

CHARM SCIENCES, INC.
ROSA FAST5 ZEARALENONE QUANTITATIVE TEST

TABLE OF CONTENTS	PAGE
GENERAL INFORMATION.....	1
PREPARATION OF TESTING MATERIALS AND EQUIPMENT.....	2
EXTRACTION PROCEDURES	3
SAMPLE PREPARATION	4
TEST PROCEDURES.....	4
SUPPLEMENTAL ANALYSIS.....	7
REPORTING AND CERTIFYING TEST RESULTS.....	7
STORAGE CONDITIONS AND PRECAUTIONS	7
EQUIPMENT AND SUPPLIES.....	8
REVISION HISTORY.....	9
FLOW CHARTS	10

GENERAL INFORMATION

The Charm ROSA FAST5 Zearalenone Quantitative Test kit is an immunoreceptor assay utilizing ROSA (Rapid One Step Assay) lateral flow technology. Zearalenone is extracted from the sample using 70% methanol (MeOH) in water. Zearalenone interacts with colored beads in the lateral flow test strip and the color intensity in the test and control zones is measured by the ROSA-M Reader or Charm EZ-M reader and displayed as parts per billion (ppb) zearalenone.

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 current policies and/or instructions 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 FAST5 Zearalenone Quantitative Test
Product Number:	LF-ZEARQ-FAST5
Effective Date of Instructions:	01/26/2016
Instructions Revision Number	5
Conformance Range:	100 – 1000 ppb
Number of Analyses to Cover Conformance Range:	2
Type of Service:	Quantitative
Supplemental Analysis:	No
Approved Commodities:	Corn, barley, brewer's rice, distillers dried grain with solubles (DDGS), flaking corn grits, milled rice, oats, rough rice, sorghum, wheat, wheat bran, wheat flour
Extraction method:	Shake vigorously 50 gram sample with 100 milliliters (mL) 70% methanol/30% distilled or deionized water (v/v) for 1 minute. For distillers dried grains with solubles (DDGS) shake vigorously 50 gram sample with 150 mL 70% methanol/30% distilled or deionized water (v/v) for 1 minute. For wheat bran shake vigorously 50 gram sample with 200 mL 70% methanol/30% distilled or deionized water (v/v) for 1 minute.
Test Format:	Lateral flow strip
Detection Method:	ROSA-M Reader, Model # LF-ROSAREADER-M-NB and Charm EZ-M reader, Model # LF-ROSA-EZ-M

PREPARATION OF TESTING MATERIALS AND EQUIPMENT

a. Test Strips:

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 and unused test strips should be discarded.

b. ZEAR 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 (18 °C to 30 °C).

c. Preparation of 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).

- (1) Using a 1000 mL graduated cylinder, measure 700 mL methanol and place it into a clean carboy with spigot.
- (2) Using a 500 mL graduated cylinder, measure 300 mL distilled or deionized water and add to the methanol. Shake until it is completely 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 this solution at room temperature in a tightly closed container until needed. Mix again before use.

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

d. Negative Control

Prepare negative control by adding 100 microliters (µL) extraction solvent to 1.0 mL ZEAR Dilution Buffer in a clean micro-centrifuge tube, cap, mix, and label.

e. Positive Control

Reconstitute the dry positive control by adding 300 µL extraction solvent followed by 3.0 mL ZEAR Dilution Buffer. Shake well and allow to stand at room temperature for 10 minutes. Mix again before use.

f. Reader and Test Strip Performance Testing:

- (1) Equipment Setup
 - (a) **ROSA-M Reader:** Enter performance mode in ROSA-M Reader by selecting ZEAR channel in 3-line mode (ZEAR flashing) and sequentially pressing ESC, 5, ENTER. Follow ROSA-M Reader prompts to test calibration strips (LOWCAL and HIGHCAL) and controls (NEGCONTROL and POSCONTROL).
 - (b) **Charm EZ-M reader:** Enter performance mode in Charm EZ-M reader by selecting Perf. Mon. from the Main Menu, followed by Perf. Test. Follow the reader prompts to test calibration strips (LO CAL and HI CAL) and controls (NEG CTRL and POS CTRL). Select ZEARQ-FAST5 from the TESTS list if prompted.

