

Test Kit Instruction

May 11, 2015

Romer Labs FLUROQUANT AFLA IAC

FORWARD

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 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 PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@udsa.gov.

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1. GENERAL INFORMATION

The FluoroQuant Afla IAC (FQ AFLA IAC) test kit is a rapid, quantitative fluorometric test for detection of total aflatoxins (B1, B2, G1, and G2) using an immunoaffinity column for sample purification. Samples are extracted with methanol (MeOH)/water, then diluted and passed over an immunoaffinity column. The column is rinsed and then eluted with methanol. Developer solution is added to the eluent, which causes aflatoxin to fluoresce. The sample is then read in a calibrated FQ Reader fluorometer.

Approved Test Kit Information	
Test Kit Vendor:	<i>Romer Labs, Inc. (636) 583-8600</i>
Test Kit Name:	FluoroQuant Afla IAC Test Kit (FQ Afla IAC)
Product Number:	COKFA4010
Effective Date of Instructions:	05/11/2015
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, brown rice, corn flour, corn germ meal, corn meal, corn screenings, corn/soy blend, distillers dried grains with soluble (DDGS), flaking corn grits, milled rice, oats, popcorn, rough rice, rye, sorghum/milo, soybeans, and wheat.
Extraction method:	Blend 50-gram sample and 10 gram NaCl with 200 milliliters (mL) 80% MeOH/20% deionized or distilled water (v/v) for 1 minute.
Test Format:	Immunoaffinity cleanup with fluorometric detection
Detection Method:	FQ Reader (Product Model No. EQFFM3000)

2. PREPARATION OF TESTING MATERIALS AND EXTRACTION SOLVENT

All reagents and kit components must be at room temperature 20-24°C (68-75°F) before use.

a. Calibration of FQ Reader Fluorometer.

Calibration of FQ Reader Fluorometer should be completed at least once in every 24 hours as follows:

- (1) Turn on the FQ Reader fluorometer. The Test Kit Selection Screen will appear (no warm-up period is required). The test kit selection screen shows all test kits that are available for reading on the instrument.
- (2) Touch "FluoroQuant Afla IAC". The Commodity selection screen will appear automatically.
- (3) Ensure that the correct Optical Kit is installed in the FQ Reader for the test to be run. The FQ Module A should be installed for use with FluoroQuant Afla test kits.
- (4) Touch "IAC Method 1". The Calibration screen will appear automatically following the selection of the commodity to be analyzed.
- (5) Insert High calibrator when instructed; close the lid and touch "OK".
- (6) Insert Low calibrator when instructed; close the lid and touch "O"
- (7) The Home screen appears following a successful calibration. The FQ Reader will display the message "Calibration Complete! Ready to Begin Testing".
- (8) Insert Control (yellow) calibrator and close the lid when calibration is completed. Read the control calibrator and check the value against the range listed on the card. The value must be within the range listed on the card in the calibrator box to proceed with running samples.

b. Preparation of Developer Solution.

- (1) Transfer the contents of one developer concentrate vial into 25 mL of distilled or deionized water using the disposable transfer pipette. If the developer concentrate is not yellow / brown when pulled into the disposable pipette, discard and use another vial.
- (2) Rinse the empty vial three times with the prepared developer, returning the rinse to the 50mL amber bottle each time.
- (3) Developer must be made fresh a minimum of every 24 hours.
- (4) It is acceptable to make a "double batch" (2 vials to 50 mL water) if a large number of samples are anticipated.

c. Preparation of Extraction Solvent - 80% MeOH/20% deionized or distilled water (v/v).

Note: Extraction solution may be purchased pre-mixed. One can also prepared by following the procedure below.

- (1) Using a 1000 mL graduated cylinder, measure 800 mL of methanol (ACS grade) and place it into a clean carboy with spigot.
- (2) Using a 250 mL graduated cylinder, measure 200 mL deionized or distilled water and add into the methanol and shake until it is completely mixed.
- (3) Label the container with date of preparation and initials of technician who prepared the solvent.
- (4) Store this extraction solvent at room temperature in a tightly closed container until needed. This solvent is good for 12 months.

d. Preparation of Washing Solvent - 16% MeOH/84% Water (v/v).

- (1) Prepare washing solvent by adding 40 mL of the extraction solvent to 160 mL distilled or deionized water in the amber bottle fitted with 2 mL dispenser.
- (2) Other volumes may be made, as long as the ratio above is kept constant.
- (3) Washing solvent must be made fresh after 24 hours if it is stored in the 2 mL dispenser bottle.

3. SAMPLE PREPARATION AND EXTRACTION PROCEDURES

Extraction Procedure for Corn, Brown Rice, Corn Flour, Corn Germ Meal, Corn Meal, Corn Screenings, Corn/Soy Blend, DDGS, Flaking Corn Grits, Milled Rice, Oats, Popcorn, Rough Rice, Rye, Sorghum/Milo, Soybeans, Wheat.

- a. Weigh out 50 grams (+/- 0.1 g) of ground sample into a glass or polypropylene blender jar.
- b. Add 10 grams of sodium chloride (ACS Grade) to the sample in the jar.
- c. Add 200 mL of extraction solvent and close the jar securely using a blender blade assembly.
- d. Blend the sample on high for 1 minute.
- e. Filter the sample using coffee filter into a glass or polypropylene container and labeled with the sample identification (do not use polyethylene container or any other type of plastic).
- f. The filtered extract is now ready for assay.

4. TEST PROCEDURES

a. Analysis Procedure.

