

CHARM SCIENCES
ROSA FAST5 ZEARALENONE QUANTITATIVE TEST

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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 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 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:	Charm Sciences, Inc. 1-978-687-9200
Test Kit Name:	ROSA FAST5 Zearalenone Quantitative Test
Product Number:	LF-ZEARQ-FAST5
Effective Date of Instructions:	11/28/2014
Instructions Revision Number:	0
Conformance Range:	100 – 1000 ppb
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	No
Approved Commodities:	Corn, barley, brewer's rice, distillers dried grain with solubles, flaking corn grits, milled rice, oats, rough rice, sorghum, wheat, wheat bran, wheat flour
Extraction method:	Shake 50 gram sample with 100 milliliters (mL) 70% methanol and shake for 1 minute. For distillers dried grain with solubles, use 150 mL 70% methanol and shake for 1 minute. For wheat bran, use 200 mL 70% methanol and shake for 1 minute.
Test Format:	Lateral Flow Strip
Detection Method:	ROSA-M Reader, Model # LF-ROSAREADER-M-NB Charm EZ-M, Model # LF-ROSA-EZ-M

PREPARATION OF TESTING MATERIALS

a. Test Strips

- (1) Store refrigerated in tightly closed 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 to 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 have turned purple or pink.
- (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.

b. ZEAR Dilution Buffer

- (1) Use buffer supplied with each test kit only.
- (2) Dispense buffer into a clean micro-centrifuge tube for each sample to be tested (see Sample Preparation section).
- (3) Use pre-dispensed tubes and buffer solution at room temperature (18 to 30°C).
- (4) Store buffer bottle and pre-dispensed tubes refrigerated when not in use.

c. Preparation of Extraction Solution 70% MeOH/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) Add 300 mL distilled or deionized water to the methanol and 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 and Positive Control

- (1) Negative Control
 - (a) To prepare negative control, add 100 microliters (μL) 70% methanol (extraction solution) to 1.0 mL ZEAR Dilution Buffer in a micro-centrifuge tube. Cap, mix, and label.
 - (b) To run, use 300 μL of negative control as your test sample in Sample Analysis found in Test Procedures section.
- (2) Positive Control
 - (a) Zearalenone Positive Control is supplied dry. Store refrigerated.
 - (b) To prepare positive control, reconstitute the dry positive control by adding 300 μL 70% methanol (extraction solution) followed by 3.0 mL ZEAR Dilution Buffer. Shake well. Allow to stand for 10 minutes at room temperature before use. Mix again before use.
 - (c) Store reconstituted positive control refrigerated (0 to 7° C) for up to 1 week or aliquot and freeze within 6 hours of reconstitution at (-15° C or below) for up to 2 months. Thaw slowly (overnight in refrigerator or with cool water) and shake well before use.
 - (d) Store thawed positive control refrigerated and use within 24 hours of thawing. DO NOT REFREEZE.
 - (e) To run, use 300 μL of positive control as your test sample in Sample Analysis found in Test Procedures section.

e. Equipment Preparation

- (1) ROSA Incubator must be clean and level. The ROSA Incubator temperature must be at $45\pm 1^\circ\text{C}$ (the temperature indicator should match the incubator temperature). ROSA Incubator may take more than 10 minutes to reach proper temperature, depending on the ambient temperature.

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:** Enter performance mode in Charm EZ-M by selecting Perf. Mon. from the Main Menu, followed by Perf. Test. Follow Charm EZ-M 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 ROSA-M Reader or Charm EZ-M 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. 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.

EXTRACTION PROCEDURES

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

- (1) Obtain a representative sample according to official procedures.
- (2) Grind/mill to a 20-mesh particle size according to official procedures.
- (3) Mix thoroughly and transfer 50 grams ground sample into a clean extraction container.
- (4) Add 100 mL 70% methanol.
- (5) Shake vigorously for 1 minute by hand.
- (6) Clarify sample extract.
 - (a) Allow sample to settle for 1 minute to obtain settled extract.
 - (b) After settling, centrifuge to clarify extract. Using transfer pipet, add 1 to 1.5 mL sample extract into a clean micro-centrifuge tube and label. Centrifuge in mini-centrifuge for 10 seconds.
- (7) Prepare additional sample extracts (up to 4 for quad incubator) following steps 1 – 6.
- (8) Proceed to Sample Preparation section.

