

Test Kit Instruction

February 26, 2021

Charm Sciences, Inc.
ROSA FAST Aflatoxin 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 816-702-3817 or email at 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 816-702-3923 or email at Patrick.J.McCluskey@usda.gov.

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Contents

FORWARD.....	1
1. GENERAL INFORMATION	3
2. PREPARATION OF TESTING MATERIALS AND EQUIPMENT	4
3. EXTRACTION PROCEDURE	5
4. SAMPLE PREPARATION FOR QUANTITATION	5
5. TEST PROCEDURES	6
6. REPORTING AND CERTIFYING TEST RESULTS	8
7. STORAGE CONDITIONS AND PRECAUTIONS	8
8. EQUIPMENT AND SUPPLIES	9
9. REVISION HISTORY	10
10. FLOW CHARTS	11

1. GENERAL INFORMATION

ROSA FAST Aflatoxin Quantitative Test is an immunoreceptor assay utilizing ROSA (Rapid One Step Assay) lateral flow technology. Aflatoxins are extracted from the sample using 70% methanol/30% water. Aflatoxins interact 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 billion (ppb) aflatoxins.

Approved Test Kit Information	
Test Kit Vendor:	Charm Sciences, Inc. 978-687-9200
Test Kit Name:	ROSA FAST Aflatoxin Quantitative Test
Product Number:	LF-AFQ-FAST
Effective Date of Instructions:	02/26/2021
Conformance Range:	5.0 – 300 ppb
Number of Analyses to Cover Conformance Range:	3
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), barley (with hull, including malting barley), brown rice, corn germ meal, corn gluten meal, corn/soy blend, distillers dried grain (DDG), distillers dried grain with solubles (DDGS), hominy (including hominy grits), millet, oats (whole oats with hull), oat groats, popcorn, rough rice, rye (or rye berries, including whole grain rye flour, rye meal, cracked rye, and rye chops), sorghum, soybean hulls, soybean meal, soybean (including whole soybean and full-fat soy flour), wheat (including whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings)
Extraction Method:	Vigorously shake 50-gram sample with 100 milliliters (mL) 70% methanol/30% distilled or deionized water (v/v) by hand for 1 minute. For corn germ meal, DDG, DDGS, oats, soybean hulls, and soybean, vigorously shake 50-gram sample with 150 mL 70% methanol/30% distilled or deionized water (v/v) by hand for 1 minute.
Test Format:	Lateral flow strip
Detection Method:	Reflectance
Reader:	Charm EZ-M, Model LF-ROSA-EZ-M

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 AFQ 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 Extraction Solvent [70% Methanol/30% Water (v/v)]

The extraction solvent used in the method is a methanol/water mixture consisting of 70% methanol (reagent grade or better) and 30% distilled or deionized water (v/v); prepare if not purchased premade.

- (1) Measure 700 mL methanol (using 1000 mL graduated cylinder) and place it into a clean container.
- (2) Measure 300 mL distilled or deionized water (using 1000 mL graduated cylinder) and add to the methanol and shake until mixed.
- (3) Label the container stating the mixture 70% methanol/30% water (v/v), date of preparation, and initials of technician who prepared the solution.
- (4) Store extraction solvent at room temperature in the tightly closed container until needed. Mix again before use.

NOTE: To prepare smaller or larger amounts of extraction solvent use the ratio of 7 parts methanol to 3 parts distilled or deionized water.

d Negative Control.

- (1) Prepare Negative Control by adding 100 microliters (µL) extraction solvent (using 100 µL pipette) to 1000 µL AFQ Dilution Buffer (using 100-1000 µL pipette) in a micro-centrifuge tube. Cap, mix (shake vigorously for 5 seconds), and label as Negative Control.
- (2) Use Negative Control in TEST PROCEDURES section.

e Positive Control.

- (1) Reconstitute the dry Positive Control (provided with test kit) by adding 300 µL extraction solvent (using 300 µL pipette) followed by 3.0 mL AFQ Dilution Buffer (using 100-1000 µL pipette). 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.

f 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 **AFQ-FAST (3MIN)** 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 2 ppb
 - (b) Positive Control: 12 ppb to 28 ppb

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.

g. ROSA Incubator.

