

RIDASCREEN FAST FUMONISIN QUANTITATIVE TEST KIT

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GENERAL INFORMATION

The RIDASCREEN FAST Fumonisin test kit is a competitive enzyme immunoassay for quantitative analysis of fumonisins in corn and additional commodities. The test kit is limited to providing fumonisins measurements between 0.5 – 5 parts per million (ppm).

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 Mycotoxin Handbook 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 Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division by phone at 816-659-8403 or email at Patrick.J.McCluskey@usda.gov.

Approved Test Kit Information

Test Kit Vendor:	<i>R-Biopharm Inc 1-877-789-3033</i>
Test Kit Name:	RIDASCREEN FAST Fumonisin
Product Number:	R5602
Effective Date of Instructions:	11/19/2015
Instructions Revision Number	1
Conformance Range:	0.5 – 5 ppm
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	Yes
Approved Commodities:	Barley, corn, corn germ, corn gluten meal, corn meal, corn/soy blend, distiller dried grains, distiller dried grains with solubles, milled rice, oats, sorghum, soybeans, and wheat.
Extraction method:	Shake vigorously 3 minutes by hand or by mechanical shaker with similar shaking action.
Test Format:	Microtiter well plate assay
Detection Method:	Stat Fax Reader, Model 303 Plus.

PREPARATION OF TESTING MATERIALS

Wash Solution:

- (1) To prepare the Wash Solution, dissolve the contents of the packet containing the buffer salt in 1 liter of distilled or deionized water. Document the technician's name, preparation date, and expiration date on wash solution bottle. A removable gum-label affixed to bottle for documentation is recommended.
- (2) Swirl to mix before use. When stored properly (at 36 - 46° F) the solution has a shelf life of four weeks.
- (3) Alternative Preparation of Wash Solution:
 - (a) Dissolve the contents of the packet in only 100 mL of distilled or deionized water to obtain a 10 fold concentrated washing buffer. This solution expires after approximately 8 weeks when store at room temperature (68 - 77° F).
 - (b) Use 1 part of the concentrated washing buffer and dissolve with 9 parts of distilled or deionized water to obtain the ready to use wash solution.

Example: 100 mL of this concentrated washing buffer should be mixed with 900 mL of distilled water to get 1000 mL of wash solution.

EXTRACTION AND SAMPLE PREPARATION PROCEDURES

Extraction and sample preparation procedures are for the following commodities: Barley, corn, corn germ, corn gluten meal, corn meal, corn/soy blend, dried distiller grains, dried distiller grains with solubles, milled rice, oats, sorghum, soybeans, and wheat.

1. Place a sheet of filter paper (Whatman #1 folded or equivalent) into a clean suitable container.
2. Label a clean collection container with the sample identification.
3. Place a 50-gram (+/- 0.2) portion of ground sample in a Whirl-Pak bag.
4. Add 250 mL of 70% methanol/30% water (v/v) solution and shake vigorously 3 minutes by hand or by mechanical shaker with similar shaking action for 3 minutes.
5. Filter the extract through the Whatman No. 1 filter paper into a clean collection container.
6. Dilute the filtered extract 1:14 v/v (1 + 13) ratio using 100 μ L of the filtered extract with 1.3 mL of distilled or deionized water (**final volume 1.4 mL or 1400 μ L**) into a separate clean container.
7. This is the **diluted extract** and ready for testing.
8. Proceed to the test procedures.

TEST PROCEDURES

1. Test Procedures:

- a. Allow reagents and antibody wells to reach room temperature (68° - 77° F) prior to running the test.
- b. Insert a sufficient number of wells into the microwell holder for all control standards and samples to be tested. (For example: to test 11 samples, use 16 wells - 5 for the standards and 11 for the test samples).

NOTE: Do not run more than 2 strips (11 samples) per set of control standards.

- c. Using a new pipette tip for each standard and test sample, pipette 50 microliters (µL) of standard and prepared diluted test sample extract into each designated/separate wells.
- d. Using repeating pipettor add 50 µL of enzyme conjugate (red-capped bottle) into each well.
- e. Using repeating pipettor add 50 µL of the anti-fumonisin antibody solution (black-capped bottle) into each well.
- f. Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for 10-15 seconds without spilling reagents.
- g. Incubate for 10 minutes (± 1 minute) at room temperature.
- h. Dump the contents of the wells. Turn the wells upside down and tap on a paper towel until all the remaining liquid has been removed.
- i. Using a wash bottle, fill each well with distilled/deionized water. Empty the wells again and remove all remaining liquid. Repeat this step 2 more times (total of 3 washes).
- j. Using repeating pipettor add 100 µL of substrate/chromogen to each well.
- k. Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for 10-15 seconds without spilling reagents.
- l. Incubate for 5 minutes (± 0.5 minutes) at room temperature in the dark. Cover the wells with a paper towel to protect them from light sources.
- m. Using repeating pipettor add 100 µL of stop solution to each well.
- n. Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for 10-15 seconds without spilling reagents.
- o. Measure absorbance at 450 nm using the Stat-Fax Reader, Model 303 Plus. Results must be read within 10 minutes.

