

# Test Kit Instruction

November 6, 2020

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## **Charm Sciences, Inc. ROSA DONQ2 Quantitative Test**

### **FORWARD**

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. 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 Mycotoxin Handbook for information on use of this test kit in official inspections including sampling, general sample preparation, 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).

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## 1. GENERAL INFORMATION

ROSA DONQ2 Quantitative Test is an immunoreceptor assay utilizing ROSA (Rapid One Step Assay) lateral flow technology. Deoxynivalenol (DON) or vomitoxin is extracted from the samples using water. DON interacts with colored beads in the lateral flow test strip and the color intensity in the test and control zones is measured by the Charm EZ-M reader and interpreted as parts per million (ppm) DON.

Approved Test Kit Information	
<b>Test Kit Vendor:</b>	<i>Charm Sciences, Inc. 978-687-9200</i>
<b>Test Kit Name:</b>	ROSA DONQ2 Quantitative Test
<b>Product Number:</b>	LF-DONQ2
<b>Test Format:</b>	Lateral Flow Strip
<b>Reader:</b>	Charm EZ-M, Model# LF-ROSA-EZ-M
<b>Detection Method:</b>	Reflectance
<b>Effective Date of Instructions:</b>	11/06/2020
<b>Conformance Range:</b>	0.50 ppm – 30 ppm
<b>Number of Analyses to Cover Conformance Range:</b>	2
<b>Type of Service:</b>	Quantitative
<b>Approved Commodities:</b>	Corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), wheat (including whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings), barley (with hull, including malting barley), brown rice, buckwheat, corn bran, corn germ meal, corn gluten feed (including corn gluten feed meal), corn gluten meal, distillers dried grain with solubles (DDGS), hominy, malted barley (including malted barley flour), milled rice (including brewer's rice and glutinous rice), oats (whole oats with hull), rice bran, rough rice, rye (or rye berries, including whole grain rye flour, rye meal, cracked rye, and rye chops), sorghum, soybean meal, triticale, wheat bran (including wheat bran aleurone)
<b>Extraction Method:</b>	For samples ground so that at least 90% passes a No. 20 sieve, shake vigorously by hand for 1 minute. For samples ground so that 60 – 89% passes a No. 20 sieve, shake vigorously by hand for 3 minutes.

## 2. PREPARATION OF TESTING MATERIALS AND EQUIPMENT

### a. Test Strips.

Remove from the container only the number of test strips to be used in 1 day. Keep these test strips at room temperature (18 °C to 30 °C) during daily use for up to 12 hours; discard the unused test strips after the 12-hour period.

### b. DONQ2 Dilution Buffer.

(1) Dispense buffer into a clean micro-centrifuge tube and label for each sample to be tested.

(2) Use pre-dispensed buffer tubes and buffer solution at room temperature.

### c. Negative Control.

(1) DONQ2 Dilution Buffer is used as a Negative Control.

(2) Use **Negative Control** in TEST PROCEDURES section.

### d. Positive Control.

(1) Reconstitute the dry Positive Control (provided with test kit) by adding 6.0 milliliters (mL) (using the 1000 µL variable volume pipette) DONQ2 Dilution Buffer. Cap, shake well, and allow to stand for 10 minutes at room temperature before use. Mix before use.

(2) Use reconstituted **Positive Control** in TEST PROCEDURES section.

### e. Reader and Test Strip Performance Testing.

(1) Enter performance mode in Charm EZ-M reader by selecting Perf. Mon. from the Main Menu, followed by Perf. Test.

(a) Follow the system prompts to test calibration strips (LO CAL and HI CAL).

(b) Follow the system prompts to test controls (NEG CTRL and POS CTRL); select DONQ2 from the TESTS list if prompted.

(2) Test calibration strips daily to verify Charm EZ-M reader performance. Calibration strips must test/perform in the specified ranges; only use calibration strips that match the serial number of the Charm EZ-M reader.

(3) Test Negative Control and Positive Control weekly to verify test strip performance. Valid control ranges are:

(a) Negative Control: less than or equal to 0.1 ppm

- (b) Positive Control: 0.5 ppm to 1.5 ppm

**If calibration strips or controls do not perform in specified ranges, discontinue use and contact Charm Sciences for assistance. Notify your monitoring field office or TSD with any documented information for quality control purposes.**

f. ROSA Incubator.

