

Program Notice

FGIS-PN-12-05

06/25/12

CHARM SCIENCES ROSA® FAST5 DON QUANTITATIVE TEST METHOD

1. PURPOSE

This program notice is revised to add/offer supplemental analysis test procedures for the Charm ROSA® FAST5 DON quantitative test kit product number LF-DONQ-FAST5. The test method is approved for official DON testing of wheat, corn, barley, malted barley, wheat bran, wheat flour, wheat midds, milled rice, rough rice, sorghum, oats, and distiller's dried grain with solubles at 0.5-5.0 parts per million (ppm).

2. REPLACEMENT HIGHLIGHTS

This program notice supersedes FGIS PN-12-01, Charm Sciences® FAST5 DON Quantitative Test Method, dated 03/08/12. The program notice is being revised to include supplemental analysis procedures for quantitation greater than 5.4 ppm using a diluted sample, and minor editorial changes.

3. BACKGROUND

The Policies, Procedures, and Market Analysis Branch (PPMAB) and Technology and Science Division (TSD) have an ongoing evaluation of new and recertified mycotoxin test methods. In an effort to offer official use of this new TSD approved test method in a timely manner PPMAB has decided to issue this program notice prior to the release of the revised DON handbook. The following test procedures are approved for use by official testing locations.

4. TEST INSTRUCTIONS

a. General Information.

The Charm Sciences ROSA® FAST5 DON Quantitative test kit is an immunoreceptor assay utilizing ROSA lateral flow test strip technology. DON is extracted from a sample using distilled or deionized water. The extracted DON interacts with the colored beads and the intensity of the color in the test zone is read as parts per billion (ppb) by the ROSA-M Reader.

To convert the ppb to parts per million (ppm), divide ppb concentration by 1000, (e.g., 500 ppb/1000 = 0.5 ppm).

Approved Test Kit Information					
Test Kit Vendor	Charm Sciences Inc. 1-978-687-9200				
Test Kit Name	Charm ROSA® FAST5 DON Quantitative Test Method				
Product Number	Conformance Limit		Type of Service	Extraction Solution	Supplemental Analysis
LF-DONQ-FAST5	Min	Max	Quantitative	Deionized or Distilled Water	Yes
QAC Number VOMS	0.5 ppm	5 ppm			
Grain/Commodities Approved for					
Wheat, Corn, Barley, Malted Barley, Sorghum, Milled Rice, Rough Rice, Oats, Wheat Bran, Wheat Flour, Wheat Midds, and Distiller's Dried Grain with Solubles.					

b. Preparation of Testing Materials.

(1) Negative Control.

The DONQ-FAST5 Dilution Buffer is used as a Negative Control. To run the Negative Control, use 300 µl following Sample Analysis section j.

Note: Negative control should read less than 100 ppb.

(2) Positive Control.

The 1000 ppb DON Positive Control is supplied dry. It is to be stored under refrigeration.

- (a) To reconstitute positive control add 3.0 ml DONQ-FAST5 Dilution Buffer, and shake/mix well for 30 seconds.
- (b) Allow to stand for 10 minutes at room temperature before use.
- (c) Mix again before using.
- (d) Reconstituted Positive Control is equivalent to Diluted Extract prepared from 1000 ppb in wheat.
- (e) To run the Positive Control, use 300 µl of reconstituted/prepared positive control as the diluted extract following Sample Analysis section j.
- (f) Store reconstituted Positive Control refrigerated for up to 1 week, or aliquot and freeze within 6 hours of reconstitution at - 15°C or below for up to 2 months.

- (g) Thaw frozen Positive Control slowly (overnight in refrigerator or with cool water) and shake well before use.
- (h) Store thawed Positive Control refrigerated and use within 24 hours of thawing. DO NOT REFREEZE.

Note: Negative and Positive Control test sample should be run weekly using the Performance Monitoring Mode to verify performance of equipment and test strips. See Performance Testing Procedures section g.

c. Equipment Preparation.

