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1. General Information

RIDASCREEN FAST Aflatoxin ECO assay is a competitive enzyme immunoassay with aqueous extraction for the quantitative determination of aflatoxin. The basis of the test is the antigen-antibody reaction. The microtiter wells are coated with capture antibodies directed against anti-aflatoxin antibodies. Aflatoxin standards or sample solutions, aflatoxin enzyme conjugate and anti-aflatoxin antibodies are added. Free aflatoxin and aflatoxin enzyme conjugate compete for the aflatoxin antibody binding sites (competitive enzyme immuno-assay). At the same time, the anti-aflatoxin antibodies are also bound by the immobilized capture antibodies. Any unbound enzyme conjugate is then removed in a washing step. Substrate/chromogen is added to the wells, bound enzyme conjugate converts the chromogen into a blue product. The addition of the stop solution leads to a color change from blue to yellow. The measurement is made photometrically at 450 nm. The absorbance is inversely proportional to the aflatoxin concentration in the sample.

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@ams.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 PPMB by phone at 816-659-8403 or email at Patrick.J.McCluskey@ams.usda.gov

Approved Test Kit Information

Test Kit Vendor:	<i>R-Biopharm Inc. 877-789-3033</i>
Test Kit Name:	RIDASCREEN FAST Aflatoxin ECO
Product Number:	R5201
Effective Date of Instructions:	11/20/2017
Instructions Revision Number:	1
Conformance Range:	5.0 – 300 ppb
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Approved Commodities:	Corn (including dent or field corn, cracked corn, corn grits or polenta, and corn screenings), corn gluten meal, corn/soy blend, corn starch, malted barley, milled rice (including brewer's rice and glutinous rice), popcorn, sorghum, soybeans (including whole soybean and full-fat soy flour), and wheat (including whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings).
Extraction method:	Vigorously shake 50 grams sample with 250 mL of ECO Extraction Buffer for three minutes by hand.
Test Format:	Competitive enzyme immunoassay.
Detection Method:	Stat Fax Reader, Model 303 Plus

2. Preparation of Testing Materials

A. Wash Solution

Dissolve the contents of the buffer salt packet in 1 liter of distilled or deionized water. Swirl to mix until salt is fully dissolved. Prepare the solution in or transfer it to an appropriately-sized squeeze bottle for use.

The Wash Solution expires after 4 weeks when stored at 2 - 8 °C (36 - 46 °F).

Document the preparer's ID, preparation date, and expiration date on the Wash Solution bottle.

B. ECO Extraction Buffer

Transfer contents of one ECO extractor packet to a suitable container and dissolve completely in 600 ml of distilled or deionized water.

Note: Larger quantities may be prepared at one time as long as the ratio of water to ECO extractor (600 mL per packet) remains constant.

The ECO Extraction Buffer expires after 4 weeks when stored at 2 - 8 °C (36 - 46 °F).

Document the preparer's ID, preparation date, and expiration date on the ECO Extraction Buffer container.

3. Sample Preparation and Extraction Procedures (5 – 300 ppb)

Collect and prepare all test samples according to accepted sampling techniques (see Mycotoxin Handbook).

Bring all reagents and samples to room temperature (20 - 25 °C / 68 - 77 °F) before use, and perform the sample preparation at room temperature.

- A. Sample extraction for corn (including dent or field corn, cracked corn, corn grits or polenta, and corn screenings), corn gluten meal, corn starch, malted barley, milled rice (including Brewer's Rice and Glutinous Rice), popcorn, sorghum, soybeans ((including whole soybean and full-fat soy flour), and wheat (including whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings).

1. Weigh 50 ± 0.2 grams ground sample into a Whirl Pak bag.
2. Using a 250 mL graduated cylinder, add 250 mL of ECO Extraction Buffer and close the bag securely to prevent spillage.
3. Shake vigorously by hand for three minutes.
4. Let the extract settle for 1 minute, then filter into a culture tube or other appropriate container using an R-Biopharm filter syringe. The filtrate is the **filtered extract**.

This **filtered extract** can be used within 60 minutes.

- B. Sample extraction for **corn/soy blend** samples.

1. Weigh 50 ± 0.2 grams ground sample into a Whirl Pak bag.
2. Using a 250 mL graduated cylinder, add 250 mL of ECO Extraction Buffer and close the bag securely to prevent spillage.
3. Shake vigorously by hand for three minutes.
4. Let the extract settle for 1 minute, transfer to a 1.5 mL micro centrifuge tube and centrifuge in mini centrifuge for 1 minute at 7000rpm. Then filter the supernatant into a culture tube or other appropriate container using an R-Biopharm filter syringe. The filtrate is the **filtered extract**.

Pipette 1 mL of the filtered extract and pipette 1 mL of distilled or deionized water (using a new pipette tip) into a clean separate test tube. Cap the tube and mix well by inverting up and down five to six times. This is the **diluted extract** and is ready for testing.

4. Test Procedures (quantitation range 5 – 300 ppb)

Bring all reagents to room temperature (20 - 25 °C / 68 - 77 °F) before use, and perform the test at room temperature.

Do not analyze more than three test strips per run.