- (1) ROSA Incubator must be clean and level.
- (2) 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).

3. EXTRACTION PROCEDURE

- a. Weigh 50 ± 0.2 grams ground sample into a clean extraction container or Whirl-pak bag.
- b. **For corn** (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), **barley** (with hull, including malting barley), **brown rice, corn gluten meal, corn/soy blend, hominy** (including hominy grits), **millet, oat groats, popcorn, rough rice, rye** (or rye berries, including whole grain rye flour, rye meal, cracked rye, and rye chops), **sorghum, soybean meal, and wheat** (including whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings): add 100 mL extraction solvent (using 100 mL graduated cylinder).
For corn germ meal, DDG, DDGS, oats (whole oats with hull), **soybean hulls, and soybean**: add 150 mL extraction solvent (using 250 mL graduated cylinder).
- c. Shake vigorously by hand for 1 minute (use within 30 minutes).
- d. Transfer 1 mL to 1.5 mL extract (using transfer pipette) 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).
- e. Repeat steps for additional samples.

4. SAMPLE PREPARATION FOR QUANTITATION

NOTE: Laboratories may initially test the Second Diluted Extract if levels typically reported in their market area are within the 20 ppb to 100 ppb quantitation range.

a. **Prepare Diluted Extract for 5 ppb to 20 ppb quantitation.**

- (1) Pipet 1000 μL AFQ Dilution Buffer (using 100-1000 μL pipette) into a clean micro-centrifuge tube.
- (2) Pipet 100 μL centrifuged extract (using 100 μL pipette) to the micro-centrifuge tube containing 1000 μL AFQ Dilution Buffer, cap, mix (shake vigorously for 5 seconds), and label as **Diluted Extract**.

For barley (with hull, including malting barley), **corn gluten meal, corn/soy blend, oats** (whole oats with hull), **oat groats, popcorn, rye** (or rye berries, including whole grain rye flour, rye meal, cracked rye, and rye chops), **and wheat** (including whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings), filter Diluted Extract.

- (a) Draw Diluted Extract into 1 mL syringe and connect Minisart RC15 syringe filter.
 - (b) Pass Diluted Extract through Minisart RC15 syringe filter, collect **Filtered Diluted Extract** in a clean micro-centrifuge tube, and label.
- (3) Repeat for additional samples.

- (4) Use **Diluted Extract** and **Filtered 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 20 ppb to 100 ppb quantitation.

- (1) Pipet 1000 µL AFQ Dilution Buffer (using 100-1000 µL pipette) into a clean micro-centrifuge tube.
- (2) Pipet 300 µL **Diluted Extract** or **Filtered Diluted Extract** (using the 300 µL pipette) to micro-centrifuge tube containing 1000 µL AFQ Dilution Buffer, cap, mix (shake vigorously for 5 seconds), and label as **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.

c. Prepare Third Diluted Extract for 100 ppb to 300 ppb quantitation.

- (1) Pipet 1000 µL AFQ Dilution Buffer (using 100-1000 µL pipette) into a clean micro-centrifuge tube.
- (2) Pipet 300 µL **Second Diluted Extract** (using 300 µL pipette) to micro-centrifuge tube containing 1000 µL AFQ Dilution Buffer, cap, mix (shake vigorously for 5 seconds), and label as **Third Diluted Extract**.
- (3) Repeat for additional samples.
- (4) Use **Third 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 °C ± 1 °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) Hold the pipette vertically and slowly add 300 µL test sample (using 300 µL pipette for Diluted Extract and Filtered Diluted Extract, Second Diluted Extract, Third Diluted Extract, or control) into the sample compartment at the ROSA Incubator line.
- (6) Reseal the tape over the sample pad compartment.

NOTE: 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 test strip(s):
 - (a) Corn and controls: 3 minutes
 - (b) All other commodities: 5 minutes

- (9) Remove strip from the ROSA Incubator.