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

d. DONQ-FAST5 Dilution Buffer.

- (1) Use DONQ-FAST5 Dilution Buffer supplied with each test kit only.
- (2) Dispense 1.0 ml of DONQ-FAST5 Dilution Buffer into a micro-centrifuge tube for each sample to be tested. If using dispenser, prime with DONQ-FAST5 Dilution Buffer solution before dispensing. After dispensing, flush dispenser with deionized or distilled water, then flush out remaining water with air. Store dispenser in clean container.
- (3) Use pre-dispensed tubes and buffer solution at room temperature (18 to 30°C).
- (4) Store DONQ-FAST5 Dilution Buffer bottle and pre-dispensed tubes refrigerated.

e. Test Strips.

- (1) Store refrigerated, tightly closed in supplied container.
- (2) To open, 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.
- (3) In high humidity, limit condensation by opening container after it has warmed to room temperature, estimated between 20 - 30 minutes from the time the container was removed from the refrigerator.

- (4) Inspect/verify desiccant indicator. Beads inside desiccant packets should be blue. Do not use test strips if the blue beads turn purple or pink.

Note: If blue desiccant packets turn white or pink, performance test the strips with Negative and Positive Controls before continued use. Contact Charm Sciences for further instructions.

- (5) Remove from the container only the number of test strips to be used in one day, document time of removal. Keep these test strips at room temperature during daily use for up to 12 hours. Unused test strips should be discarded.

f. Extraction Solution.

- (1) Obtain distilled or deionized water for extraction.
- (2) Clearly label and store at room temperature in a tightly sealed container.
- (3) Dispose in accordance with all applicable local, state, and federal disposal regulations.

g. Performance Testing Procedures.

- (1) Enter performance mode by selecting DON channel in 3 line mode on the ROSA-M-Reader by pressing ESC, then the number 5, then ENTER. Follow ROSA-M-Reader prompts.
- (2) Test calibration strips daily to verify ROSA-M-Reader performance. Calibration strips must test/perform in the specified ranges.
- (3) Test Negative Control and Positive Control weekly to verify performance of equipment and test strips.
- (4) Valid Control Ranges are:
 - Negative Control must read less than or equal to 100 ppb.
 - Positive Control must read between 500 to 1500 ppb.
- (5) 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.

h. Extraction Procedures.

- (1) Obtain a representative sample according to official procedures for the requested commodity.

Commodity	Official Sample Size	Ground Sample size	Sample Basis
Wheat, Barley, Malted Barley	200 grams	100 grams	Dockage and Stone free
Corn	1000 grams	1000 grams	BCFM included
Milled Rice	200 grams	100 grams	Milled basis
All other approved commodities	200 grams	100 grams	Sample as whole

- (2) Grind/mill to the optimum particle size. See DON handbook chapter 3 (sample preparation) for further information.
- (3) Mix thoroughly and transfer 50 grams of ground sample into a clean extraction container.

Note: For **corn** cut down to a representative 50 gram test portion using a GIPSA approved divider (i.e. Boerner or riffle).

- (4) Add 250 ml of the deionized or distilled water.
- (5) Blend or shake vigorously for 1 to 2 minutes. Allow sample to settle for 1 minute to obtain sample extract.

Note: Extraction Procedures for Distiller's Dried Grains, with Solubles.

- (a) Transfer 10 to 15 ml of sample extract to a 50 ml conical tube.
- (b) Using a transfer pipet add 30 percent of KOH one drop at a time to the conical tube, mix sample extract, and monitor pH with pH strips or pH meter.
- (c) Add the 30 percent KOH one drop at a time (as needed) until pH is between 6.5 and 7.5, before proceeding.
- (6) Filter or Centrifuge to clarify sample extract.
- (a) To filter, place a Whatman 2V or equivalent filter paper into a funnel, pour sample extract into filter, and collect clarified extract.