- (2) Test calibration strips daily to verify performance of ROSA-M Reader or Charm EZ-M reader. Calibration strips must test/performance in the specified ranges.
- (3) Test negative control and positive control weekly to verify test strip performance. Valid control ranges are:
 - (a) Negative Control: less than 15 ppb
 - (b) Positive Control: 150 to 350 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:

ROSA Incubator must be clean and level. The ROSA Incubator temperature must be at 45 ± 1 °C (the temperature indicator should match the incubator temperature).

EXTRACTION PROCEDURES

a. 2:1 Extraction Procedure for corn, barley, brewer's rice, flaking corn grits, milled rice, oats, rough rice, sorghum, wheat, wheat flour:

- (1) Weigh 50.0 ± 0.2 grams ground samples into a clean extraction container.
- (2) Add 100 mL extraction solvent.
- (3) Shake vigorously for 1 minute by hand.
- (4) Allow sample to settle for 1 minute to obtain settled extract (can be used only for next 30 minutes).
- (5) Transfer 1 to 1.5 mL settled extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (can be used only for next 2 hours).
- (6) Repeat for additional samples (up to four (4) samples for each quad ROSA Incubator).

b. 3:1 Extraction Procedure for distillers dried grain with solubles:

- (1) Weigh 50.0 ± 0.2 grams ground samples into a clean extraction container.
- (2) Add 150 mL extraction solvent.
- (3) Shake vigorously for 1 minute by hand.
- (4) Allow sample to settle for 1 minute to obtain settled extract (can be used only for next 30 minutes).
- (5) Transfer 1 to 1.5 mL settled extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (can be used only for next 2 hours).
- (6) Repeat for additional samples (up to four (4) samples for each quad ROSA Incubator).

c. 4:1 Extraction Procedure for wheat bran:

- (1) Weigh 50.0 ± 0.2 grams ground samples into a clean extraction container.

- (2) Add 200 mL extraction solvent.
- (3) Shake vigorously for 1 minute by hand.
- (4) Allow sample to settle for 1 minute to obtain settled extract (can be used only for next 30 minutes).
- (5) Transfer 1 to 1.5 mL settled extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (can be used only for next 2 hours).
- (6) Repeat for additional samples (up to four (4) samples for each quad ROSA Incubator).

SAMPLE PREPARATION

This test kit uses different testing sensitivity ranges (Diluted Extract and Second Diluted Extract) for reporting zearalenone measurements for grains and commodities.

a. Sample Preparation of Diluted Extract for 100 to 350 ppb quantitation.

- (1) Pipet 1.0 mL ZEAR Dilution Buffer into a clean micro-centrifuge tube.
- (2) Pipet 100 μ L clarified extract to a predisposed (1.0 mL ZEAR Dilution Buffer) micro-centrifuge tube, cap, mix and label. This sample is the Diluted Extract.
- (3) Repeat for additional samples.
- (4) Filter each Diluted Extract by drawing into 1 mL syringe, passing through Minisart RC15 syringe filter, and collecting filtered Diluted Extract in a clean micro-centrifuge tube. Label each micro-centrifuge tube.

NOTE: For wheat bran, filter Diluted Extract using a GF/CA syringe filter.

b. Sample Preparation of Second Diluted Extract for 300 to 1000 ppb quantitation.

- (1) Pipet 1.0 mL ZEAR Dilution Buffer into a clean micro-centrifuge tube.
- (2) Pipet 300 μ L filtered Diluted Extract to a predisposed (1.0 mL ZEAR Dilution Buffer) micro-centrifuge tube, cap, mix, and label. This sample is the Second Diluted Extract.
- (3) Repeat for additional samples.