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

- (1) Obtain a representative sample according to official procedures.
- (2) Grind/mill to a 20-mesh particle size according to official procedures.
- (3) Mix thoroughly and transfer 50 grams ground sample into a clean extraction container.
- (4) Add 150 mL 70% methanol.
- (5) Shake vigorously for 1 minute by hand.
- (6) Clarify sample extract.
 - (a) Allow sample to settle for 1 minute to obtain settled extract.
 - (b) After settling, centrifuge to clarify extract. Using transfer pipet, add 1 to 1.5 mL sample extract into a clean micro-centrifuge tube and label. Centrifuge in mini-centrifuge for 10 seconds.
- (7) Prepare additional sample extracts (up to 4 for quad incubator) following steps 1 – 6.

(8) Proceed to Sample Preparation section.

c. Extraction Procedure for: wheat bran.

- (1) Obtain a representative sample according to official procedures.
- (2) Grind/mill to a 20-mesh particle size according to official procedures.
- (3) Mix thoroughly and transfer 50 grams ground sample into a clean extraction container.
- (4) Add 200 mL 70% methanol.
- (5) Shake vigorously for 1 minute by hand.
- (6) Clarify sample extract.
 - (a) Allow sample to settle for 1 minute to obtain settled extract.
 - (b) After settling, centrifuge to clarify extract. Using transfer pipet, add 1 to 1.5 mL sample extract into a clean micro-centrifuge tube and label. Centrifuge in mini-centrifuge for 10 seconds. This is the clarified extract.
- (7) Prepare additional sample extracts (up to 4 for quad incubator) following steps 1 – 6.
- (8) Proceed to Sample Preparation section.

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 100 μ L of clarified extract to a predispensed (1.0 mL ZEAR Dilution Buffer) micro-centrifuge tube, cap, mix and label.
- (2) Repeat for additional samples.
- (3) Filter each Diluted Extract by drawing into 1 mL syringe, passing through Minisart RC15 syringe filter, 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.

- (4) Use filtered Diluted Extract as your test sample in Sample Analysis found in Test Procedures section. This filtered Diluted Extract can also be used for Second Diluted Extract.

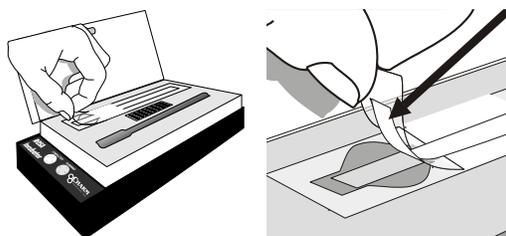
b. Sample Preparation of Second Diluted Extract from the Diluted Extract for 300 to 1000 ppb quantitation.

- (1) Pipet 300 μ L of filtered Diluted Extract to a predisposed (1.0 mL ZEAR Dilution Buffer) micro-centrifuge tube, cap, mix, and label.
- (2) Repeat for additional samples.
- (3) Use Second Diluted Extract as your test sample in Sample Analysis found in Test Procedures section.

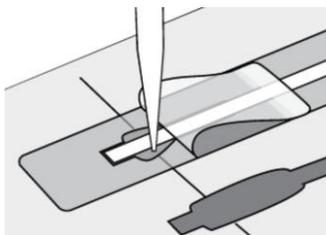
TEST PROCEDURES

a. Sample Analysis

- (1) Check that the ROSA Incubator temperature is $45 \pm 1^\circ \text{C}$.
- (2) Label test strip(s) to identify sample.
- (3) Open the incubator lid and place test strip in the ROSA Incubator with the flat side facing upward.
- (4) While holding the test strip flat in the incubator, use tab to expose sample compartment by peeling tape back to “Peel to Here” line. Avoid lifting the test strip and sponge under tape and bending back the white wick and sponge under the tape.



- (5) Holding the pipet vertically, slowly pipet 300 μ L test sample (Diluted Extract, Second Diluted Extract, or control) into the sample compartment at the ROSA Incubator line (as shown).