- (b) To centrifuge, transfer sample extract (1.0 to 1.5 ml using a transfer pipet) to a labeled micro-centrifuge tube and centrifuge for 10 seconds to obtain clarified extract.

Note: Filtering for Wheat Midds.

- (c) Filter each extract by drawing into 1 ml syringe and passing through a glass fiber/cellulose acetate (GF/CA) syringe filter.

- (d) Collect filtered extract in a clean micro-centrifuge tube.

- (7) Prepare additional sample extracts (up to 4 for Quad ROSA incubator) following steps (1) - (6).

i. Sample Preparation.

- (1) Sample Preparation of the Diluted Extract for 0.5 to 1.4 ppm quantitation.

- (a) Pipet 100 μ l of the **clarified sample extract** to a pre-dispensed (1.0 ml DONQ Fast5 Dilution Buffer) micro-centrifuge tube, cap, mix and label. This is the first diluted extract.

- (b) Repeat for additional samples.

- (2) Sample Preparation of the Second Diluted Extract from First Diluted Extract for 1 to 5 ppm quantitation.

- (a) Pipet 300 μ l of the **first diluted extract** to a pre-dispensed (1.0 ml DONQ Fast5 Dilution Buffer) micro-centrifuge tube, cap, mix, and label. This is the second diluted extract.

- (b) Repeat for additional samples.

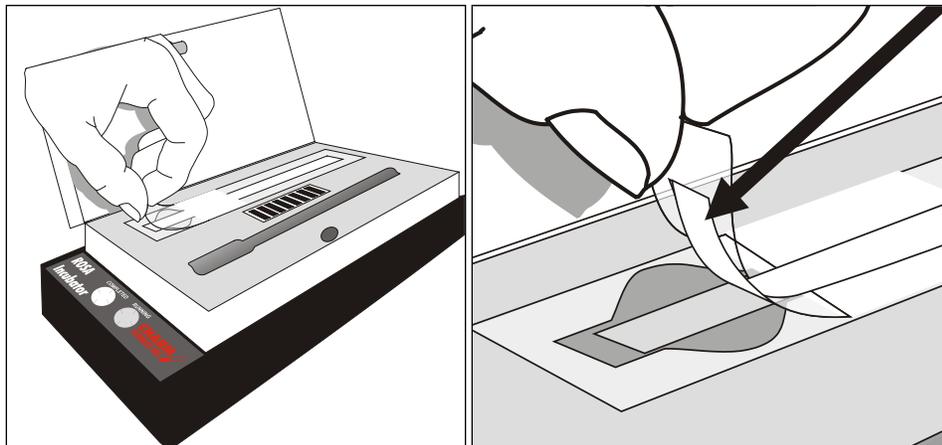
Note: Laboratories may initially test the second diluted extract if levels typically reported in their market area are within the 1 ppm to 5 ppm testing range.

j. Sample Analysis.

- (1) Label test strip(s) to identify sample.

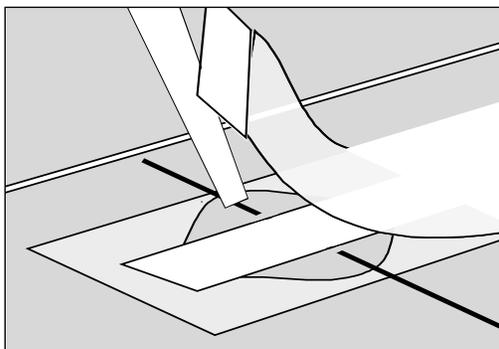
- (2) Open the incubator lid and place test strip in the ROSA-M Incubator with the flat side facing upward.

- (3) While holding the strip flat on the incubator, use tab to expose a 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.

