

CHARM SCIENCES, INC. ROSA WET-S5 FUMONISIN QUANTITATIVE TEST

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GENERAL INFORMATION

ROSA WET-S5 Fumonisin Quantitative Test is an immunoreceptor assay utilizing ROSA (Rapid One Step Assay) lateral flow technology and Water Extraction Technology (WET) that eliminates the use of organic solvents (methanol, ethanol, etc.). WET uses a non-hazardous extraction powder added to the sample followed by water to extract fumonisins into the aqueous solvent. Fumonisin 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) fumonisins.

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.

Refer to the Mycotoxin Handbook 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 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.

Approved Test Kit Information

Test Kit Vendor:	<i>Charm Sciences, Inc. 978-687-9200</i>
Test Kit Name:	ROSA WET-S5 Fumonisin Quantitative Test
Product Number:	LF-FUMQ-WETS5
Test Format:	Lateral flow strip
Reader:	Charm EZ-M reader, Model LF-ROSA-EZ-M
Detection Method:	Reflectance
Effective Date of Instructions:	08/19/2016
Instructions Revision Number	0
Conformance Range:	0.50 ppm to 30 ppm
Number of Analyses to Cover Conformance Range:	2
Type of Service:	Quantitative
Approved Commodities:	Corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), corn germ meal, distillers dried grains with solubles (DDGS), oats, popcorn, sorghum, wheat
Extraction method:	Shake vigorously by hand for 1.5 minutes.

PREPARATION OF TESTING MATERIALS AND EQUIPMENT

1. 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.
2. FUMQ-WETS5 Dilution Buffer:
 - a. Dispense buffer into a clean micro-centrifuge tube and label for each sample to be tested.
 - b. Use pre-dispensed buffer tubes and buffer solution at room temperature.
3. EP Control:
 - a. Prepare EP Control by dissolving one packet of WET-S Extraction Powder in 150 milliliters (mL) deionized or distilled water by gently swirling until extraction powder is dissolved.
 - b. Use to prepare Positive Control
4. Negative Control:
 - a. Use FUMQ-WETS5 Dilution Buffer as Negative Control in TEST PROCEDURES section.
 - b. Use Negative Control in TEST PROCEDURES section.
5. Positive Control:
 - a. Reconstitute the dry Positive Control by adding 300 microliters (µL) EP Control followed by 2.7 mL FUMQ-WETS5 Dilution Buffer. Cap, shake well and allow to stand at room temperature for 10 minutes. Mix before use.
 - b. Use Positive Control to prepare Positive Control Extract.
6. Positive Control Extract:
 - a. Prepare Positive Control Extract by adding 300 µL reconstituted Positive Control to 900 µL FUMQ-WETS5 Dilution Buffer in a clean micro-centrifuge tube, cap, mix (shake vigorously for 5 seconds) and label.
 - b. Use Positive Control Extract (within 4 hours of preparation) in TEST PROCEDURES section.
7. Reader and Test Strip Performance Testing:
 - a. Enter performance mode in Charm EZ-M reader by selecting Perf. Mon. from the Main Menu, followed by Perf. Test.
 - (1) Follow the system prompts to test calibration strips (LO CAL and HI CAL).
 - (2) Follow the system prompts to test controls (NEG CTRL and POS CTRL); select FUMQ-WETS5 from the TESTS list if prompted.
 - b. Test calibration strips daily to verify Charm EZ-M reader performance. Calibration strips must test/perform in the specified ranges.
 - c. Test Negative Control and Positive Control Extract weekly to verify test strip performance. Valid control ranges are:
 - (1) Negative Control: less than 100 ppb (0.1 ppm)

- (2) Positive Control Extract: 1400 to 2600 ppb (1.4 to 2.6 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.

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

EXTRACTION PROCEDURES

- 3:1 Extraction Procedure for corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), oats, popcorn, sorghum, and wheat:**
 - Weigh 50.0 ± 0.2 grams ground samples into a clean extraction container.
 - Add contents of one (1) packet of WET-S Extraction Powder.
 - Add 150 mL deionized or distilled water.
 - Shake vigorously for 1.5 minutes by hand.
 - 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 within 2 hours).
 - Repeat for additional samples.
- 5:1 Extraction Procedure for corn germ meal and DDGS:**
 - Weigh 50.0 ± 0.2 grams ground samples into a clean extraction container.
 - Add contents of two (2) packets of WET-S Extraction Powder.
 - Add 250 mL deionized or distilled water.
 - Shake vigorously for 1.5 minutes by hand.
 - 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 within 2 hours**).
 - Repeat for additional samples.