TEST PROCEDURES

a. Sample Analysis:

- (1) Check that the ROSA Incubator temperature is 45 ± 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 pipet vertically and slowly pipet 300 μ L test sample (diluted extract or control) into the sample compartment at the ROSA Incubator line.
- (6) Reseal the tape over the sample pad compartment.

NOTE: When performing multiple tests using a ROSA Incubator:

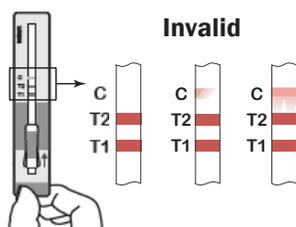
- (a) Peel, pipet, and reseal before starting next strip.
- (b) Complete all test strips (up to four (4) with quad incubator) within 1 minute.
- (7) Close lid on the ROSA Incubator.
- (8) Incubate for 5 minutes.
- (9) Remove strip from the ROSA Incubator.

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

- (a) Wipe foreign matter (dust, etc.) from the test strip(s).
- (b) Inspect and read test strip(s) within 2 minutes of incubation completion. When running multiple test strips in the ROSA Incubator, remove one strip for visual inspection and interpretation at a time.
- (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 ROSA-M Reader or Charm EZ-M reader.
- (3) If test strip is INVALID, re-test the diluted extract or control.

c. Interpretation:

- (1) ROSA-M Reader
 - (a) Insert a clean and 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.

- (b) Read results on ZEAR channel in 3-line mode (ZEAR flashing) using the appropriate MATRIX. If desired, enter Sample and/or Operator. Press ENTER to read.
1. For **corn, barley, brewer's rice, flaking corn grits, milled rice, oats, rough rice, sorghum, wheat, and wheat flour** use:
 - **MATRIX 00:** Assay of Diluted Extract for 100 to 350 ppb quantitation.
 - **MATRIX 01:** Assay of Second Diluted Extract for 300 to 1000 ppb quantitation.
 2. For **DDGS** use:
 - **MATRIX 02:** Assay of Diluted Extract for 100 to 350 ppb quantitation.
 - **MATRIX 03:** Assay of Second Diluted Extract for 300 to 1000 ppb quantitation.
 3. For **wheat bran** use:
 - **MATRIX 04:** Assay of Diluted Extract for 100 to 350 ppb quantitation.
 - **MATRIX 05:** Assay of Second Diluted Extract for 300 to 1000 ppb quantitation.

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

A + sign on a READING value indicates that the concentration of the sample is greater than the sensitivity range of the sample dilution. For example, a Diluted Extract READING of "+351^{ppb}" indicates a value greater than 350 ppb. For quantitation greater than 350 ppb, Second Diluted Extract is prepared and analyzed.

(2) Charm EZ-M reader (Read only mode)

- (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 ZEARQ-FAST5 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:** Assay of Diluted Extract for 100 to 350 ppb quantitation.
 - **2ND DE:** Assay of Second Diluted Extract 300 to 1000 ppb quantitation.

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

A + sign on a READING value indicates that the concentration of the sample is greater than the sensitivity range of the sample dilution. For example, a Diluted Extract READING of "+350 PPB" indicates a value greater than 350 ppb. For quantitation greater than 350 ppb, Second Diluted Extract is prepared and analyzed.

SUPPLEMENTAL ANALYSIS

There is no Supplemental Analysis for this test kit.

REPORTING AND CERTIFYING TEST RESULTS

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division 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

a. Storage Conditions:

- (1) Store test strips refrigerated in tightly closed supplied container.
- (2) Store dilution buffer bottle and pre-dispensed micro-centrifuge tubes refrigerated.
- (3) Store reconstituted positive control refrigerated (0 °C to 7 °C) for up to one week or aliquot (at least 0.5 ml) to clean micro-centrifuge tubes, label, and freeze within six hours of reconstitution (-15 °C or below) 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.**

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, estimated between 20 to 30 minutes from the time the container was removed from the refrigerator.
 - (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.
- (2) Use ZEAR Dilution Buffer supplied with each test kit only.
- (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 ± 1 °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.