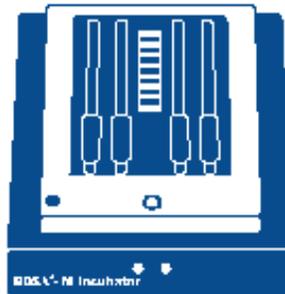


- (4) Holding the pipet vertically slowly pipet test sample for requested quantitation level or control into the side of sample compartment at the ROSA Incubator line (as shown).
- (a) 300 μ l of **first diluted extract**, for 0.5 to 1.4 ppm quantitation.
 - (b) 300 μ l of **second diluted extract**, for 1 to 5 ppm quantitation.
 - (c) 300 μ l of **supplemental diluted extract, negative or positive control**.

NOTE: Pipet very slowly.



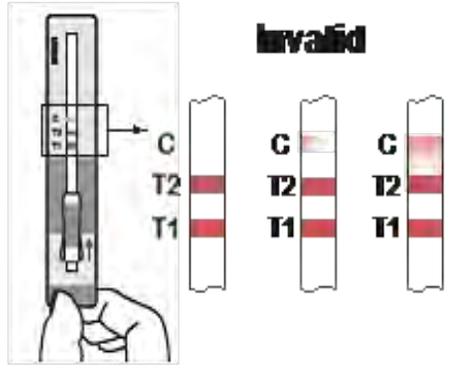
- (5) Reseal the tape over the sample pad compartment. When performing multiple test(s) using a ROSA Incubator.
 - Peel, pipet, and reseal before starting next strip.
 - Complete all test strips within 1 minute.
- (6) Close lid on the incubator and tighten the latch. Timer starts and a red light will automatically illuminate.



LF-INC4-5-45D: Quad incubator, 5 minute timer with display, set for 45°C.

- (7) Incubate for 5 minutes, but not more than 7 minutes. At 5 minutes a beeper and alternating yellow and red blinking lights will start to flash.
- (8) Remove strip(s) from the ROSA Incubator. Do not squeeze sample compartment. Hold test strip vertically with sample compartment in the down position until interpreted. After strip removal, lower lid but do not latch.
- (9) While holding test strip vertically with sample compartment in the down position determine test validity. Read within **2 minutes** of incubation completion.

If test is invalid re-test the diluted extract or control.

 <p style="text-align: center;">Invalid</p>	<p>The test is INVALID if any of the following are observed:</p> <ol style="list-style-type: none">1. C (Control) line is missing.2. T1, T2 (Test) or C line is smeared or uneven.3. T1, T2 or C line is obscured by diluted extract or Control.4. Beads do not flow past T1, T2 or C lines. <p>If test is INVALID re-test the diluted extract or Control.</p> <p>DO NOT PUT INVALID TEST STRIPS IN ROSA-M READER</p>
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k. Interpreting the Lateral Flow Test Strip using the ROSA-M Reader.

- (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-ROSAREADER-M-NB: ROSA-M Reader supplied with calibrators.

- (2) Read result on DON Channel (3-Line Mode) using the appropriate **MATRIX**. If desired, enter **Sample** and/or **Operator**. Press **ENTER** to read.

NOTE: Use the following table to determine the Matrix number to be used.

Matrix 00 Assay for analysis of first diluted extract for 0 to 1.4 ppm quantitation.

Matrix 01 Assay for analysis of second diluted extract for 1 to 5 ppm quantitation.

Matrix 02 Assay for analysis of the supplemental diluted extract for 1 to 5 ppm (Uncorrected DON) quantitation

- (3) **Reading 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).

A + sign on a READING value indicates that the concentration of the sample is greater than the Sensitivity range.

Second Diluted Extract: If test analysis using the second diluted extract reports a reading of less than 1000 ppb on **MATRIX 01** it indicates a value below the detection range. Re-test using the **first diluted extract** and run another test strip to report quantitative results for 0 to 1400 ppb, using the **MATRIX 00** setting. Proceed to Test Procedures (3) and follow the instruction using 300 µl of the **first diluted extract**.