- A. Insert a sufficient number of microtiter wells into the microwell holder for all standards and samples to be tested (six wells for standards, plus one for each test sample). Record standard and sample positions.
- B. Using a 50 μ L pipette, add 50 μ L of each standard (white capped bottle) or prepared sample into separate wells. Use a new pipette tip for each standard or sample.
- C. Add 50 μ L of conjugate (red capped bottle) to each well using a repeater pipette with a 2.5 mL tip on setting 1.
- D. Add 50 μ L of antibody (black capped bottle) to each well using a repeater pipette with a 2.5 mL tip on setting 1. Mix gently by shaking the plate manually and incubate for 5 min at room temperature (20 - 25 °C / 68 - 77 °F).
- E. Dump the liquid out of the wells into a sink. Tap the micro well holder upside down onto a clean filter towel (three times in a row) to remove all remaining liquid from the wells.
- F. Using a wash bottle fill the wells with the Wash Solution in such a way so that the wells do not overflow with Wash Solution.

- G. Empty the wells again and remove all remaining liquid. Repeat the washing step two more times.
- H. Add 100 μ L of substrate/chromogen (brown capped bottle) to each well using a repeater pipette with a 5.0 mL tip on setting 1. Mix gently by shaking the plate manually and incubate for 3 minutes at room temperature (20 - 25 °C / 68 - 77 °F) **in the dark** (covered with paper to protect from light).
- J. After the 3-minutes incubation, uncover the wells and add 100 μ L of stop solution (yellow capped bottle) to each well using a repeater pipette with a 5.0 mL tip on setting 1. Mix gently by sliding the plate back and forth on a flat surface for 10-15 seconds.
- K. Measure the absorbance at 450 nm using the Awareness Technology Stat-Fax 303 Plus. Read within 10 minutes after addition of stop solution.

5. Reading the Results

- A. Using the Stat-Fax 303 Plus Microwell Reader:
 - 1) Turn on the reader with the switch on the back.
 - 2) Press "Menu" and "99", then Enter, to see a list of available tests.
 - 3) Enter the number for "Aflatoxin ECO" from the printout and press Enter.
 - 4) Place the first (or only) well strip in the far right column of the carrier with the first standard at the top.
 - 5) Align the carrier to the far left for strip 1, then press Enter. The carrier will move the strip inside the reader to be read.
 - 6) After reading of the first (or only) strip is complete, the display will read: "Accept curve Y/N."
 - a. An r-value of ≥ 0.980 is required for acceptable results. Proceed only if this condition is met. If the r-value is not acceptable, stop here and repeat the test from the beginning.
 - b. If you are only running one strip, the test is now complete. Press the clear button twice to return to the start menu.
 - c. If you have an additional strip(s) to run, manually move the carrier to the right so that the second strip (center of carrier tray) or third strip (left

side of carrier tray) of wells is aligned with the notch in the center. Press Enter to begin reading the aligned strip.

- d. Once the last strip is read, press the clear button twice to return to the start menu.

6. Reporting and Certifying Test Results

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

7. 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 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. Avoid exposure to direct light.
- 4) Do not analyze or read more than 24 wells at a time.
- 5) Follow proper pipetting techniques, including priming pipette tips by filling and dispensing solution once before use.
- 6) Use of incubation times other than those specified may give inaccurate results.
- 7) Bring kits to room temperature (20 - 25 °C / 68 - 77 °F) prior to use.
- 8) Return all reagents to 2 - 8 °C (35 - 46 °F) immediately after use.
- 9) Avoid prolonged storage of kits at ambient temperatures.
- 10) Treat all used liquids, including sample extracts and labware as if contaminated with aflatoxin. Gloves and other protective apparel should be worn at all times.
- 11) To avoid cross-contamination, use new pipette tips for each measurement.

8. Equipment and Supplies

A. Materials provided in test kits

- 1) One Microtiter plate with 48 wells (6 strips with removable wells) each coated with capture antibodies
- 2) Six white-capped bottles of 0, 3.0, 10.0, 30.0, 100.0, and 300 ppb aflatoxin standards
- 3) One red-capped bottle of 6 mL conjugate
- 4) One black-capped bottle of 6 mL antibody
- 5) One brown-capped bottle of 10 mL substrate/chromogen
- 6) One yellow-capped bottle of 14 mL stop solution
- 7) One packet of washing buffer (salt)
- 8) Two packets of ECO extractor buffer

B. Materials required but not provided

- 1) Awareness Technology Stat-Fax Model 303 reader with 450-nm filter
- 2) 50 μ L and 1000 μ L pipette and pipette tips
- 3) Graduated cylinders (plastic or glass): 250 mL and 1 L.
- 4) R-Biopharm filter syringes (Catalog No. X300)
- 5) Balance and Timer
- 6) Repeating pipette and 2.5/5.0 mL tips.
- 7) Paper towels, Kay dry paper or equivalent absorbent material.
- 8) Waterproof marker
- 9) Wash bottle
- 10) Deionized or distilled water and
- 11) Extraction material for FGIS extraction

- 12) Additional ECO extractor packets. These are required when performing the FGIS extraction or when more than 1200 mL of Extraction Buffer will be needed per kit. (Catalog No. 5005Z01-01)
- 13) Mini centrifuge (if testing corn/soy blend samples) (Catalog No. 1205R44)
- 14) 1.5mL micro centrifuge tubes (if testing corn/soy blend samples) (Catalog No. 9230-N15)

9. Revision History

Revision 1 (11/20/2017)

- Added corn gluten meal, corn/soy blend, corn starch, malted barley, milled rice, popcorn, sorghum, soybeans, and wheat as approved additional commodities. The test procedure for additional commodities was added.

Revision 0 (4/17/2017)