

R-Biopharm
RIDASCREEN FAST Aflatoxin SC

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GENERAL INFORMATION

The RIDASCREEN FAST Aflatoxin Single Control (SC) test method, product number R9002, is a competitive enzyme immunoassay for the quantitative analysis of aflatoxins in corn, barley, corn bran, corn flour, corn germ, corn gluten meal, corn grits, corn meal, corn screenings, corn/soy blend, corn starch, distillers dried grains (DDG), distillers dried grains with solubles (DDGS), malted barley, milled rice, oats, popcorn, rice bran, rough rice, rye, sorghum, soybeans, wheat, wheat flour, and wheat middlings.

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 (PPMB) 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 PPMB by phone at 816-891-8403 or email at Patrick.J.McCluskey@usda.gov.

Approved Test Kit Information

Test Kit Vendor:	<i>R-Biopharm Inc. 877-789-3033</i>
Test Kit Name:	RIDASCREEN FAST Aflatoxin SC
Product Number:	R9002
Effective Date of Instructions:	11/13/2014
Instructions Revision Number	1
Conformance Range:	5 – 100 ppb
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	Yes
Approved Commodities:	Corn, barley, corn bran, corn flour, corn germ, corn gluten meal, corn grits, corn meal, corn screenings, corn/soy blend, corn starch, distillers dried grains (DDG), distillers dried grains with solubles (DDGS), malted barley, milled rice, oats, popcorn, rice bran, rough rice, rye, sorghum, soybeans, wheat, wheat flour, and wheat middlings.
Extraction method:	Blend 50-gram sample with 250 mL of 70% methanol/30% water (v/v) using a blender set to high speed for 2 minutes.
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. For example 100 mL of this concentrated washing buffer should be mixed with 900 mL of distilled water to get 1000 mL of wash solution.

SAMPLE PREPERATION AND EXTRACTION PROCEDURES

Extraction Procedure for corn, barley, corn bran, corn flour, corn germ, corn gluten meal, corn grits, corn meal, corn screenings, corn/soy blend, corn starch, distillers dried grains, distillers dried grains (DDG) with solubles (DDGS), malted barley, milled rice, oats, popcorn, rice bran, rough rice, rye, sorghum, soybeans, wheat, wheat flour, and wheat middlings.

- (1) Weigh 50 ± 0.2 grams ground samples into a blender jar.
- (2) Add 250 mL of 70% methanol/30% water (v/v) and close the jar securely to prevent spillage.
- (3) Blend on high setting for 2 minutes.
- (4) Filter the extract through Whatman #1 filters into a clean container that is labeled with a sample ID number.
- (5) Dilute 1 part of the filtered extract with 1 parts of distilled/deionized water. (e.g., mix 1 mL filtered extract with 1 mL water). This is the **diluted filtrate extract** and is ready for testing.
- (6) Use 50 μ L of the diluted filtrate extract per well for testing.

TEST PROCEDURES

a. Analysis Procedure.

- (1) Allow reagents and antibody wells to reach the room temperature (68 - 77° F) prior to running the test.
- (2) Only one control standard (zero standard) is included in the test kit. The standard curve (B/Bo) is provided with the certificate of the test kit.
- (3) Insert a sufficient number of wells into the microwell holder for control standard and samples to be tested. (For example: to test 15 samples use 16 wells - 1 for the control standard and 15 for the test samples).

NOTE: Do not run more than 2 strips (one standard and 15 samples) per run.

- (4) Using a new pipette tip for the zero (0) control standard and each test sample, pipette 50 μ L of standard and diluted filtrate extract (prepared from each sample) to separate wells.
- (5) Add 50 μ L of enzyme conjugate (red capped bottle) into each well using a repeating pipettor with a 2.5 mL pipette tip on setting 1.
- (6) Add 50 μ L of Anti-aflatoxin antibody (black capped bottle) into each well using a repeating pipettor with a 2.5 mL pipette tip on setting 1.
- (7) Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for **10-15 seconds** without spilling contents of the wells.
- (8) Incubate for **10 minutes** at room temperature.
- (9) Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.
- (10) Using a wash bottle, fill each well with washing buffer solution. Empty the wells again and remove all remaining liquid. Repeat this step 2 more times (total of 3 washes).
- (11) Add 100 μ L of substrate/chromogen (brown cap brown plastic bottle) to each well using a repeating pipettor with a 2.5mL pipette tip on setting 2.
- (12) Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for **10-15 seconds** without spilling contents of the wells.
- (13) Cover the wells with a paper towel to protect them from light sources, and incubate for **5 minutes** (\pm 0.5 minutes) at room temperature (64 – 86° F).
- (14) Add 100 μ L of stop solution (yellow cap-brown glass bottle) to each well using a repeating pipettor with a 2.5 mL pipette tip on setting 2.

- (15) Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for **10-15 seconds** without spilling reagents.
- (16) Measure absorbance at 450 nm using the Awareness Technology Stat-Fax Model 303 PLUS (results must be read within 10 minutes).

b. Reading Results with the Stat-Fax Reader Model 303 PLUS.