Note: Applicants may request qualitative certification in lieu of retesting of results outside of the first diluted or second diluted extract test sample sensitivity ranges/concentrations.

1. 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 less than 0.6 ppm, 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 greater than 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the DON handbook for more detailed certification procedures.

m. Supplemental Analysis.

The following describes procedures for diluting and testing samples that have reported/tested greater than 5400 ppb (5.4 ppm) DON using the ROSA FAST5 DON quantitative test kit.

A Supplemental Diluted Extract is prepared by diluting the Second Diluted Extract with DONQ-FAST5 Dilution Buffer and test on another test strip to determine the uncorrected DON sample concentration.

- (1) Prepare Second Diluted Extract according to section i “Sample Preparation”.
- (2) Prepare Supplemental Diluted Extract from the second diluted extract to dilute the sample concentration.
 - (a) Pipet 1 ml of DONQ-FAST5 Dilution Buffer into a micro-centrifuge tube.
 - (b) Pipet the volume of the Second Diluted Extract needed into the micro-centrifuge tube with the DONQ-FAST5 Dilution Buffer cap, mix, and label. This is the Supplemental Diluted Extract.
 - (c) Determine/record the Dilution Factor used to prepare the dilution required to determine the corrected final sample concentration. The Dilution Factor (**DF**) is equal to the sum of the volume of the DONQ-FAST5 Buffer plus the volume of the Second Diluted Extract divided by the volume of the Second Diluted Extract. See chart below for examples:

Dilution Factor	DONQ-FAST5 Dilution Buffer	Second Diluted Extract Volume	Divided by the Second Diluted Extract Volume
4.33 =	1 ml	(plus) 300 µl (0.3 ml)	(divided by) 0.3 ml
11 =	1 ml	(plus) 100 µl (0.1 ml)	(divided by) 0.1 ml

- (d) Follow instruction in Sample Analysis section j for analysis of the Supplemental Diluted Extract using 300 µl of Supplemental Diluted Extract.
- (e) Read results on DON (3-line mode) with **MATRIX 02**.

Valid Supplemental Diluted Extract **READINGS** must be within 1000 to 5400 ppb (1 to 5.4 ppm) detection range.

- A reading less than 1000 ppb (1 ppm) is below the detection range. Prepare another Supplemental Diluted Extract (step (b) with a lower Dilution Factor, and run another test strip to quantitate.
- A reading greater than 5400 ppb (5.4 ppm) indicates that the concentration of the sample is greater than the test range. Prepare another Supplemental Diluted Extract (step b) with a higher Dilution Factor, and run another test strip to quantitate.
- (f) The number/result displayed on the ROSA-M Reader is the **Uncorrected DON Concentration** in the sample.
- (g) To covert the uncorrected DON results to the final **Corrected DON Concentration** in the sample. Follow instruction below:

Multiply the result displayed on ROSA-M Reader by the Dilution Factor used to prepare the Supplemental Diluted Extract.

For Example:

If the Uncorrected DON result is 2000 ppb (as displayed on the ROSA-M Reader) and the Dilution Factor is 11 the Corrected DON result concentration is 22,000 ppb ($2000 \text{ ppb} \times 11 = 22,000 \text{ ppb}$) or 22 ppm ($2.0 \text{ ppm} \times 11 = 22 \text{ ppm}$).

Note: It is recommended that locations document the volume of second diluted extract used to determine the DF for informational purposes and quality assurance needs.

n. Cleaning Labware.

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

o. 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.

p. Equipment and Supplies.

Materials supplied in test kits can be purchased that contain 20, 100, or 500 strips and include Control and DONQ-FAST5 Dilution Buffer.