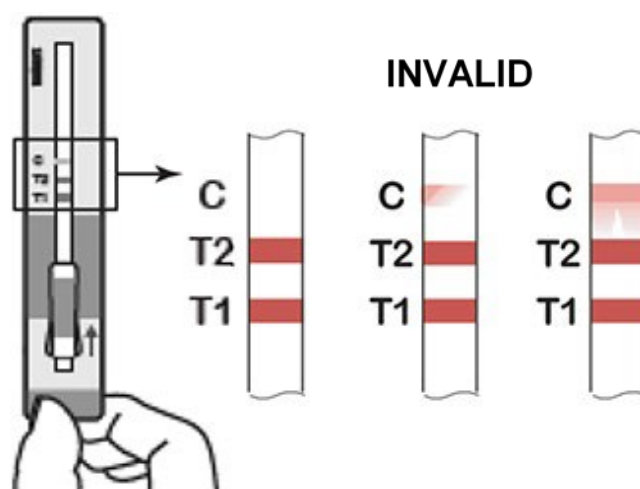
Do not squeeze sample compartment. Hold test strip vertically with sample compartment in the down position until interpreted.

NOTE: When running multiple test strips in ROSA Incubator, remove one strip at a time.

- (a) Wipe foreign matter (dust, etc.) from the test strip.
- (b) Inspect and read the test strip 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 **AFQ-FAST (3MIN)** for corn only and **AFQ-FAST (5MIN)** for all other commodities from the TESTS list with COMMODITY (select SPECIAL 1 for oat groats) and DILUTION selected for sample. If desired, enter OPERATOR ID, SAMPLE ID, and/or LOT NUMBER. Close door to read.
- DE: Diluted Extract and Filtered Diluted Extract for 5 ppb to 20 ppb quantitation
 - 2ND DE: Second Diluted Extract for 20 ppb to 100 ppb quantitation
 - 3RD DE: Third Diluted Extract for 100 ppb to 300 ppb quantitation

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 aflatoxin (ppb) in the sample.

A Diluted Extract **READING** of “+30 ppb” 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 20 ppb indicates a value below the sensitivity range of the sample dilution; perform assay with Diluted Extract using another test strip.

A Second Diluted Extract **READING** greater than 120 ppb 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 75 ppb indicates a value below the sensitivity range of the sample dilution; perform assay with Second Diluted Extract using another test strip.

A Third Diluted Extract **READING** greater than 300 ppb indicates that the sample concentration is greater than the sensitivity range of the sample dilution; report test results as greater than 300 ppb on the work record and certify as “Aflatoxins exceed 300 ppb”.

6. REPORTING AND CERTIFYING TEST RESULTS

Refer to the Mycotoxin Handbook for reporting and certification of test results. For questions regarding reporting and certifying test results, contact Patrick McCluskey (816-702-3923 or 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 Aflatoxin B1 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 AFQ Dilution Buffer supplied with each test kit only at room temperature. Keep buffer at room temperature during daily use for up to 12 hours; place back in refrigerator after daily use.
- (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-AFQ-FAST-20K/-20ESK
 - (a) One container of 20 AFQ-FAST test strips
 - (b) Aflatoxin B1 Positive Control(s)
 - 1 One control in LF-AFQ-FAST-20K
 - 2 Two controls in LF-AFQ-FAST-20ESK
 - (c) One AFQ Dilution Buffer
- (2) LF-AFQ-FAST-100K/-100ESK
 - (a) One container of 100 AFQ-FAST test strips
 - (b) Aflatoxin B1 Positive Control(s)
 - 1 One control in LF-AFQ-FAST-100K
 - 2 Five controls in LF-AFQ-FAST -100ESK
 - (c) One AFQ Dilution Buffer
- (3) LF-AFQ-FAST-500K/-500ESK
 - (a) Five containers of 100 AFQ-FAST test strips
 - (b) Aflatoxin B1 Positive Controls
 - 1 Five controls in LF-AFQ-FAST-500K
 - 2 Twenty-five controls in LF-AFQ-FAST-500ESK
 - (c) Five AFQ Dilution Buffers

b. Materials required, but not provided.