- (1) ROSA Incubator must be clean and level.
- (2) The ROSA Incubator temperature must be at  $45^{\circ}\text{C} \pm 1^{\circ}\text{C}$  (the temperature indicator should match the incubator temperature).

### 3. EXTRACTION PROCEDURE

The sample to be tested should be collected and prepared according to accepted sampling techniques (see Mycotoxin Handbook).

a. Procedure for corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), wheat (including whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings), barley (with hull, including malting barley), brown rice, buckwheat, corn bran, corn germ meal, corn gluten feed (including corn gluten feed meal), corn gluten meal, hominy, malted barley (including malted barley flour), milled rice (including brewer's rice and glutinous rice), oats (whole oats with hull), rice bran, rough rice, rye (or rye berries, including whole grain rye flour, rye meal, cracked rye, and rye chops), sorghum, soybean meal, triticale, wheat bran (including wheat bran aleurone).

- (1) Weigh  $50 \pm 0.2$  grams ground samples into a clean extraction container.
- (2) Using a 250 mL graduated cylinder, add 250 mL deionized or distilled water.
- (3) For samples ground so that at least 90% passes a No. 20 sieve, shake vigorously by hand for 1 minute. For samples ground so that 60 – 89% passes a No. 20 sieve, shake vigorously by hand for 3 minutes.
- (4) Using a 3 mL transfer pipet, transfer 1 mL to 1.5 mL extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (centrifuge within 30 minutes of extraction and use centrifuged extract within 2 hours).
- (5) Repeat steps for additional samples.

b. Procedure for DDGS.

- (1) Weigh  $50 \pm 0.2$  grams ground samples into a clean extraction container.
- (2) Using a 250 mL graduated cylinder, add 250 mL deionized or distilled water.

- (3) For samples ground so that at least 90% passes a No. 20 sieve, shake vigorously for 1 minute. For samples ground so that 60 – 89% passes a No. 20 sieve, shake vigorously for 3 minutes.
- (4) Adjust pH of an aliquot of extract by adding 10-30% KOH or NaOH dropwise until pH is between 6.5 and 7.5; monitor pH with pH strips or pH meter.
- (5) Using a 3 mL transfer pipet, transfer 1 mL to 1.5 mL extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (centrifuge within 30 minutes of extraction and use centrifuged extract within 2 hours).
- (6) Repeat steps for additional samples.

#### 4. SAMPLE PREPARATION FOR QUANTITATION

a. Prepare Diluted Extract for 0.5 ppm to 5 ppm quantitation.

- (1) Pipet 1000 microliters ( $\mu\text{L}$ ) DONQ2 Dilution Buffer using the 1000  $\mu\text{L}$  variable volume pipette into a clean micro-centrifuge tube.
- (2) Pipet 50  $\mu\text{L}$  centrifuged extract using the 50  $\mu\text{L}$  fixed volume pipette to the micro-centrifuge tube containing 1000  $\mu\text{L}$  DONQ2 Dilution buffer, cap, mix (shake vigorously for 5 seconds), and label. This tube contains the **Diluted Extract**.
- (3) Repeat for additional samples.
- (4) Use Diluted Extract (use within 6 hours of preparation) as your test sample in Sample Analysis found in TEST PROCEDURES section.

b. Prepare Second Diluted Extract for 5 ppm to 30 ppm quantitation.

- (1) Pipet 1000  $\mu\text{L}$  DONQ2 Dilution Buffer using the 1000  $\mu\text{L}$  variable volume pipette into a clean micro-centrifuge tube.
- (2) Pipet 200  $\mu\text{L}$  **Diluted Extract** using the 1000  $\mu\text{L}$  variable volume pipette to micro-centrifuge tube containing 1000  $\mu\text{L}$  DONQ2 Dilution Buffer, cap, mix (shake vigorously for 5 seconds), and label. This tube contains the **Second Diluted Extract**.
- (3) Repeat for additional samples.
- (4) Use Second Diluted Extract (use within 6 hours of preparation) as your test sample in Sample Analysis found in TEST PROCEDURES section.