SAMPLE PREPARATION FOR QUANTITATION

- Preparation for corn, oats, popcorn, sorghum, and wheat:
 - Prepare Diluted Extract.
 - Pipet 900 μL FUMQ-WETS5 Dilution Buffer into a clean micro-centrifuge tube.
 - Pipet 100 μL centrifuged extract to micro-centrifuge tube containing 900 μL FUMQ-WETS5 Dilution Buffer, cap, mix (shake vigorously for 5 seconds), and label. This tube contains the Diluted Extract; Diluted Extract is not assayed.
 - Prepare Second Diluted Extract for 0.5 to 5 ppm quantitation.

- (1) Pipet 900 μL FUMQ-WETS5 Dilution Buffer into a clean micro-centrifuge tube.
 - (2) Pipet 100 μL Diluted Extract to micro-centrifuge tube containing 900 μL FUMQ-WETS5 Dilution Buffer, cap, mix (shake vigorously for 5 seconds), and label. This tube contains the Second Diluted Extract.
 - c. Prepare Third Diluted Extract for 5 to 30 ppm quantitation.
 - (1) Pipet 900 μL FUMQ-WETS5 Dilution Buffer into a clean micro-centrifuge tube.
 - (2) Pipet 100 μL Second Diluted Extract to micro-centrifuge tube containing 900 μL FUMQ-WETS5 Dilution Buffer, cap, mix (shake vigorously for 5 seconds), and label. This tube contains the Third Diluted Extract.
 - d. Repeat for additional samples.
 - e. Use Second Diluted Extract or Third Diluted Extract (**within 6 hours after preparation**) as your test sample in Sample Analysis found in TEST PROCEDURES section.
2. Preparation for corn germ meal and DDGS:
 - a. Prepare Diluted Extract.
 - (1) Pipet 1000 μL FUMQ-WETS5 Dilution Buffer into a clean micro-centrifuge tube.
 - (2) Pipet 200 μL centrifuged extract to micro-centrifuge tube containing 1000 μL FUMQ-WETS5 Dilution Buffer, cap, mix (shake vigorously for 5 seconds), and label. This tube contains the Diluted Extract; Diluted Extract is not assayed.
 - b. Prepare Second Diluted Extract for 0.5 to 5 ppm quantitation.
 - (1) Pipet 900 μL FUMQ-WETS5 Dilution Buffer into a clean micro-centrifuge tube.
 - (2) Pipet 100 μL Diluted Extract to micro-centrifuge tube containing 900 μL FUMQ-WETS5 Dilution Buffer, cap, mix (shake vigorously for 5 seconds), and label. This tube contains the Second Diluted Extract.
 - c. Prepare Third Diluted Extract for 5 to 30 ppm quantitation.
 - (1) Pipet 900 μL FUMQ-WETS5 Dilution Buffer into a clean micro-centrifuge tube.
 - (2) Pipet 100 μL Second Diluted Extract to micro-centrifuge tube containing 900 μL FUMQ-WETS5 Dilution Buffer, cap, mix (shake vigorously for 5 seconds), and label. This tube contains the Third Diluted Extract.
 - d. Repeat for additional samples.
 - e. Use Second Diluted Extract or Third Diluted Extract (**within 6 hours after preparation**) as your test sample in Sample Analysis found in TEST PROCEDURES section.

TEST PROCEDURES

1. Sample Analysis:
 - a. Check that the ROSA Incubator temperature is 45 ± 1 $^{\circ}\text{C}$.
 - b. Label test strip(s) to identify sample.
 - c. Place test strip in the ROSA Incubator with the flat side facing upward.

- d. 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.
- e. Holding pipet vertically, slowly pipet 300 μ L test sample (Second or Third Diluted Extract or control) into the sample compartment at the ROSA Incubator line.
- f. Reseal the tape over the sample pad compartment.

When performing multiple tests (two maximum) using a ROSA Incubator:

- (1) Peel, pipet, and reseal before starting next strip.
 - (2) Complete procedure for all test strips within 30 seconds.
- g. Close lid on the ROSA Incubator.
 - h. Incubate for 5 minutes.
 - i. Remove strip from the ROSA Incubator.