- (1) 100 μL pipette and pipette tips (Charm order code: PIP-100UL-1STOP and 100-ULT-X1 (rack of 96 tips))
- (2) 300 μL pipette and pipette tips (Charm order code: PIP-300UL-1STOP-M and 1-MLT-96 (rack of 96 tips))
- (3) 100-1000 μL variable volume pipette and pipette tips (Charm order code: PIP-100-1000UL-1STOP and 1-MLT-96 (rack of 96 tips))
- (4) 100 mL, 250 mL, and 1000 mL graduated cylinders (Charm order code: GRAD-CYL-100/-250/-1000)
- (5) Charm EZ-M reader (Charm order code: LF-ROSA-EZ-M)
- (6) Container for storing 70% methanol/30% water
- (7) Deionized or distilled water
- (8) Extraction containers or Whirl-Pak bags (Charm order code: WHIRLPK-50 (50/PK))

- (9) FGIS-approved scale (balance) with minimum division of 0.1 g (see FGIS Mycotoxin Handbook for details)
- (10) Methanol (reagent grade or better; Sigma order code: 179337-4L) or 70% methanol (Charm order code: MEOH-4L-4PK: (4/PK))
- (11) Micro-centrifuge tubes (Charm order code: CEN-2-0ML-TUBES-100 (100/PK))
- (12) Mini-centrifuge (Charm order code: MINICEN-110V)
- (13) Printer for Charm EZ-M reader (Charm order code: PRN-THERM-CITIZEN)
- (14) ROSA Incubator (Charm order code: LF-INC4-3-45D or LF-INC2-5-45D)
- (15) Sample grinder (see FGIS Mycotoxin Handbook for details)
- (16) Transfer pipette (Charm order code: PIP-3ML-100K (100/PK))
- c. Materials required, but not provided, for commodities requiring filtration of Diluted Extract.
 - (1) Minisart RC15 syringe filters (Charm order code: FIL-0_45UM-RC15 (20/PK))
 - (2) Syringes (Charm order code: SYRINGE-1ML-PK-NS (20/PK))

9. REVISION HISTORY

Effective: 02/26/2021


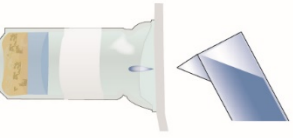
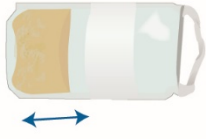
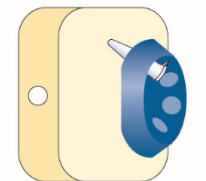
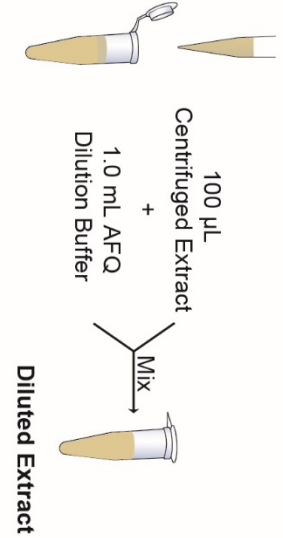
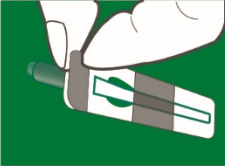
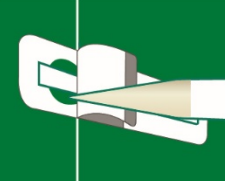

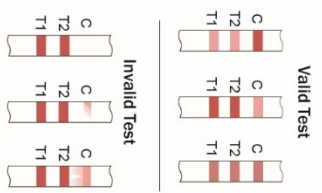
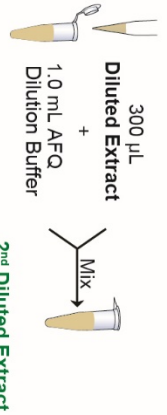
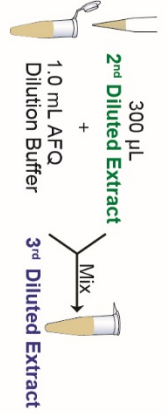
Refer to FGIS Test Kit Instructions for Complete Test Procedure

