

CHAPTER 16

CHARM SCIENCES, INC. ROSA® DONQ TEST KIT

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16.1 GENERAL INFORMATION

The ROSA® LF- DONQ test kit uses lateral flow test strip technology that provides quantitative (0.5 ppm to 5 ppm) test results for wheat, corn, barley, malted barley, milled rice, rough rice, sorghum, wheat flour, wheat midds, and oats.

16.2 PREPARATION OF TESTING MATERIALS

NOTE: A Negative and Positive Control test sample should be run periodically using the Performance Monitoring Mode (see section 16.4 d) 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 DONQ Dilution Buffer for the Negative Control. **Negative control should read less than 100 ppb.**

b. Positive Control.

- (1) Reconstitute/prepare the 1.0 ppm DON Positive Control by adding 3.0 ml of DONQ Dilution Buffer and mix for 30 seconds.
- (2) Allow to stand for 10 minutes at room temperature before use.
- (3) Mix again before using.
- (4) Use 300 µl of reconstituted/prepared positive control as your diluted extract and test following Test Procedures section 16.4 (b).

Note: Positive control should read between 500-1500 ppb. A valid positive test result must be received before official testing. If Calibration Strips or Controls do not perform in specified ranges, discontinue use and notify service area Field Office Location, TSD, or Charm Sciences.

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 $45 \pm 1^{\circ}\text{C}$ (temperature indicator should be green).
 - (2) Incubator must be clean and level.

- d. DONQ Dilution Buffer.
 - (1) Pre-dispense 1.0 ml of DONQ Dilution Buffer into a micro-centrifuge tube for each sample to be tested.
 - (2) Use this solution at room temperature.
 - (3) Store DONQ Dilution Buffer and any unused predispensed tubes at 32-45 °F.

- e. Test Strips.
 - (1) Remove ROSA® DONQ 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.

16.3 EXTRACTION PROCEDURES

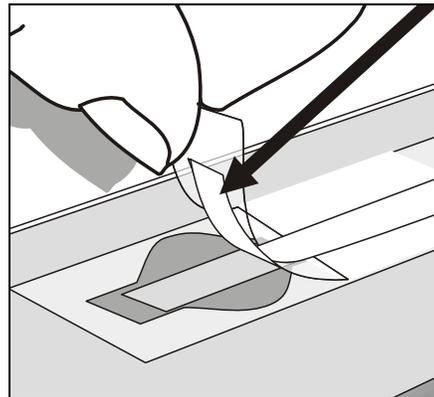
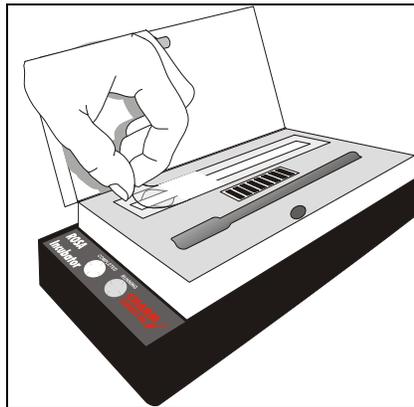
- a. Transfer 50 grams of ground sample into a clean extraction container.
- b. Add 250 ml of the deionized or distilled water.
- c. Blend for 1 minute or shake for 2 minutes. Allow sample to settle for 1 minute to obtain sample extract.

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.

16.4 TEST PROCEDURES

a. Sample Preparation.

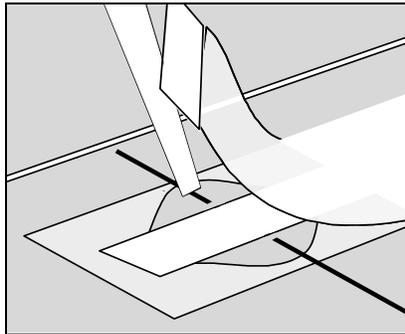
- (1) Pipette 100 μ l of sample extract to a predisposed (1.0 ml DONQ Dilution Buffer), labeled micro-centrifuge tube, cap, and mix. This is the diluted extract.
- (2) Label the test strip to identify sample.
- (3) Open the incubator lid and place test strip in the ROSA-M Incubator with the flat side 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 Analysis.

- (1) Pipette 300 μ l of diluted extract or control into the side of sample compartment at the position indicated by the silver line on the incubator.

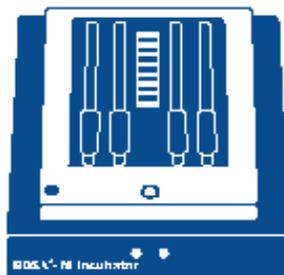
NOTE: Pipette very slowly.