- (1) Stat-Fax Model 303 PLUS Microwell Reader.
 - (a) Press Menu, the prompt should read: “Select Test” press 1, then ENTER.
 - (b) The concentrations and B/BO% should now be printing.
- (2) Display will read: “New B/BO Number Y/N (Yes/No). Press "N" if the B/BO matches the QC sheet in the test kit in use. Press “Y” if the B/BO on the printout does not match the QC sheet in the kit.
- (3) If “Y” was pressed for new B/BO, it will now display: Cal 2 B/BO%= ____ simply insert the B/BO number from the QC sheet for standard 2 and press ENTER.
- (4) When completed the reader will print “Test is Updated”.

Note: Please verify new B/BO number entered on the printout match test kit QC sheet.

- (5) If “N” was pressed for new B/BO, or you just finished updating the B/BO , it will now display: “Set carrier to 1; press Enter”
- (6) Place the wells in the far right column of the carrier with the zero (0) standard being at the top.
- (7) Align carrier to the far left for column 1. Then press ENTER.
- (8) The reader is now reading the first eight wells. Once complete the display will read: “Plot Curve Y/N Select N.
- (9) 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.

- (13) Additional information regarding reader setting and operation should be obtained from R-Biopharm and readers manual.

SUPPLEMENTAL ANALYSIS

1. Dilution of the Sample Extract.

If quantitative results are above the testing limits (i.e., 100 ppb) of the test kit, test results are reported as exceeding the limit. To determine and report an aflatoxin level higher than 100 ppb, the sample extract must be diluted so that a value between 5 and 100 ppb is obtained.

The final aflatoxin concentration is calculated by multiplying the results with the diluted extract by the dilution factor.

2. Example.

If the original analysis reported the aflatoxin value at greater than 100 ppb, the sample extract would be diluted using the following procedures in order to obtain a true value.

- a. Prepare a 35% methanol dilution solvent by adding equal portions of distilled or deionized water and 70/30 methanol/water extraction solvent. Example: 10 mL of water plus 10 mL of 70/30 methanol mixture will provide 20 mL of 35% methanol in water.
- b. Dilute 200 μ L (0.2 mL) of the diluted extract (obtained from the original extract as applicable) with 1.8 mL of the dilution solvent mixture from step “a” above. The total volume is 2 mL. This is a 1:9 dilution, Dilution Factor (DF) is 10. Proceed to sample test analysis section.
- c. Multiply the analytical results obtained by ten (**10**) to obtain the actual aflatoxin concentration. For example, if 25 ppb was the original value obtained with the diluted extract, the actual concentration in the original sample was 250 ppb (25 ppbX10).
- d. A final result of less than 53 ppb is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the procedure for the Diluted Extract (non-supplemental analysis) and only perform the supplemental analysis again if the value is greater than 100 ppb.

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 (816-891-8403 or Patrick.J.McCluskey@udsa.gov).

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions

The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 36° F and 46° F.

b. Precautions

- (1) Do not interchange individual reagents between kits of different lot numbers.
- (2) Do not use the test kits beyond the noted expiration date.
- (3) The substrate/chromogen solution is light sensitive, therefore, avoid exposure to direct light.

EQUIPMENT AND SUPPLIES

a. Materials Provided in Test Kits (48 well kit).

- (1) 1 Microtiter plate with 48 wells (6 strips with removable wells each) coated with capture antibodies.
- (2) 1 Afla standard solutions of 1.3 mL 0 ppm (zero standard).
- (3) 1 red-capped bottle of 3 mL peroxidase conjugated aflatoxin solution.
- (4) 1 black-capped bottle of 3 mL anti- aflatoxin antibody.
- (5) 1 brown-capped brown plastic bottle of 6 mL substrate/chromogen, stained red.
- (6) 1 yellow-capped brown glass bottle of 6 mL stop solution.
- (7) 1 packet of washing buffer (salt).

b. Materials Required but not Provided.

- (1) Awareness Technology Inc. Stat-Fax Model 303 PLUS with 450-nm filter.
- (2) RIDASOFT Win Software. (Optional)
- (3) 50 µL, 100 µL, and 1000 µL Pipettor and pipette tips.
- (4) Graduated cylinders (plastic or glass): 250 mL and 1 L.
- (5) Blender.
- (6) Filter funnel.

- (7) Whatman #1 filter paper or equivalent.
- (8) Balance.
- (9) Repeating pipettor.
- (10) Paper towels, Kay dry paper or equivalent absorbent material.
- (11) Waste receptacle.
- (12) Timer: 3 channel minimum.
- (13) Waterproof marker, Sharpie or equivalent.
- (14) Wash bottle.
- (15) Deionized or distilled water.
- (16) Methanol.

REVISION HISTORY

Revision 1 (11/13/14)

Twenty four additional commodities were approved (barley, corn bran, corn flour, corn germ, corn gluten meal, corn grits, corn meal, corn screens, corn/soy blend, corn starch, distillers dried grains, distillers dried grains (DDG) with solubles (DDGS), malted barley, milled rice, oats, popcorn, rice bran, rough rice, rye, sorghum, soybeans, wheat, wheat flour, and wheat middlings) for RIDASCREEN FAST Aflatoxin SC test. The test procedure for these additional commodities has been incorporated in this revision.