

U.S. DEPARTMENT OF AGRICULTURE
Grain Inspection, Packers and Stockyards Administration
Federal Grain Inspection Services

CERTIFICATE NO. FGIS 2003-102

CERTIFICATE OF CONFORMANCE

Quantitative Test Kit for Fumonisin for Corn, Corn Meal, Corn Soy Blend, Corn Germ Meal, Sorghum, Popcorn, and Flaking Corn Grits.

For: VICAM
Model: FumoniTest 200

Submitted by:
VICAM
313 Pleasant Street
Watertown, MA 02472
Tel: 1-800-338-4831
Contact: Nancy Zabe

Standard Features and Options

<u>Model</u>	<u>Sample Preparation</u>	<u>Extraction Method</u>	<u>Temperature Range</u>	<u>Fumonisin Range</u>
FumoniTest Test Kit	Clean, Ground Sample so that 95% passes through a 20 mesh sieve.	50 g sample Methanol/Water (80/20) (See Attachment 2)	18-30 °C (64-86 °F)	0.5 -5.0 ppm

Test kits must be operated according to the instructions in Attachment 2.

This quantitative fumonisin test kit underwent an initial Type Evaluation under the authority of Section 7B (c) of the United States Grain Standards Act, as amended, and was found to meet all design and test performance criteria as defined in "Design Criteria and Test Performance Specifications for Quantitative Fumonisin B1 (FB1), B2 (FB2), and B3 (FB3) Test Kits" April 2001 Revision.

Evaluation tests that were passed are summarized in Attachment 1. For further information, contact:

USDA, Grain Inspection, Packers and Stockyards Administration
Technical Services Division
Analytical, Reference, and Testing Services Branch
10383 N. Ambassador Drive
Kansas City, MO 64153-1394 Tel: (816) 891-0470

Director
Technical Service Division

Date: _____

Note: The mention of firm name or trade products does not imply that they are endorsed or recommended by the United States Department of Agriculture over other firms or similar products not mentioned.

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ATTACHMENT 1

Manufacturer: VICAM
313 Pleasant Street
Watertown, MA 02472

TEST 1: TIME REQUIRED FOR COMPLETION OF ANALYSIS

Data submitted by the manufacturer indicated that the analysis time required for one sample was less than the maximum limit of 30 minutes.

TEST 2: CAPABILITY FOR ANALYZING FOR FUMONISINS

Data submitted by the manufacturer showed that the test kit was capable of detecting fumonisins at 0.5 ppm.

TEST 3: IDENTIFIED COMMODITIES

Data submitted by the manufacturer showed that the test kit met the established accuracy and precision specifications at 0.5, 1.0, 2.5, and 5.0 ppm combined fumonisins for each of the commodities listed below:

CORN, CORN MEAL, CORN SOY BLEND, CORN GERM MEAL, SORGHUM, POPCORN, AND FLAKING CORN GRITS.

TEST 4: AVOIDANCE OF TOXIC OR HAZARDOUS SUBSTANCES

Data submitted by the manufacturer showed that this test kit meets safety criteria.

TEST 5: ACCURACY AND PRECISION OF TEST KIT WITH CORN SAMPLES

Data submitted by test kit manufacturer showed that this test kit met all of the accuracy and precision specifications for ground corn spiked at 0.5, 1.0, 2.5, and 5.0 ppm. Personnel from the Analytical, Reference, and Testing Services Branch (ARTS), Technical Services Division, Grain Inspection, Packers and Stockyards Administration (GIPSA) verified performance at 1 and 4 ppm.

TEST 6: ACCURACY OF TEST KIT ON NATURALLY CONTAMINATED CORN SAMPLES

Data submitted by test kit manufacturer on two naturally contaminated corn samples containing approximately 1 and 4 ppm fumonisin, met the performance criteria when test kit results were compared with results from a standard reference method. GIPSA personnel using a naturally contaminated corn sample containing approximately 2.0 ppm verified these data.

TEST 7: LIMIT OF DETECTION OF TEST KIT FOR EACH COMMODITY TESTED

Data supplied by the manufacturer showed that this test kit had a limit of detection of less than or equal to 0.5 ppm fumonisin for each of the commodities listed under Test 3.