- (2) Reseal the tape over the sample pad compartment. When testing multiple samples, complete the peel, pipette, 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-INC4-10-45D: Quad incubator, 10-minute timer with display, set for 45° C for Test Strips.

- (4) Incubate for 10 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.

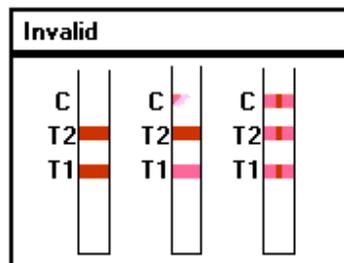
c. Visually Inspecting the Lateral Flow Test Strip.

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 results are for illustration purposes only.

(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).



d. 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 +/- 200 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 (3-Line Mode) using **MATRIX 00** (0.5 to 5 ppm DON). If desired enter **Sample** and/or **Operator**. Press **ENTER** to read.
- (3) **Result:** The ROSA-M Reader interprets the strip and displays the concentration in ppb. The reading must be converted to ppm by dividing the ppb concentration by 1000 (e.g., 500 ppb = 0.5 ppm).

16.5 SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the conformance limit. If the applicant wishes to obtain an official result above the conformance limit, the sample extract must be diluted so that a quantified value between 0.5 – 5 ppm is obtained. The final DON concentration will be interpreted using the ROSA-M Reader Channel (3-Line Mode) with **MATRIX 02**.

For Example: If the original analysis reported DON results at 9.0 ppm and the test kit conformance limit value is 5 ppm, in order to obtain a true value proceed as follows.

1. **First Diluted Extract** is obtained by following steps in sections 16.3 Extraction Procedures, and 16. 4 a Test Procedures, section (1).
2. **Second Diluted Extract** is prepared from the First Diluted Extract by pipetting 300 μ l (0.300 ml) of the original diluted extract to a pre-dispensed (**1.0 ml DON Dilution Buffer**) micro-centrifuge tube, cap mix, and label. Repeat for additional diluted extracts.

3. **Third Diluted Extract** is prepared from the Second Diluted Extract for **0.5 – 5 ppm quantitation**. Pipette 100 µl (0.100 ml) of Second Diluted Extract to a pre-dispensed (**1.0 ml DON Dilution Buffer**) micro-centrifuge tube, cap, mix, and label. Repeat for additional Second Diluted extracts.
4. Follow the steps in Section 16.4 Test Procedures, sections b., using 300 µl (0.300 ml) of the Third Diluted Extract as test sample.
5. Insert a clean and valid test strip into the **ROSA-M Reader**. Slide into slot, with sample compartment in the up position, until it stops. Read results on **DON** Channel (3-Line Mode), with **MATRIX 02**. If desired, enter **SAMPLE**, and/or **OPERATOR**. Press **ENTER** to read.
6. **READING:** The number displayed on the ROSA-M Reader is the concentration of **DON (ppm)** in the sample. There is no conversion needed.

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the conformance limits of test kit in use.

16.6 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.

16.7 CLEANING LABWARE

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

16.8 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.

16.9 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits.

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

(1) LF-DONQ-20

- (a) 1 package containing 20 ROSA® DONQ strips packed in a moisture-resistant container.
- (b) 1 - 1.0 ppm DON Control.
- (c) 1 DONQ Dilution Buffer.

(2) LF-DONQ-100

- (a) 1 package containing 100 ROSA® DONQ strips packed in a moisture-resistant container.
- (b) 1 - 1.0 ppm DON ppm Control.
- (c) 1 DONQ Dilution Buffer.

- (3) LF-DONQ-500
 - (a) 5 packages containing 100 ROSA® DONQ strips packed in a moisture-resistant container.
 - (b) 5 - 1.0 ppm DON Controls.
 - (c) 5 DONQ Dilution Buffers.

b. Materials Required but not Provided:

- (1) Sample grinder.
- (2) Balance.
- (3) Deionized or Distilled water.
- (4) Sample extraction containers.
- (5) 1.0 ml pipettor and pipette tips.
- (6) 300 µl pipettor and pipette tips.
- (7) 100 µl pipettor and pipette tips.
- (8) 250 ml graduated cylinder.
- (9) 1.5 ml micro-centrifuge tubes.

c. Optional Equipment and Supplies:

- (1) Mini-centrifuge.
- (2) Whatman 2V filter paper or equivalent.
- (3) Filter funnel.

16.10 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.