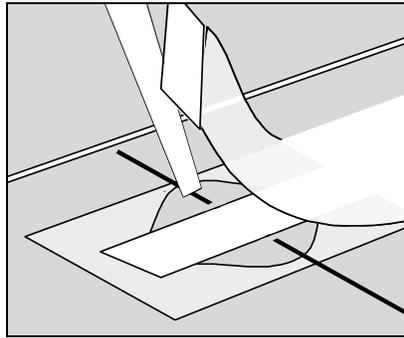
- (1) Pipet 50 μ l of clarified extract to a predisposed (1.0 ml DON Dilution Buffer), labeled micro-centrifuge tube, cap, and mix. This is the diluted extract.
- (2) Label the test strip to identify sample.
- (3) Open the incubator lid and place test strip in the ROSA-M Incubator with the flat side facing upward.
- (4) While holding the strip flat on the incubator, use tab to peel tape back to the indicated line exposing the sample pad. Avoid bending back the white wick and sponge under the tape.



c. Sample Analysis.

- (1) Pipet 300 µl of diluted extract into the side of sample compartment at the position indicated by the silver line on the incubator.

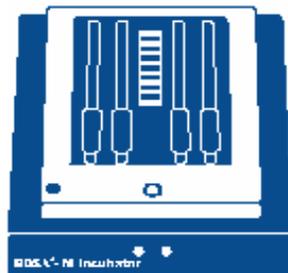
NOTE: Pipet very slowly.



- (2) Reseal the tape over the sample pad compartment. When testing multiple samples, complete the peel, pipet, and reseal steps on each strip before going to the next strip.

NOTE: Add diluted extract to all strips within 1 minute. If a quad incubator is used, 4 samples can be incubated simultaneously.

- (3) Close lid on the incubator and tighten the latch. The solid red timer light will automatically start when the lid is closed.



LF-INC4-45D: Quad incubator, 3-minute timer with display, set for 45° C for Test Strips

- (4) Incubate for 3 minutes. After the incubation step is complete, a beeper will sound and the yellow “test complete” light will begin to flash.
- (5) Remove strips and interpret the results. **Strips must be removed from the incubator and read within 2 minutes of incubation completion.** After strip removal, lower, but do not latch the incubator lid.

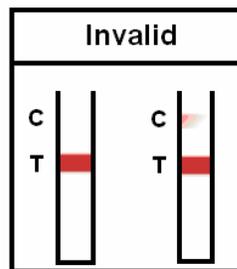
d. Visually Interpreting the Lateral Flow Test Strip.

Development of a Control Line indicates that the strip has functioned properly. Any strip that does not develop a Control Line should be discarded. A second preparation of the extract (using a fresh dilution) should be made and tested using another strip.

Note: The examples shown below depicting invalid, negative, and positive results are for illustration purposes only. Do not use these color bars as actual intensity measurement for determining if the sample is positive or negative.

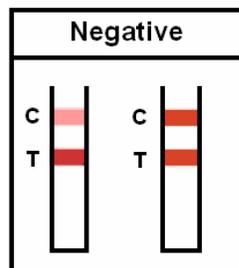
(1) Invalid Result.

A test is invalid if a Control Line is missing, smeared, or uneven, or if the Test Line is uneven. It is invalid if the diluted extract is obscuring either the Control (C) or Test Line (T).



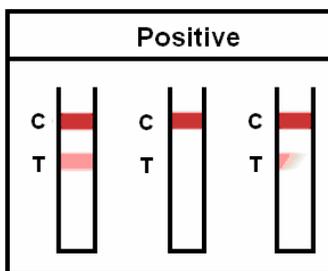
(2) Negative Result.

A sample containing DON residues less than or equal screening level will develop a Test Line that is darker or equal in intensity to the Control Line in the test area.



(3) Positive Result.

A sample containing DON residues in excess of screening level will develop a Test Line that is lighter in intensity than the Control Line



e. Interpreting the Lateral Flow Test Strip using the ROSA-M Reader.

NOTE: Periodically enter Performance Mode in reader by selecting DON channel and pressing ESC and then 5. Follow reader prompts. Run each of the calibration strips to verify reader performance. The strips should read +/- 2 ppb from the average written on the strips themselves.

- (1) Insert a clean valid test strip into the ROSA-M Reader. Slide the strip into the slot, with the sample compartment in the up position, until it stops.



LF-ROSA READER-M: ROSA-M Reader supplied with calibrators.

- (2) Read result on **DON** Channel (2-Line Mode) using **MATRIX 00** (Screening 0.5 ppm DON) or **MATRIX 01** (screening 1.0 ppm DON). If desired enter **Sample** and/or **Operator**. Press **ENTER** to read.
- (3) **Result:** The ROSA-M Reader interprets the strip and displays either **NEGATIVE** or **POSITIVE**.

13.5 REPORTING AND CERTIFYING TEST RESULTS

- a. When the screening level is 0.5 ppm report results on the pan ticket and inspection log as being equal to or less than 0.5 ppm (≤ 0.5 ppm), or as exceeding 0.5 ppm (> 0.5 ppm), as applicable.
- b. When the screening level is 0.5 ppm certify results as being equal to or less than 0.5 ppm or exceeding 0.5 ppm, as applicable.
- c. When the screening level is 1.0 ppm report results on the pan ticket and inspection log as being equal to or less than 1.0 ppm (≤ 1.0 ppm), or as exceeding 1.0 ppm (> 1.0 ppm), as applicable.
- d. When the screening level is 1.0 ppm certify results as being equal to or less than 1.0 ppm or exceeding 1.0 ppm, as applicable.
- e. Refer to the Certification section of the handbook for more detailed certification procedures.

13.6 CLEANING LABWARE

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

13.7 WASTE DISPOSAL

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

13.8 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits.

Kits can be purchased that contain 20, 100, or 500 strips and include Control and DON Dilution Buffer.

(1) LF-DONPN-20 –

- (a) 1 package containing 20 ROSA® DON P/N strips packed in a moisture-resistant container.
- (b) 1 - 0.5 ppm DON Control.
- (c) 1 DON Dilution Buffer

(2) LF-DONPN-100 –

- (a) 1 package containing 100 ROSA® DON P/N strips packed in a moisture-resistant container.
- (b) 1 - 0.5 DON ppm Control.
- (c) 1 DON Dilution Buffer.

(3) LF-DONPN-500 –

- (a) 5 packages containing 100 ROSA® DON P/N strips packed in a moisture-resistant container.
- (b) 5 - 0.5 ppm DON Controls.
- (c) 5 DON Dilution Buffers.

b. Materials Required but not Provided:

- (1) Sample grinder.
- (2) Balance.
- (3) Methanol - Reagent grade or better.
- (4) Deionized or Distilled water.
- (5) Sample extraction containers.

- (6) 1.0 ml pipettor and pipette tips.
 - (7) 300 μ l pipettor and pipette tips.
 - (8) 100 μ l pipettor and pipette tips.
 - (9) 50 μ l pipettor and pipette tips.
 - (10) 250 ml graduated cylinder.
 - (11) 1.5 ml micro-centrifuge tubes.
- c. Optional Equipment and Supplies:
- (1) Mini-centrifuge.
 - (2) Whatman 2V filter paper or equivalent.
 - (3) Filter funnel.

13.9 STORAGE CONDITIONS

a. Storage Conditions.

Test kits should be refrigerated between 32°- 45°F.

b. Precautions.

- (1) Do not use the test kits beyond the noted expiration date.
- (2) Prolonged exposure to high temperatures may adversely affect the test results.
- (3) Do not open the desiccated canister until ready to use the strips.