TEST 8: SENSITIVITY OF ELECTRONIC EQUIPMENT TO ELECTROMAGNETIC FIELDS

The BBI Source Scientific Model No. VICAM4 and BBI Source Scientific MF-2000 Model No. 9906-1 fluorimeters the manufacturer offered with this test kit meets all of the electromagnetic field sensitivity specifications required by GIPSA.

TEST 9: TEMPERATURE SENSITIVITY

Data submitted by the manufacturer showed that this test kit gave results within acceptable limits at temperatures ranging from 18° to 30° C.

TEST 10: STABILITY

Data submitted by the manufacturer supported storage and stability claims.

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ATTACHMENT 2

Manufacturer: VICAM
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Calibration Settings: use FumoniTest™ calibration standards

<u>Instrument</u>	<u>Green</u>	<u>Red</u>	<u>Yellow</u>
Vicam Series 4	-0.50	6.0	2.8 ± 0.3
MF-2000™	Overlay FumoniTest™		2.4 - 3.1

Setup:

1. Calibrate fluorometer.
2. Prepare methanol: water (80:20) solution every week or as needed.
3. Prepare 1X PBS every week or as needed. (10X concentrate Vicam part #G1113: dilute 100 mL 10X concentrate with 900 ml purified water)
4. Prepare 1X 0.1% Tween-20/2.5% PEG/PBS Wash Buffer every week or as needed. (5X concentrate Vicam part # G5014: dilute 200mL 5X concentrate with 800mL purified water)
5. Prepare developer A and B mixture every day (Add 20µl developer B, Vicam part # G5004 to 15mL bottle developer A, Vicam part # G5005).
6. Make sure that reagent blank (1 ml methanol + 1.0 ml Developer A and B mixture) reads 0 ppm on a calibrated fluorometer.
7. Make sure that 2 ml PBS in a cuvette reads 0 ppm on a calibrated fluorometer.

Sample Extraction:

1. Weigh 50g ground sample with 5g salt and place in blender jar.
2. Add to jar 100 ml methanol:water (80:20).
3. Cover blender jar and blend at high speed for 1 minute.
4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean container.

Extract Dilution

1. Pipet or pour 10.0 ml filtered extract into a clean vessel.
2. Dilute extract with 40 ml of **0.1% Tween-20/2.5%PEG/PBS** Wash Buffer. Mix well.
3. Filter dilute extract through 1.5 µm microfibre filter (Vicam part # 31955) into a clean vessel or directly into glass syringe barrel using markings on side of barrel to measure 10 ml.

Column Chromatography

1. Pass 10 ml diluted extract (10ml = 1g sample equivalent) completely through **FumoniTest 200™** immunoaffinity column at a rate of about 1-1.5 drops/second until air comes through column.
2. Superwash* the column with 10 ml of **0.1% Tween-20/2.5%PEG/PBS** Wash Buffer through the column at a rate of 1-2 drops/second.

*Superwash: take column off the glass syringe barrel and put 1 ml of the wash solution directly into the FumoniTest 200™ Column, then attach column to the syringe barrel and fill syringe barrel with 10 ml of wash buffer.

3. Superwash* the column on **the second clean** syringe barrel with 10 ml of PBS (**NOT 0.1% Tween-20/2.5%PEG/PBS**) through the column at a rate of 1-2 drops/second until air comes through column.

4. Elute affinity column by passing 1.0 ml HPLC grade methanol through column at a rate of 1 drop/second and collect all of the sample eluate (1.0 ml) in a glass cuvette.
5. Add 1.0 ml of Developer A and B mixture to cuvette. Mix well and place cuvette in a calibrated fluorometer. Read fumonisin concentration after 240 seconds.