- p. Document test results on the work record.

2. **Reading the Results:**

- (1) Stat-Fax Model 303 PLUS Microwell Reader.
 - (a) Press Menu, the prompt should read: "Select Test" press 1, then ENTER.
- (2) Place the wells in the far right column of the carrier with the first standard being at the top.
- (3) Align carrier to the far left for column 1. Then press ENTER.
- (4) The reader is now reading the first eight wells. Once complete the display will read: "Plot Curve Y/N Select N."
- (5) Display will now read: "Accept Curve Y/N."

If you are only running one strip, the test is now complete (press the clear button twice). If you have an additional strip to run, select yes. Move the carrier to the right so that the wells are aligned with notch in the center. Now press ENTER.

- (10) The reader is now reading the second set of eight wells.
- (11) Once the last strip is read, press the clear button twice.
- (12) Test is now complete.

REPORTING AND CERTIFYING TEST RESULTS

Refer to the current Mycotoxin Handbook for reporting and certification of test results. For questions regarding the Mycotoxin Handbook instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@usda.gov).

SUPPLEMENTAL ANALYSIS PROCEDURES

Supplemental analysis (**for corn only**) is a procedure followed when a result is observed above the upper limit of the concentration range used in GIPSA's test kit performance evaluation.

The range for performance evaluation of quantitative Fumonisin test kits is 0.5 – 5 ppm. Therefore, supplemental analysis would be performed for a result above 5 ppm. In supplemental analysis, the extract is diluted so the resulting concentration is between the lower and upper limits of the test kit evaluation range (i.e., 0.5 – 5 ppm for Fumonisin), and a correction for dilution is applied to derive at the final result. Supplemental analysis is performed only at the request of the applicant.

Example:

If the original analysis reported the Fumonisin value at 9.0 ppm and the conformance limit is 5 ppm, in order to obtain a true value, dilute 1 ml of the **diluted extract** (tested previously that gave the Fumonisin value at 9.0 ppm) with 2 ml of the extraction solvent (distilled/deionized water). This is the **supplemental diluted extract** (the total volume is 3 ml). This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end, Dilution Factor is 3). Mix thoroughly and run the test. Multiply the analytical result obtained by 3 to obtain the actual Fumonisin concentration. For example, if 3.1 ppm was the value obtained with the **supplemental diluted extract**, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

EQUIPMENT AND SUPPLIES

1 Materials Provided in Test Kits:

- a. 1 Microtiter plate and 48 antibody coated wells.
- b. 5 fumonisin standard solutions of 1.3 mL each; 0, 0.222, 0.666, 2.0, and 6.0 ppm fumonisin in water.
- c. 1 red-capped bottle of 3 mL peroxidase conjugated fumonisin solution.
- d. 1 black-capped bottle of 3 mL anti-fumonisin antibody.
- e. 1 brown bottle with 6 mL of substrate/chromogen.
- f. 1 yellow-capped bottle with 14 mL of stop reagent.

2 Materials Required but not Provided:

- a. ACS Grade methanol.
- b. Deionized or distilled water.
- c. 250-mL graduated cylinder.
- d. Disposable p 125-mL container.
- e. Whatman #1 filter paper or equivalent.
- f. Sample collection tubes.
- g. (Optional)-Mechanical shaker.
- h. Stat-Fax Model 303 PLUS, with a 450-nm filter.
- i. Repeating pipettor and tips.
- j. 50 µL, 100 µL, and 1000 µL pipettor and pipette tips.

- k. Paper towels, Kay dry paper, or equivalent absorbent material.
- l. Wash bottle.
- m. Whirl Pak bags

STORAGE CONDITIONS AND PRECAUTIONS

1. The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 35° - 46 ° F.
2. Return wells not required for testing to the original foil bag and reseal them together with the desiccant drying agent provided to refrigerated storage immediately after use.
3. The substrate/chromogen is light sensitive, therefore, avoid exposure to direct light. Avoid direct sunlight during all incubations. Covering the microtiter plates is recommended.
4. Do not allow microwells to dry up totally between working steps. Avoid prolonged intervals between the working steps.
5. Carefully follow the washing sequence when washing microwells as recommended in test instructions.
6. Do not interchange individual reagents between test kits of different lot numbers.

REVISION HISTORY

Revision 0 (07/15/2015)

Revision 1 (11/19/2015)

- The RIDASCREEN FAST Fumonisin test kit was recertified.
- Extraction procedure has been changed from two minutes blending to three minutes vigorous shaking.
- Twelve additional commodities (barley, corn germ, corn gluten meal, corn meal, corn/soy blend, distiller dried grains, distiller dried grains with solubles, milled rice, oats, sorghum, soybeans, and wheat) were approved for RIDASCREEN FAST Fumonisin test kit. The test procedure for these additional commodities has been incorporated in this revision.