## 5. TEST PROCEDURES

### a. Sample Analysis.

- (1) Check that the ROSA Incubator temperature is  $45^{\circ}\text{C} \pm 1^{\circ}\text{C}$ .
- (2) Label test strip(s) to identify sample.
- (3) Place test strip in the ROSA Incubator with the flat side facing upward.
- (4) Hold the test strip flat in the ROSA Incubator and use tab to expose sample compartment by peeling tape back to “Peel to Here” line. Avoid lifting the test strip and sponge under tape and bending back the white wick and sponge under the tape.
- (5) Holding the pipette vertically, slowly pipet 300  $\mu\text{L}$  test sample (**Diluted Extract, Second Diluted Extract or Control**) using the 300  $\mu\text{L}$  fixed volume pipette into the sample compartment at the ROSA Incubator line.
- (6) Reseal the tape over the sample pad compartment.

**Incubate no more than two test strips in a single ROSA incubator at a time:**

**(a) Peel, pipet, and reseal before starting next strip.**

**(b) Complete procedure for both test strips within 30 seconds.**

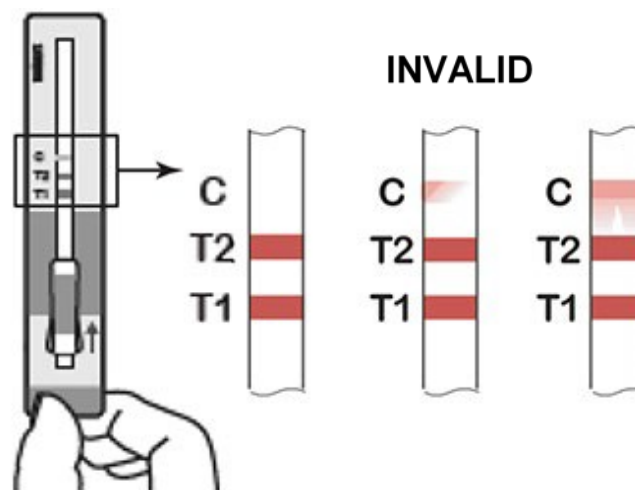
- (7) Close lid on the ROSA Incubator.
- (8) Incubate for 2 minutes.
- (9) Remove strip from the ROSA Incubator.

Do not squeeze sample compartment. Hold test strip vertically with sample compartment in the down position until interpreted.

- (a) Wipe foreign matter (dust, etc.) from the test strip.
- (b) Inspect and read the test strip(s) within 30 seconds of incubation completion.
- (c) Lower ROSA Incubator lid; do not re-latch.

b. Visual Inspection.

- (1) The test strip is INVALID if any of the following are observed:
  - (a) C (Control) line is missing.
  - (b) T1, T2 (Test) or C line is smeared or uneven.
  - (c) T1, T2, or C line is obscured by diluted extract or control.
  - (d) Beads do not flow past T1, T2 or C lines.



- (2) Do not put INVALID test strips in the Charm EZ-M reader.
- (3) If test strip is INVALID, re-test the diluted extract or control.

c. Interpretation.

- (1) Insert a clean and valid test strip into the Charm EZ-M reader. Slide the strip into the slot with the sample compartment in the down position until it stops.
- (2) Read results on DONQ2 from the TESTS list with COMMODITY and DILUTION selected for sample. If desired, enter OPERATOR ID, SAMPLE ID, and/or LOT NUMBER. Close door to read.
  - DE: Diluted Extract for 0.5 ppm to 5 ppm quantitation.
  - 2ND DE: Second Diluted Extract for 5 ppm to 30 ppm quantitation.

**Note: For controls, see Reader and Test Strip Performance Testing in PREPARATION OF TESTING MATERIALS AND EQUIPMENT section.**



- (3) **READING:** The number displayed is the concentration of DON (ppm) in the sample.

A Diluted Extract **READING** greater than 5.4 ppm indicates that the sample concentration is greater than the sensitivity range of the sample dilution; prepare Second Diluted Extract and perform assay with another test strip.

A Second Diluted Extract **READING** less than 3.5 ppm indicates a value below the sensitivity range of the sample dilution; perform assay with another strip using Diluted Extract.

A Second Diluted Extract **READING** greater than 30 ppm indicates that the sample concentration is greater than the sensitivity range of the sample dilution; report test results as greater than 30 ppm on the work record and certify as "DON exceeds 30 ppm".