- (1) Pipet 500 microliter (μL) of sample extract into a clean cuvette and discard the pipette tip.
- (2) Using a 2 mL dispenser water bottle, add 2.0 mL of distilled or deionized water.
- (3) Pierce the top seal of an AflaStar IAC column with the pointed filter column and remove the bottom cap from the IAC column.
- (4) Place the entire 2.5 mL diluted sample into the barrel of the pointed filter column.
- (5) Insert syringe plunger and stopper assembly on top of the column.
- (6) Push the extract gradually through the column, at a rate of about 1-2 drops per second. (Alternate method: Use manual or powered vacuum column stand to push extract through column).
- (7) Place 2 mL of distilled or deionized water into the cuvette that was used for diluting the sample. Pour this water onto the pointed filter column and push completely through the IAC column at a rate of about 1-3 drops per second.
- (8) Remove the pointed filter column from the IAC column and discard the pointed filter column.
- (9) Rinse the IAC column with 2 mL of washing solvent.
- (10) Repeat this procedure one more time with 2 mL of washing solvent, so that a total of 4 mL of the washing solvent have been passed through the column.
- (11) Place a clean cuvette under the IAC column.
- (12) Add 2 mL of HPLC-grade methanol (“eluent” in kit) onto IAC column and elute the column very cautiously at a rate of about 1-2 drops per second. Higher rate of elution might affect the result.

b. Reading the Results.

- (1) The Home screen of the FQ Reader will display the test kit in use (center gray box at top), the commodity for which the instrument has been calibrated (green box at top right), and the results of any measurements that have been performed (up to the previous 20 measurements).
- (2) Touch "Sample ID" to name the next sample to be analyzed (optional). Using the keypad, enter the sample name into the name field. Touch "Save" to save the sample ID.
- (3) Add 1000 μ L of premade developer solution to the purified extract.
- (4) Cap the cuvette and vortex for 5 seconds.
- (5) Wipe the cuvette with lint-free paper or cloth (included with FQ Reader) and insert into the calibrated fluorometer.
- (6) The FQ Reader will begin a 30 seconds countdown when the lid is closed. If the countdown does not begin, touch the large green read button in the upper right hand corner of the screen to begin. (The first reading after calibration or after the fluorometer has awakened from sleep mode will not begin the countdown automatically.)
- (7) After a preprogrammed 30 second delay, the results will be displayed and printed in part per billion.

Note: Once the developer is added to the IAC purified sample, it must be mixed and analyzed immediately.

5. SUPPLEMENTAL ANALYSIS

Supplemental analysis (for corn only) is not required up to 1000 ppb quantitation of aflatoxins. However, any samples greater than 1000 ppb aflatoxins can be quantified by diluting the sample extract as described below.

- (1) Dilute the sample extract 1 to 10 with extraction solvent (e.g., add 1mL filtered extract into 9 mL extraction solvent. Dilution factor is 10.
- (2) Follow the analysis procedure for corn under the "TEST PROCEDURE" on page 7.
- (3) Multiply the final result by 10.
- (4) This gives an upper range of 10,000 ppb.

6. 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@udsa.gov).

7. STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions.

- (1) Store test kits at 18-25°C (64-77°F) when not in use, and do not use beyond the expiration date. The IAC columns should be stored refrigerated at 2-8°C. Do not freeze and do not leave in direct sunlight.
- (2) Cuvettes should remain sealed in original box until ready for use. The sealing film on top of the cuvettes should remain in place during storage to prevent dust from entering.

b. Precautions.

- (1) All reagents must be at room temperature before the assay is run.
- (2) Do not re-use IAC columns.
- (3) Consider all materials, containers and devices that are exposed to the sample to be contaminated with toxin. Wear protective gloves and safety glasses when using the kit.
- (4) The components in this test kit have been quality control tested as a standard batch unit. Do not mix components from different lot numbers.

8. EQUIPMENT AND SUPPLIES

a. Materials Supplied with the Kit.

- (1) 1 box containing 50, 12 x 75 mm cuvettes
- (2) 1 bag of cuvette caps; 2 bags of pipette tips
- (3) 1 bag of coffee filter paper; 1 bag of 8 disposable transfer pipets
- (4) 1 bag of 25 pointed filter columns; 2 bottles of eluent (HPLC Grade MeOH)
- (5) 2 bottles of NaCl (ACS Grade); 1 box of 8 vials of developer
- (6) 1 box of 25 Aflatoxin IAC columns

b. Materials Required but not Provided with Kit.

- (1) Extraction solvent
- (2) EQMMS2010: Romer Series II Mill or equivalent
- (3) EQOLE1010: Balance, 400 grams
- (4) EQOLE1020: Blender; EQOLE1025: Blender jars – 1/2 pint (8 oz.)
- (5) EQOLE1050: Graduated cylinder: to hold 100 mL
- (6) EQOLE1130: Extraction jars or containers
- (7) EQOLE1301: Timer; EQOLE1350: Funnel

c. Assay Procedure.

- (1) Distilled or deionized water
- (2) EQOLE1050: Fixed 1000 μ L pipette; EQOLE1160: Fixed 500 μ L pipette
- (3) EQOLE1210: Test tube rack; EQOLE1241: Repipettor
- (4) EQOLE1247: 2 mL repipettor for washing solvent
- (5) EQOLE1248: 2 mL repipettor for DI Water (with clear bottle; optional).
- (6) EQOLE1335: Vortex mixer; EQFFM3010: FQ Reader with printer
- (7) COKFA2040: High, Low, and Control fluorometer calibrator ampoules
- (8) COKFA1081: Syringe plunger and stopper assembly OR COKFA1085: Optional plunger stand

9. REVISION HISTORY

Revision 1 (05/11/2015)

- The test kit was approved for use in quantifying the total aflatoxins up to 1000 ppb by a single test.
- The additional test procedure was provided for testing samples containing more than 1000 ppb total aflatoxins.

Revision 0 (2/23/2015)