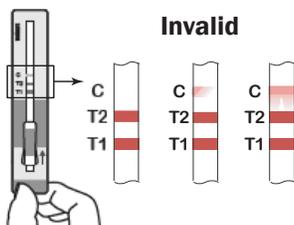


- (a) 300 μ L Diluted Extract for 100 to 350 ppb quantitation.
 - (b) 300 μ L Second Diluted Extract for 300 to 1000 ppb quantitation.
 - (c) 300 μ L negative control or positive control.
- (6) Reseal the tape over the sample pad compartment. When performing multiple test(s) using a ROSA Incubator:
 - (a) Peel, pipet, and reseal before starting next strip.

- (b) Complete all test strips (up to four with quad incubator) within 1 minute.
- (7) Close lid on the incubator and tighten the latch. Timer starts and a red light will automatic illuminate.
- (8) Incubate for 5 minutes. At 5 minutes a beeper and alternating yellow and red blinking lights will start to flash.
- (9) Remove strip(s) from the ROSA Incubator. Do not squeeze sample compartment. Hold test strip vertically with sample compartment in the down position until and 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 of Test Strip

- (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 to not flow past T1, T2 or C lines.



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

c. Test Strip 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. **MATRIX 00:** Assay of Diluted Extract for 100 to 350 ppb quantitation.
 - 2. **MATRIX 01:** 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” on MATRIX 00 indicates a value greater than 350 ppb. For quantitation greater than 350 ppb, Second Diluted Extract is prepared and analyzed.

(2) **Charm EZ-M**

- (a) Insert a clean and valid test strip into the Charm EZ-M. 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.

1. **DE:** Assay of Diluted Extract for 100 to 350 ppb quantitation.
2. **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 by phone at 816-659-8403 or Patrick.J.McCluskey@udsa.gov.

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions

- (1) Store test strips refrigerated in tightly closed supplied container.
- (2) Store dilution buffer and predispensed micro-centrifuge tubes refrigerated.
- (3) Reconstituted positive control can be refrigerated for up to 1 week and freeze within 6 hours of reconstitution at -15°C or below for up to 2 months.

b. Precautions

- (1) Debris on test strips may alter the ROSA-M Reader or Charm EZ-M optics. Keep equipment clean. Wipe dust and liquid off test strips before inserting into reader.
- (2) ROSA Incubator must be clean and level. ROSA Incubator temperature must be $45\pm 1^{\circ}\text{C}$. 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.

EQUIPMENT AND SUPPLIES

Materials supplied in test kits can be purchased that contain 20, 100, or 500 strips and include positive control and ZEAR Dilution Buffer.

a. LF-ZEARQ-FAST5-20K

- (1) 1 container of 20 ZEARQ-FAST5 test strips
- (2) 1 Zearalenone Positive Control
- (3) 1 ZEAR Dilution Buffer

b. LF-ZEARQ-FAST5-100K

- (1) 1 container of 100 ZEARQ-FAST5 test strips
- (2) 1 Zearalenone Positive Control
- (3) 1 ZEAR Dilution Buffer

c. LF-ZEARQ-FAST5-500K

- (1) 5 containers of 100 ZEARQ-FAST5 test strips
- (2) 5 Zearalenone Positive Control
- (3) 5 ZEAR Dilution Buffer

d. Equipment

- (1) Sample grinder
- (2) Balance
- (3) Mini-centrifuge
- (4) ROSA-M Reader or Charm EZ-M
- (5) ROSA Incubator
- (6) Printer for ROSA-M Reader or Charm EZ-M (optional)

e. Material Required but not Provided

- (1) Methanol (ACS reagent grade or better)
- (2) Deionized or distilled water
- (3) 1000 mL graduated cylinder
- (4) Storage bottle
- (5) Sample extraction containers
- (6) 100 to 1000 μ L variable volume pipet or 1.0 mL pipet and pipet tips
- (7) 300 μ L pipet and pipet tips
- (8) 100 μ L pipet and pipet tips
- (9) 250 mL graduated cylinder
- (10) Micro-centrifuge tubes
- (11) Transfer pipets (VWR, part # 16001-188)
- (12) 1 mL non-sterile syringes
- (13) Minisart RC15 syringe filters (Sartorius Minisart RC 15, Part No. 17762)
- (14) GF/CA syringe filters (required for wheat bran only, Phenomenex Part No. AF0-8A09-12)

REVISION HISTORY

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.