## **6. REPORTING AND CERTIFYING TEST RESULTS**

Refer to the Mycotoxin Handbook for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or [Patrick.J.McCluskey@usda.gov](mailto:Patrick.J.McCluskey@usda.gov)).

## **7. STORAGE CONDITIONS AND PRECAUTIONS**

### **a. Storage Conditions.**

- (1) Store test strips refrigerated (0 °C to 7 °C) in tightly closed supplied container.
- (2) Store dilution buffer bottle and pre-dispensed micro-centrifuge tubes refrigerated.
- (3) Store dry 1000 ppb DON Positive Control refrigerated.
- (4) Store reconstituted Positive Control refrigerated for up to 1 week or aliquot (at least 0.5 mL) to clean micro-centrifuge tubes, label, and freeze (-15 °C or below) within 6 hours of reconstitution for up to 2 months. Thaw slowly (overnight in refrigerator or with cool water) and shake well before use. Store thawed Positive Control refrigerated and use within 24 hours of thawing; **DO NOT REFREEZE.**

### **b. Precautions.**

- (1) **Test Strips.**
  - (a) To open test strip canister, remove and save plastic lid with foil-lined foam insert to reseal container. Lift foil tab and peel foil seal off container. Discard foil seal.
  - (b) In high humidity, limit condensation by opening container after it has warmed to room temperature.

- (c) Inspect/verify desiccant indicator. Beads inside desiccant packets should be blue. Do not use test strips if the blue beads have turned purple or pink.
  - (d) Re-shape dented sample compartments to fit into ROSA Incubator.
- (2) Use DONQ2 Dilution Buffer supplied with each test kit only at room temperature. Keep buffer at room temperature during daily use for up to 12 hours.
- (3) Do not use the test kits beyond the noted expiration date.
- (4) Debris on test strips may alter the reader optics. Keep equipment clean. Wipe dust and liquid off test strips before inserting into reader.
- (5) ROSA Incubator must be clean and level. ROSA Incubator temperature must be  $45^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . The temperature indicator should match the ROSA Incubator temperature. A daily thermometer check is recommended. Keep ROSA Incubator lid lowered, but not latched, unless performing test procedure. ROSA Incubator may take 10 minutes to reach proper temperature depending on ambient temperature.
- (6) Charm EZ-M reader must be clean and level. Keep reader lid closed unless performing procedure.

## 8. EQUIPMENT AND SUPPLIES

### a. Test Strips.

- (1) LF-DONQ2-20K/-20ESK
  - (a) One container of 20 DONQ2 test strips
  - (b) 1000 ppb DON Positive Control
    - 1 One control in LF-DONQ2-20K
    - 2 Two controls in LF-DONQ2-20ESK
  - (c) One DONQ2 Dilution Buffer
- (2) LF-DONQ2-100K/-100ESK
  - (a) One container of 100 DONQ2 test strips
  - (b) 1000 ppb DON Positive Control
    - 1 One control in LF-DONQ2-100K
    - 2 Five controls in LF-DONQ2-100ESK
  - (c) One DONQ2 Dilution Buffer

(3) LF-DONQ2-500K/-500ESK

- (a) Five containers of 100 DONQ2 test strips
- (b) 1000 ppb DON Positive Control
  - 1 Five controls in LF-DONQ2-500K
  - 2 Twenty-five controls in LF-DONQ2-500ESK
- (c) Five DONQ2 Dilution Buffers

b. Materials required but not provided.

- (1) 50 µL pipette and pipet tips (Charm order code: PIP-50UL and 100-ULT-X1 (rack of 96 tips))
- (2) 300 µL pipette and pipet tips (Charm order code: PIP-300UL-1STOP-M and 1-MLT-96 (rack of 96 tips))
- (3) 100 to 1000 µL variable volume pipette and pipet tips (Charm order code: PIP-100-1000UL-1STOP and 1-MLT-96 (rack of 96 tips))
- (4) 250 mL graduated cylinder (Charm order code: GRAD-CYL-250ML)
- (5) FGIS-approved scale (balance) with minimum division of 0.1 g
- (6) Charm EZ-M reader (Charm order code: LF-ROSA-EZ-M)
- (7) Deionized or distilled water
- (8) Extraction containers or Whirl-Pak bags (Charm order code: WHIRLPK-50 (50/PK))
- (9) Micro-centrifuge tubes (Charm order code: CEN-2-0ML-TUBES-100 (100/PK))
- (10) Mini-centrifuge (Charm order code: MINICEN-110V)
- (11) Printer for Charm EZ-M reader (optional Charm order code: PRN-THERM-CITIZEN)
- (12) ROSA Incubator (Charm order code: LF-INC4-2-45D or LF-INC2-2-45)
- (13) Sample grinder (see FGIS Mycotoxin Handbook)
- (14) Transfer pipets (Charm order code: PIP-3ML-100K (100/PK))