EQUIPMENT AND SUPPLIES

a. Test Strips

- (1) LF-ZEARQ-FAST5-20K
 - (a) 1 container of 20 ZEARQ-FAST5 test strips
 - (b) 1 Zearalenone Positive Control
 - (c) 1 ZEAR Dilution Buffer
- (2) LF-ZEARQ-FAST5-100K
 - (a) 1 container of 100 ZEARQ-FAST5 test strips
 - (b) 1 Zearalenone Positive Control
 - (c) 1 ZEAR Dilution Buffer
- (3) LF-ZEARQ-FAST5-500K
 - (a) 5 containers of 100 ZEARQ-FAST5 test strips
 - (b) 5 Zearalenone Positive Controls
 - (c) 5 ZEAR Dilution Buffers

b. Materials required but not provided

- (1) 100 μ L pipet and pipet tips
- (2) 300 μ L pipet and pipet tips
- (3) 100 to 1000 μ L variable volume or 1.0 mL pipet and pipet tips
- (4) 1 mL non-sterile syringes
- (5) 100 mL, 250 mL, 500 mL and 1000 mL graduated cylinder
- (6) Balance
- (7) Deionized or distilled water
- (8) GF/CA syringe filters (required for wheat bran only, Phenomenex Part No. AF0-8A09-12)
- (9) Methanol (ACS reagent grade or better)
- (10) Micro-centrifuge tubes
- (11) Mini-centrifuge
- (12) Minisart RC15 syringe filters (Sartorius Minisart RC 15, Part No. 17762)
- (13) ROSA Incubator
- (14) ROSA-M Reader or Charm EZ-M reader
- (15) Printer for ROSA-M Reader or Charm EZ-M reader (optional)
- (16) Sample grinder
- (17) Sample extraction containers
- (18) Storage bottle

- (19) Transfer pipets (optional)

REVISION HISTORY

Revision 5 (01/26/2016)

- The aliquot volume of positive control used for freezing has been changed from 1.5 mL to 0.5 mL.

Revision 4 (10/27/2015)

- Quantitation range for the second Diluted Extract was corrected from 100 to 1000 ppb to 300 to 1000 ppb.

-

Revision 3 (9/11/2015)

- Flow charts have been incorporated in to the instruction.
- Updated format of test kit instructions

Revision 2 (1/29/2015)

- Corrected MATRIX settings for distillers dried grains with solubles and wheat bran on ROSA-M Reader.

Revision 1 (1/7/2015)

- Correct Acronym of Policies, Procedures, and Market Analysis Branch (PPMAB) has been used.
- Phone number of Patrick McCluskey (816-659-8403) has been corrected.

Revision 0 (11/28/2014)

FLOW CHARTS

a. Flow chart for 2:1 Extraction Procedure and Assay for corn, barley, brewer's rice, flaking corn grits, milled rice, oats, rough rice, sorghum, wheat, and wheat flour:

Refer to Current GIPSA Test Kit Instructions for Complete Test Procedure														
<p>ROSA® FAST5 Zearelenone Quantitative Test Flow Chart- 2:1 Extraction</p> <p>Approved Commodities: Barley, Brewer's Rice, Corn, Flaking Corn Grits, Milled Rice, Oats, Rough Rice, Sorghum, Wheat, Wheat Flour</p>														
<p>See Approved Commodities Below</p>		<p>Quantitation Ranges: 100 to 350 ppb 300 to 1000 ppb</p>												
<p>Sample Preparation</p> <p>(1) Weigh 50.0 g Ground sample 50.0 g ± 0.2 g</p> <p>(2) Add Solvent 70% Methanol 100 mL</p> <p>(3) Extract Shake vigorously for 1 minute</p> <p>(4) Clarify Allow extract to settle for 1 minute Centrifuge extract for 10 seconds</p> <p>(5) Dilute 100 µL Extract + 1.0 mL ZEAR Dilution Buffer Diluted Extract</p> <p>(6) Filter Pass Diluted Extract through RC15 Filter and collect.</p>														
<p>Test Procedure</p> <p>(1) Place test strip in ROSA Incubator.</p> <p>(2) Peel tape. Pipet 300 µL filtered Diluted Extract into sample compartment. Reseal tape.</p> <p>(3) Close lid. Incubate for 5 minutes.</p>														
<p>Read Result</p> <p>(1) Inspect test strip</p> <p>(2) Read results with ROSA-M Reader or Charm EZ-M reader</p> <p>For quantitation of 300 to 1000 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>Valid Test</p> <p>Invalid Test</p>														
<p>ROSA-M Reader: Select ZEAR channel in 3-line mode (blinking) and appropriate MATRIX.</p> <p>Charm EZ-M reader: Select appropriate test (ZEARQ-FAST5), commodity and dilution. If prompted.</p> <table border="1"> <thead> <tr> <th>Sample</th> <th>Charm EZ-M Reader</th> <th>ROSA-M Reader</th> <th>Quantitation Range</th> </tr> </thead> <tbody> <tr> <td>Diluted Extract</td> <td>DILUTION DE</td> <td>MATRIX 00</td> <td>100 to 350 ppb</td> </tr> <tr> <td>2nd Diluted Extract</td> <td>DILUTION 2ND DE</td> <td>MATRIX 01</td> <td>300 to 1000 ppb</td> </tr> </tbody> </table>			Sample	Charm EZ-M Reader	ROSA-M Reader	Quantitation Range	Diluted Extract	DILUTION DE	MATRIX 00	100 to 350 ppb	2 nd Diluted Extract	DILUTION 2ND DE	MATRIX 01	300 to 1000 ppb
Sample	Charm EZ-M Reader	ROSA-M Reader	Quantitation Range											
Diluted Extract	DILUTION DE	MATRIX 00	100 to 350 ppb											
2 nd Diluted Extract	DILUTION 2ND DE	MATRIX 01	300 to 1000 ppb											
<p>© 2015 Charm Sciences, Inc. Charm, Charm EZ, and ROSA are registered trademarks of Charm Sciences, Inc. ZEAR is a trade dress of Charm Sciences, Inc.</p>														

b. Flow chart for 3:1 Extraction Procedure and Assay for DDGS:

Refer to Current GIPSA Test Kit Instructions for Complete Test Procedure

Approved Commodities:
 DDGS

See Approved Commodities Below

Quantitation Ranges:
 100 to 350 ppb
 300 to 1000 ppb

Sample Preparation

Test Procedure

Read Result

(1) Weigh



Ground sample
50.0 g ± 0.2 g

(2) Add Solvent



70% Methanol
150 mL

(3) Extract



Shake vigorously for 1 minute

(4) Clarify



Allow extract to settle for 1 minute
Centrifuge extract for 10 seconds

(5) Dilute



100 µL Extract + 1.0 mL ZEAR Dilution Buffer
MIX
Diluted Extract

(6) Filter



Pass Diluted Extract through RC15 Filter and collect

(1)



Place test strip in ROSA incubator.

(2)



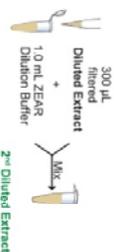
Peel tape. Paper 300 µL filtered Diluted Extract into sample compartment. Reseal tape.

(3)



Close lid. Incubate for 5 minutes.