ROSA® FAST Aflatoxin Quantitative Test Flow Chart - 3 minute incubation

Approved Commodity:
Corn

See Approved Commodity
Below

Quantitation Ranges: 5 to 20 ppb
20 to 100 ppb
100 to 300 ppb

Sample Preparation														
<p>(1) Weigh</p> <p>Ground sample 50.0 ± 0.2 g</p> 	<p>(2) Add Solvent</p> <p>70% Methanol 100 mL</p> 	<p>(3) Extract</p> <p>Shake vigorously for 1 minute</p> 												
<p>(4) Clarify</p> <p>Centrifuge extract for 10 seconds</p> 	<p>(5) Dilute</p> <p>Prepare Diluted Extract.</p> 													
Test Procedure														
<p>(1)</p> <p>Place test strip in ROSA Incubator.</p> 	<p>(2)</p> <p>Peel tape. Pipet 300 µL Diluted Extract into sample compartment. Reseal tape.</p> 	<p>(3)</p> <p>Close lid. Incubate for 3 minutes.</p> 												
Read Result														
<p>(1) Inspect test strip</p> <p>(2) Read result with Charm EZ-M reader</p> <p><i>Charm EZ-M reader:</i> Select appropriate test (AFQ-FAST (3MIN)), commodity and dilution if prompted.</p> <table border="1"> <thead> <tr> <th>Sample</th><th>Dilution</th><th>Quantitation Range</th></tr> </thead> <tbody> <tr> <td>Diluted Extract</td><td>DE</td><td>5 to 20 ppb</td></tr> <tr> <td>2nd Diluted Extract</td><td>2ND DE</td><td>20 to 100 ppb</td></tr> <tr> <td>3rd Diluted Extract</td><td>3RD DE</td><td>100 to 300 ppb</td></tr> </tbody> </table> 	Sample	Dilution	Quantitation Range	Diluted Extract	DE	5 to 20 ppb	2 nd Diluted Extract	2ND DE	20 to 100 ppb	3 rd Diluted Extract	3RD DE	100 to 300 ppb	<p>For quantitation of 20 to 100 ppb:</p> <p>(1) Prepare 2nd Diluted Extract</p> <p>(2) Repeat Test Procedure (steps 1, 2, 3) with 2nd Diluted Extract</p> <p>(3) Read Result</p>  <p>For quantitation of 100 to 300 ppb:</p> <p>(1) Prepare 3rd Diluted Extract</p> <p>(2) Repeat Test Procedure (steps 1, 2, 3) with 3rd Diluted Extract</p> <p>(3) Read Result</p> 	
Sample	Dilution	Quantitation Range												
Diluted Extract	DE	5 to 20 ppb												
2 nd Diluted Extract	2ND DE	20 to 100 ppb												
3 rd Diluted Extract	3RD DE	100 to 300 ppb												



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Refer to FGIS Test Kit Instructions for Complete Test Procedure


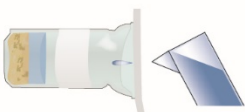

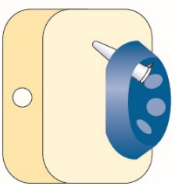
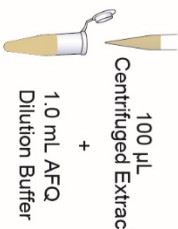


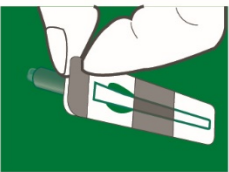
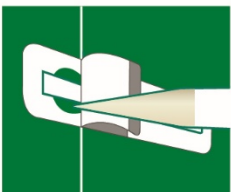