c. Materials required but not provided for testing DDGS.

- (1) 10-30% KOH (Sigma cat #221473-25G) or NaOH (Sigma cat# 221465-25G) in water (w/v)
- (2) Conical tubes (Charm order code: TST-50ML (50/PK))
- (3) pH paper (Charm order code: LA-PH-STRIPS) or pH meter

## 9. REVISION HISTORY

Effective Date: 11/06/2020

# Refer to GLPSA Test Kit Instructions for Complete Test Procedure

## ROSA® DONQ2 Quantitative Test Flow Chart

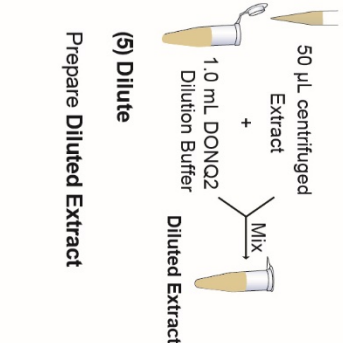
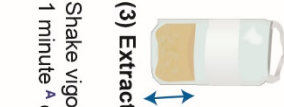
Approved Commodities:

Barley, Brown Rice, Buckwheat, Corn, Corn Bran, Corn Germ Meal, Corn Gluten Feed, Corn Gluten Meal, Distillers Dried Grain with Solubles, Hominy, Malted Barley, Milled Rice, Oats, Rice Bran, Rough Rice, Rye, Sorghum, Soybean Meal, Triticale, Wheat, Wheat Bran

See Validated Commodities Below

Quantitation Ranges: 0.5 to 5 ppm  
5 to 30 ppm

### Sample Preparation



(1) Weigh  
Ground sample  
50.0 ± 0.2 g

(2) Add Water  
Deionized or Distilled Water  
250 mL

(3) Extract  
Shake vigorously for 1 minute<sup>A</sup> or 3 minutes<sup>B</sup>

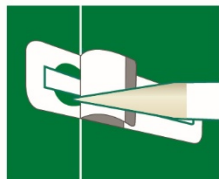
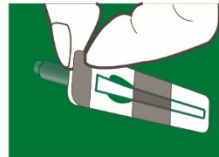
(4) Clarify  
Allow extract to settle for 1 minute. Centrifuge for 10 seconds

(5) Dilute  
Prepare Diluted Extract

<sup>A</sup>Extract for 1 minute if 90% of sample passes a No. 20 sieve.  
<sup>B</sup>Extract for 3 minutes if 60 – 89% of sample passes a No. 20 sieve.

**DDGS Only**  
Adjust Extract pH to 6.5 to 7.5 with 10-30% KOH

### Test Procedure

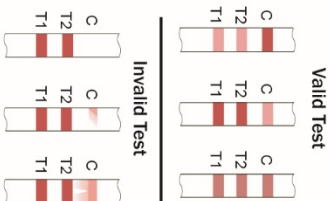


(1)  
Place test strip in ROSA incubator.

(2)  
Peel tape.  
Pipet 300 µL Diluted Extract into sample compartment.  
Reseal tape.

(3)  
Close lid.  
Incubate for 2 minutes.

### Read Result



Charm EZ-M Reader: Select appropriate test (DONQ2), commodity and dilution if prompted.		
Sample	Dilution	Quantitation Range
Diluted Extract	DE	0.5 to 5 ppm
2 <sup>nd</sup> Diluted Extract	2ND DE	5 to 30 ppm

(1) Prepare 2<sup>nd</sup> Diluted Extract  
(2) Repeat Test Procedure (steps 1, 2, 3) with 2<sup>nd</sup> Diluted Extract  
(3) Read Result



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