- (1) LF-DONQ-FAST5 20K:

- (a) 1 package containing 20 DONQ-FAST5 test strips packed in a moisture-resistant container.
 - (b) 1 - 1000 ppb DON Positive Control.
 - (c) 1 DONQ-FAST5 Dilution Buffer.
- (2) LF-DONQ-FAST5 100K:
- (a) 1 package containing 100 DONQ-FAST5 test strips packed in a moisture-resistant container.
 - (b) 1 - 1000 ppb DON ppb Positive Control.
 - (c) 2 DONQ-FAST5 Dilution Buffer.
- (3) LF-DONQ-FAST5 500K:
- (a) 5 packages containing 100 DONQ-FAST5 test strips packed in a moisture-resistant container.
 - (b) 5 - 1000 ppb DON Positive Controls.
 - (c) 10 DONQ-FAST5 Dilution Buffers.
- (4) Materials required but not provided:
- (a) Sample grinder.
 - (b) Balance.
 - (c) Deionized or Distilled water.
 - (d) Sample extraction containers.
 - (e) 1.0 ml pipettor and pipet tips.
 - (f) 300 μ l pipettor and pipet tips.
 - (g) 100 μ l pipettor and pipet tips.
 - (h) 250 ml graduated cylinder.
 - (i) 1.5 ml micro-centrifuge tubes.

- (j) Filter funnel.
- (k) Transfer pipets.
- (l) Whatman 2V filter paper or equivalent.
- (5) Material required for testing of Distillers Dried Grains with Solubles:
 - (a) LA-PH-STRIPS: pH Test Strips.
 - (b) KOH30PERCT-250ML: 30 percent Potassium Hydroxide.
 - (c) TST-50ML-25: Plastic Conical tubes (50 ml).
- (6) Material required for testing of Wheat Midds:
 - (a) FIL-0-2UM-GFCA: 0.2 μ m GF/CA filter to fit on a 1 ml syringe.
 - (b) SYRINGE-1ML-NS: 1 ml plastic non-sterile syringe.
- (7) Equipment:
 - (a) MINICEN-110/220V: Mini-centrifuge
 - (b) LF-ROSAREADER-M-NB: ROSA-M Reader supplied with calibration strips.
 - (c) LF-CALIB-RRM: ROSA-M Reader Calibration Strips.
 - (d) LF-INC4-5-45D: Quad ROSA Incubator (45°C and 5 minute timer with display.
 - (e) LF-INC2-5-45: Dual ROSA Incubator (45°C and 5 minute timer without display.

q. Storage Conditions and Precautions

- (1) Storage Conditions.

Store DONQ-FAST test strips, DONQ-FAST5 Dilution Buffer, and 1000 ppb DON Positive Control refrigerated between 32°- 45°F.
- (2) Precautions:
 - (a) Do not use the test kits beyond the noted expiration date.

- (b) Prolonged exposure to high temperatures may adversely affect the test results.
- (c) Do not open the desiccated canister until ready to use the strips.
- (d) Debris on test strips may alter the ROSA-M Reader optics. Keep equipment clean and wipe dust and liquid off test strips before inserting in ROSA-M Reader.
- (e) ROSA Incubator must be clean, level, and temperature must be $45\pm 1^{\circ}\text{C}$. The temperature indicator should match the ROSA Incubator temperature. A daily thermometer check is recommended.
- (f) Keep the ROSA Incubator lid lowered, but not latched, unless performing a test procedure.
- (g) ROSA Incubators may take more than 10 minutes to reach proper temperature, depending on ambient temperature.

Contact Charm Sciences or TSD concerning any testing problems or questions.

5. FILING

Retain a copy of this program notice with the DON Handbook until the handbook is revised to include the test method stated herein.

6. QUESTIONS

Please direct any questions concerning this program notice to Patrick McCluskey, GIPSA's Policies, Procedures, and Market Analysis Branch (PPMAB), at 816-659-8403, or email patrick.j.mccluskey@usda.gov, or Carl Jackson, PPMAB, at 202-720-8286 or email carl.jackson@usda.gov.

/s/ Robert Lijewski

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