For quantitation of 300 to 1000 ppb:



300 µL filtered Diluted Extract + 1.0 mL ZEAR Dilution Buffer
MIX
2nd Diluted Extract

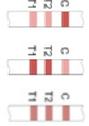
(1) Prepare 2nd Diluted Extract

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

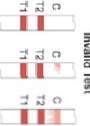
(3) Read Result

(1) Inspect test strip

Valid Test



Invalid Test



ROSA-M Reader: Select ZEAR channel in 3-line mode (blinking) and appropriate MATRIX.

Charm EZ-M reader: Select appropriate test (ZEARQ-FAST5), commodity and dilution if prompted.

Sample	Charm EZ-M Reader	ROSA-M Reader	Quantitation Range
Diluted Extract	DILUTION DE	MATRIX 02	100 to 350 ppb
2 nd Diluted Extract	DILUTION 2ND DE	MATRIX 03	300 to 1000 ppb

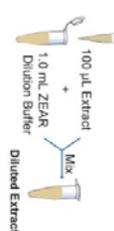
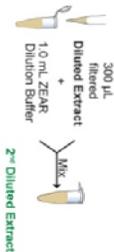
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 MKK-1025-001 Aug. 2015

Page 11 of 12

c. Flow chart for 4:1 Extraction Procedure and Assay for wheat bran:

Refer to Current GIPSA Test Kit Instructions for Complete Test Procedure		Quantitation Ranges:												
ROSA® FAST5 Zearalenone Quantitative Test Flow Chart- 4:1 Extraction Approved Commodities: Wheat Bran		See Approved Commodities Below 100 to 350 ppb 300 to 1000 ppb												
Sample Preparation	<ol style="list-style-type: none"> (1) Weigh Ground sample $50.0\text{ g} \pm 0.2\text{ g}$ (2) Add Solvent 70% Methanol 200 mL (3) Extract Shake vigorously for 1 minute (4) Clarify Allow extract to settle for 1 minute Centrifuge extract for 10 seconds (5) Dilute Prepare Diluted Extract  (6) Filter Pass Diluted Extract through GF/FCA Filter and collect. 													
Test Procedure	<ol style="list-style-type: none"> (1) Place test strip in ROSA incubator.  (2) Peel tape. Pipet 300 µL filtered Diluted Extract into sample compartment. Reseal tape.  (3) Close lid. Incubate for 5 minutes.  													
Read Result	<ol style="list-style-type: none"> (1) Inspect test strip ROSA-M Reader: Select ZEAR channel in 3-line mode (blinking) and appropriate MATRIX. Charm EZ-M reader: Select appropriate test (ZEARQ-FAST5), commodity and dilution if prompted. <table border="1" data-bbox="470 630 568 1239"> <thead> <tr> <th>Sample</th> <th>Charm EZ-M Reader DILUTION</th> <th>ROSA-M Reader DE</th> <th>Quantitation Range</th> </tr> </thead> <tbody> <tr> <td>Diluted Extract</td> <td>DE</td> <td>MATRIX_04</td> <td>100 to 350 ppb</td> </tr> <tr> <td>2nd Diluted Extract</td> <td>2ND DE</td> <td>MATRIX_05</td> <td>300 to 1000 ppb</td> </tr> </tbody> </table> 	Sample	Charm EZ-M Reader DILUTION	ROSA-M Reader DE	Quantitation Range	Diluted Extract	DE	MATRIX_04	100 to 350 ppb	2 nd Diluted Extract	2ND DE	MATRIX_05	300 to 1000 ppb	<p>For quantitation of 300 to 1000 ppb:</p> <ol style="list-style-type: none"> (1) Prepare 2nd Diluted Extract  (2) Repeat Test Procedure (steps 1, 2, 3) with 2nd Diluted Extract (3) Read Result
Sample	Charm EZ-M Reader DILUTION	ROSA-M Reader DE	Quantitation Range											
Diluted Extract	DE	MATRIX_04	100 to 350 ppb											
2 nd Diluted Extract	2ND DE	MATRIX_05	300 to 1000 ppb											

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