Assay range: 0.5 - 5 ppm

Limit of detection: 0.50 ppm

Materials required:

1. FumoniTest™ affinity column (Vicam part # G1029 = 25 per box)
2. Glass cuvette (Vicam part # 34000)
3. HPLC grade methanol
4. 1.5 µm microfibre filter paper - 11 cm (Vicam part # 31955)
5. Distilled, reverse osmosis, or deionized water
6. Phosphate buffered saline (PBS) (10X concentrate, Vicam part # G1113)
7. 0.1% Tween 20/2.5% PEG/PBS Wash Buffer (5X concentrate, Vicam part # G5014)
8. FumoniTest™ calibration standards (Vicam part # 33060)
9. Commercial blender with stainless steel container (Vicam part # 20200)
10. Micro-pipettor, 1ml (Vicam part # G4033)
11. Micro-pipettor, 20µl (Vicam part # G4031)
12. Micro-pipet tips, 50 µl (for 20 µl pipettor, 96 per box) (Vicam part # 20658)
13. Micro-pipet tips, 1 mL (96 per box) (Vicam part # 20656)
14. FumoniTest Developer A-fluorometer, 15mL (Vicam part # G5005)
15. FumoniTest Developer B (Vicam part # G5004)

Notes:

1. Always use good, clean equipment and reagents (HPLC grade methanol and distilled, reverse osmosis or deionized water). Check reagents for background fluorescence. Reagent blank (1 .0 mL methanol + 1 .0 mL Developer A and B mixture) should read 0 ppm on a calibrated fluorometer. If reagent blank reads high, read 2mL methanol in a cuvette and 2mL Developer A and B mixture in a cuvette separately. Replace reagent giving a high fluorescence reading. Cuvettes not purchased from Vicam may give background fluorescence.
2. FumoniTest developers will react with any protein . Keep developer solutions, column eluate and pipet tips used to measure elution solution and developers free from protein (i.e. finger prints).
3. Perform test from beginning to end without interruptions.
4. Use clean cuvettes and avoid contamination of eluate solution in cuvette. Wipe outside of cuvette with Kim wipe and make sure there are no particles inside cuvette before taking fluorometric readings.
5. Use only equipment specified by Vicam. Avoid contact of any test reagents or solutions (such as methanol, water, extract, column eluate or eluting solution) with rubber or soft flexible plastic. These materials may leach contaminating fluorescent materials into the sample.
6. Protect calibration standards from light and replace every year.
7. **Store developers and columns in refrigerator.** Bring to room temperature before using.
8. Developers A and B should be stored refrigerated until mixing A and B. Developer A and B mixture can be kept at room temperature. Mix new developer every two days. **Do not store developer A and B mixture in a 50mL bottle dispenser as used for AflaTest developer.** Store in developer A bottle and keep tightly capped when not in use. **Dispense using 1mL Micro-pipettor with clean pipet tips.**

Technical assistance:

For assistance please contact Vicam Technical Services by phone at 800-338-4381 (in the United States, Canada, Mexico and outside Massachusetts) or at 617-926-7045 (outside the United States and in Massachusetts) or by fax at 617-923-8055 or e-mail to techservice@vicam.com.

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ATTACHMENT 3

Manufacturer: VICAM
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Manufacturer's Notification Responsibilities

Manufacturers with test kits on the list of equipment approved for official inspection must notify the Director, Technical Services Division, USDA-GIPSA, 10383 N. Ambassador Drive, Kansas City, Missouri 64153-1394, in writing when any changes or alteration are made to the approved test kit, any reagents or equipment used in the test kit, or to any part of the analytical method. Failure to notify GIPSA of these changes will serve as grounds for immediate removal of the test kit from the approved list. Changes in packaging or other marketing information about the test kit are exempt from this requirement.

If the manufacturer wishes for an altered test kit to remain on the approved list without having to repeat all of the tests listed under Performance Specifications, she/he must submit a testing waiver request. A complete explanation of the change(s) made and how test results are affected must accompany this waiver. Data must be submitted which support the claim that the test kit still meets all Performance Standards that were potentially affected by the change that was made.

If a test waiver is not submitted, the test kit will be removed from the approved list until retesting has confirmed compliance with ALL listed Performance Standards.

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ATTACHMENT 4

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PROTOCOL & NOTIFICATION AGREEMENT STATEMENT

This is to certify that I am an Official representative of the _____ and I fully understand the conditions which GIPSA will use to determine if our quantitative Fumonisin test kit marketed under the trade name _____ will be given a Certificate of Conformance for use in the official inspection system. I approve of these conditions and I also agree to abide by the Manufacturer's Notification Responsibilities given in this document.

Name

Date

Title