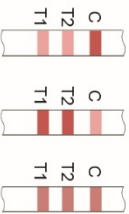
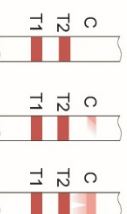
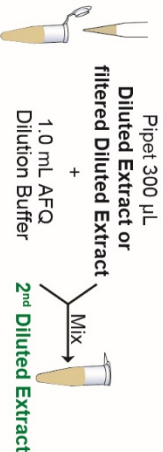
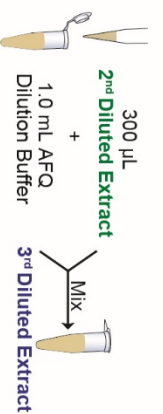
ROSA® FAST Aflatoxin Quantitative Test Flow Chart - 5 minute incubation

Approved Commodities: Barley, Brown Rice, Corn Gluten Meal, Corn/Soy Blend, Hominy, Millet, Oat Groats, Popcorn, Rough Rice, Rye, Sorghum, Soybean Meal, Wheat
^a2:1 Extraction Ratio: Barley, Brown Rice, Corn Gluten Meal, Corn/Soy Blend, Hominy, Millet, Oat Groats, Popcorn, Rough Rice, Rye, Sorghum, Soybean Meal, Wheat
^b3:1 Extraction Ratio: Corn Germ Meal, Distillers Dried Grain, Distillers Dried Grain with Solubles, Oats, Soybean Hulls, Soybeans



See Approved Commodities Below

Quantitation Ranges: 5 to 20 ppb
20 to 100 ppb
100 to 300 ppb

Sample Preparation		Test Procedure	Read Result												
<div><div><div></div><div>(1) Weigh Ground sample 50.0 ± 0.2 g</div></div><div><div></div><div>(2) Add Solvent 70% Methanol 100 mL^A/150 mL^B</div></div><div><div></div><div>(3) Extract Shake vigorously for 1 minute</div></div><div><div></div><div>(4) Clarify Centrifuge extract for 10 seconds</div></div><div><div><div><div>100 µL Centrifuged Extract + 1.0 mL AFQ Dilution Buffer</div><div>Mix</div></div><div></div><div>(5) Dilute Prepare Diluted Extract</div></div><div><div></div><div>Pass Diluted Extract through RC15 Filter</div><div>Filter for: For Barley, Corn Gluten Meal, Corn/Soy Blend, Oats, Oat Groats, Popcorn, Rye, and Wheat</div></div></div></div>		<div><div></div><div>(1) Place test strip in ROSA Incubator.</div></div> <div><div></div><div>(2) Peel tape. Pipet 300 µL Diluted Extract or filtered Diluted Extract into sample compartment. Reseal tape.</div></div> <div><div></div><div>(3) Close lid. Incubate for 5 minutes.</div></div>	<div><div><div><div></div><div>Valid Test</div></div><div><div></div><div>Invalid Test</div></div></div><div><table><thead><tr><th>Sample</th><th>Dilution</th><th>Quantitation Range</th></tr></thead><tbody><tr><td>Diluted Extract</td><td>DE</td><td>5 to 20 ppb</td></tr><tr><td>2nd Diluted Extract</td><td>2ND DE</td><td>20 to 100 ppb</td></tr><tr><td>3rd Diluted Extract</td><td>3RD DE</td><td>100 to 300 ppb</td></tr></tbody></table><div>Charm EZ-M reader: Select appropriate test (AFQ-FAST(5MIN)), commodity (select SPECIAL 1 for Oat Groats) and dilution if prompted.</div></div><div><div><div><div></div><div>(1) Prepare 2nd Diluted Extract</div><div>(2) Repeat Test Procedure (steps 1, 2, 3) with 2nd Diluted Extract</div><div>(3) Read Result</div></div><div><div><div><div></div><div>(1) Prepare 3rd Diluted Extract</div><div>(2) Repeat Test Procedure (steps 1, 2, 3) with 3rd Diluted Extract</div><div>(3) Read Result</div></div></div></div></div></div></div>	Sample	Dilution	Quantitation Range	Diluted Extract	DE	5 to 20 ppb	2 nd Diluted Extract	2ND DE	20 to 100 ppb	3 rd Diluted Extract	3RD DE	100 to 300 ppb
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