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

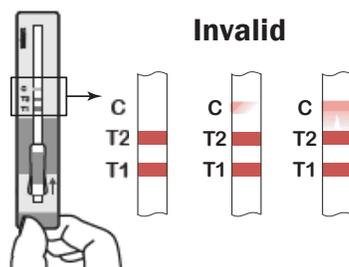
- (1) Wipe foreign matter (dust, etc.) from the test strip.
- (2) Inspect and interpret test strip.

When running multiple test strips in the ROSA Incubator, remove one strip for visual inspection and interpretation at a time and complete procedure for all test strips within 1 minute of incubation completion.

- (3) Lower ROSA Incubator lid; do not re-latch.

2. Visual Inspection:

- a. The test strip is INVALID if any of the following are observed:
 - (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.



- b. Do not put INVALID test strips in the Charm EZ-M reader.
 - c. If test strip is INVALID, re-test the diluted extract or control.
3. Interpretation:

- a. 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.
- b. Read results on FUMQ-WETS5 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.
 - 2ND DE: Assay of Second Diluted Extract for 0.5 to 5 ppm quantitation.
 - 3RD DE: Assay of Third Diluted Extract for 5 to 30 ppm quantitation.

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

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

A Second Diluted Extract **READING** greater than 5 ppm (5.4 ppm if reporting results to the tenths) indicates that the sample concentration is greater than the sensitivity range of the sample dilution; prepare Third Diluted Extract and perform assay with another test strip.

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

A Third Diluted Extract **READING** greater than 30 ppm indicates that the sample concentration is greater than the sensitivity range of the sample dilution; report test result as greater than 30 ppm on the work record and certify as “Fumonisins exceed 30 ppm”.

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).

STORAGE CONDITIONS AND PRECAUTIONS

1. Storage Conditions:
 - a. Store test strips refrigerated (0 °C to 7 °C) in tightly-closed supplied container.
 - b. Store dilution buffer bottle and pre-dispensed micro-centrifuge tubes refrigerated.
 - c. Store WET-S Extraction Powder at room temperature in supplied packet.
 - d. Store EP Control refrigerated for up to 1 week or aliquot (at least 1.5 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 EP Control refrigerated and use within 24 hours of thawing; **DO NOT REFREEZE.**
 - e. Store dry Fumonisins Positive Control refrigerated.
 - f. 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 within 6 hours of reconstitution for up to two 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.

2. Precautions:

- a. Test Strips
 - (1) 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.
 - (2) In high humidity, limit condensation by opening container after it has warmed to room temperature.
 - (3) Inspect/verify desiccant indicator. Beads inside desiccant packets should be blue. Discard test strips if the blue beads have turned purple or pink.
 - (4) Re-shape dented sample compartments to fit into ROSA Incubator.
- b. Use FUMQ-WETS5 Dilution Buffer supplied with each test kit only at room temperature. Keep buffer at room temperature during daily use for up to 12 hours.
- c. Do not use the test kits beyond the noted expiration date.
- d. Debris on test strips may alter the reader optics. Keep equipment clean. Wipe dust and liquid off test strips before inserting into reader.
- e. ROSA Incubator must be clean and level. ROSA Incubator temperature must be $45\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$. 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.
- f. Charm EZ-M reader must be clean and level. Keep reader lid closed unless performing procedure.

EQUIPMENT AND SUPPLIES

1. Test Strips

- (1) LF-FUMQ-WETS5-20K
- (2) 1 container of 20 FUMQ-WETS5 test strips
- (3) Fumonisin Positive Control(s):
 - (a) One control in LF-FUMQ-WETS5-20K
 - (b) Two controls in LF-FUMQ-WETS5-20ESK
- (4) 1 FUMQ-WETS5 Dilution Buffer
- b. LF-FUMQ-WETS5-100K
 - (1) 1 container of 100 FUMQ-WETS5 test strips
 - (2) Fumonisin Positive Control(s):
 - (a) One control in LF-FUMQ-WETS5-100K
 - (b) Five controls in LF-FUMQ-WETS5-100ESK

- (3) 4 FUMQ-WETS5 Dilution Buffers
- c. LF-FUMQ-WETS5-500K
 - (1) 5 containers of 100 FUMQ-WETS5 test strips
 - (2) Fumonisin Positive Controls:
 - (a) Five controls in LF-FUMQ-WETS5-500K
 - (b) Twenty-five controls in LF-FUMQ-WETS5-500ESK
 - (3) 20 FUMQ-WETS5 Dilution Buffers
- 2. WET-S Extraction Powder
 - a. LF-WET-EXTS-50G-20: WET-S Extraction Powder for 50 gram sample (20/pack)
 - b. LF-WET-EXTS-50G-100: WET-S Extraction Powder for 50 gram sample (100/pack)
- 3. Materials required but not provided
 - a. 100 μ L pipet and pipet tips
 - b. 200 μ L pipet and pipet tips
 - c. 300 μ L pipet and pipet tips
 - d. 100 to 1000 μ L variable volume pipet and pipet tips
 - e. 250 mL graduated cylinder
 - f. Balance
 - g. Charm EZ-M reader
 - h. Deionized or distilled water
 - i. Micro-centrifuge tubes
 - j. Mini-centrifuge
 - k. Printer for Charm EZ-M reader (optional)
 - l. ROSA Incubator
 - m. Sample extraction containers or Whirl-pak bags
 - n. Sample grinder
 - o. Transfer pipets

REVISION HISTORY

Revision 0 (08/19/2016)

FLOW CHARTS

Flow chart for 3:1 Extraction Procedure and Assay for corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), oats, popcorn, sorghum, and wheat:

Refer to GIPSA Test Kit Instructions for Complete Test Procedure

Approved Commodities:
Corn, Oats, Popcorn, Sorghum, Wheat

ROSA® WET™-S5 Fumonisin Quantitative Test Flow Chart (3:1)

See Approved Commodities Below

Quantitation Ranges: 0.5 to 5 ppm
5 to 30 ppm

See Approved 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 WET-S Extraction Powder
1 Packet for 50 g sample

(3) Add Solvent
Deionized or Distilled Water
150 mL

(4) Extract
Shake vigorously
for 1.5 minutes

(5) Clarify
Centrifuge extract
for 10 seconds

0.5 to 5 ppm

100 µL Centrifuge Extract + 900 µL FUMQ-WETS5 Dilution Buffer → Diluted Extract

followed by

100 µL Diluted Extract + 900 µL FUMQ-WETS5 Dilution Buffer → 2nd Diluted Extract

(6) Dilute
Prepare Diluted Extract followed by 2nd Diluted Extract

Test Procedure

(1) Place test strip in ROSA Incubator

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

(3) Close lid. Incubate for 5 minutes.

Read Result

(1) Inspect test strip

Valid Test

Invalid Test

(2) Read result with Charm EZ-M reader

Charm EZ-M reader: Select appropriate test (FUMQ-WETS5), commodity and dilution if prompted.

Sample	Charm EZ-M Reader DILUTION 2ND DE	Quantitation Range
2 nd Diluted Extract	DILUTION 2ND DE	0.5 to 5 ppm
3 rd Diluted Extract	DILUTION 3RD DE	5 to 30 ppm

For quantitation of 5 to 30 ppm:

100 µL 2nd Diluted Extract + 900 µL FUMQ-WETS5 Dilution Buffer → Mix → 3rd Diluted Extract

(1) Prepare 3rd Diluted Extract

(2) Repeat Test Procedure (steps 1, 2, 3) with 3rd Diluted Extract

(3) Read Result

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a. Flow chart for 5:1 Extraction Procedure and Assay for corn germ meal and DDGS:

Refer to GIPSA Test Kit Instructions for Complete Test Procedure

ROSA® WET™-S5 Fumonisin Quantitative Test Flow Chart (5:1)

Approved Commodities:
Corn Germ Meal, DDGS

See Approved 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 WET-S Extraction Powder
2 Packets for 50 g sample

(3) Add solvent
Deionized or Distilled Water
250 mL

(4) Extract
Shake vigorously
for 1.5 minutes

(5) Clarify
Centrifuge extract
for 10 seconds

(6) Dilute
Prepare Diluted Extract followed by 2nd Diluted Extract

Test Procedure

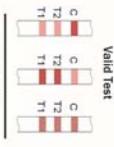
(1) Inspect test strip

(2) Read result with Charm EZ-M reader

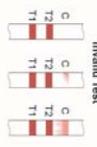
(3) For quantitation of 5 to 30 ppm:

Read Result

Valid Test



Invalid Test



Sample	Charm EZ-M Reader 2 nd Diluted Extract DILUTION 2ND DE	Quantitation Range
3 rd Diluted Extract	DILUTION 3RD DE	5 to 30 ppm

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