

CHAPTER 6

DIACHEMIX® AFLATOXIN FPA TEST KIT

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6.1 GENERAL INFORMATION

The DIACHEMIX® AFLATOXIN FPA test kit (Product #61545) uses fluorescence polarization assay technology that provides qualitative (equal to or less than 20 ppb) results in CORN. Fluorescence polarization assays were first commercialized in the 1970's and is based on measuring the polarization of light caused by changes in molecular size as a result of antigen-antibody reactions. This test kit uses a Sentry™ 100 reader (field portable) that displays results as a (millipee) mp value. (The mp value refers to the polarization measured by the reader and is pronounced “millipee”)

6.2 TESTING AREA

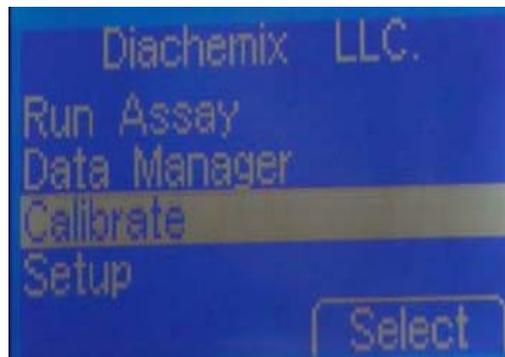
The extraction solution and other materials used in the DIACHEMIX® AFLATOXIN FPA test kit necessitates the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory ONLY. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this handbook to ensure a safe and efficient work environment.

6.3 SENTRY™ 100 SAMPLE READER CALIBRATION

The SENTRY™ 100 must be calibrated before its first use, every four to six months, when there is evidence of a performance problem. The SENTRY™ 100 is calibrated using the Calibration Kit provided with the SENTRY™ 100.

a. Turn on the SENTRY™ 100.

Wait until the “Diachemix LLC” screen is displayed. Use the up or down arrow to highlight the ‘Calibrate’ line. Press the button associated with the ‘Select’ command on the screen to move to the ‘Calibrate’ screen.



b. Blank Sample Intensity.

- (1) Use the up or down arrow if needed to highlight the 'Blank Int.' line.



- (2) Pipette 1 ml of the Blank Standard sample (buffer) into a clean 10 x75 mm glass borosilicate tube and label the tube. Insert the tube containing the Blank Standard sample in the chamber and close the top of the chamber.
- (3) Read the buffer blank by pressing the button associated with the 'Execute' or 'Read' command on the screen. 'Please Wait' will appear on the screen.
- (4) Use the up arrow to again highlight the 'Blank Int.' line.
- (5) Perform another read by again pressing the button associated with the 'Read' command on the screen again. 'Please Wait' will appear on the screen.
- (6) Repeat steps 4 and 5 twice, or as needed to assure a stable read. A stable read would be a series of consistent results (for example, 660...590...620...630). An unstable read would be a series with a substantial variance in results (for example, 660...590...79,000).

c. Low Polarization Standard.

- (1) Use the up or down arrow if needed to highlight the 'Sample Int.' line.
- (2) Pipette 1 ml of Low Polarization Standard into a clean 10 x75 mm glass borosilicate tube and label the tube. Insert the tube containing the Low Polarization Standard sample in the chamber and close the top of the chamber.

- (3) Read the Low Polarization Standard by pressing the button associated with the 'Read' command on the screen. 'Please Wait' will appear on the screen.
- (4) Record the value displayed on the 'Sample mP' line on a piece of paper.
- (5) Leave the Low Polarization Standard sample in the chamber with the top of the chamber closed.
- (6) Press the button associated with the 'Read' command on the screen and then record the resulting 'Sample mP' value. Repeat this step eight more times.
- (7) Average the resulting 'Sample mP' values from the last five of the ten reads. (Add the last five values together and divide by five.)
- (8) The average for the last five 'Sample mP' values should be between 22 to 28 mP (25 ± 3 mP).
- (9) If the average for the last five 'Sample mP' values is not between 22 to 28 mP, use the up or down arrow keys to highlight the 'G Factor' line on the screen.
- (10) Press the button associated with the 'Edit' command on the screen. Use the numeric keys to change the G Factor value displayed on the screen. The G Factor (Gain Factor) assists in calibrating the internal optics.
- (11) Every one one-thousand of a point change (0.001) in the G Factor value inversely adjusts the instrument's milliP values by 1 mP. By raising the G Factor value one one-thousand of a point (0.001) the instrument's milliP values are lowered by 1 mP. By lowering the G Factor value one one-thousand of a point (0.001) the instrument's milliP values are raised by 1 mP. Please note that the instrument automatically inserts a decimal point when all four numbers are input (for example, entering 1 0 0 2 and pressing the button associated with the 'Edit' command on the screen inputs the value '1.002').
- (12) Press the button associated with the 'Done' command on the screen to accept the edited G Factor.

- (13) Repeat steps 1 through 13 until the average for the last five 'Sample mP' values is between 22 to 28 mP (25 ± 3 mP).

d. High Polarization Sample Intensity.

- (1) Remain in the screen titled 'Calibrate', use the up or down arrow if needed to highlight the 'Sample Int.' line.
- (2) Pipette 1 ml of High Polarization Standard into a clean 10 x75 mm glass borosilicate tube and label the tube. Insert the tube containing the High Polarization Standard sample in the chamber and close the top of the chamber.
- (3) Read the High Polarization Standard by pressing the button associated with the 'Read' command on the screen. 'Please Wait' will appear on the screen.
- (4) Record the value displayed on the 'Sample mP' line on a piece of paper.
- (5) Leave the High Polarization Standard sample in the chamber with the top of the chamber closed.
- (6) Press the button associated with the 'Read' command on the screen, and then record the resulting 'Sample mP' value. Repeat this step eight more times.
- (7) Average the resulting 'Sample mP' values from the last five of the ten reads. (Add the last five values together and divide by five.) The average for the last five 'Sample mP' values should be greater than 360 mP. If the values are greater than 360 mP, the instrument has passed this quality control check. Please skip to Step 9.
- (8) The instrument has failed an important quality control check if the average for the last five 'Sample mP' values is not greater than 360 mP. If this failure does occur, repeat the High Polarization Standard calibration protocol (Steps 1 through 7 above). If the instrument fails again, then repeat the full Calibration of the Sentry™ 100. If the High Polarization Standard fails again during the full calibration process, contact Diachemix® Technical Assistance and provide a description of the problem, including the 'Sample mP' values from the Calibration process.
- (9) Carefully pour the Buffer, Low Polarization Standard and High Polarization Standard from each sample tube back into its appropriate bottle.

- (10) Press the button associated with the 'Back' command to return to the main screen.

6.4 PREPARATION OF TESTING MATERIALS

a. Reagents.

Store all FPA reagents in a refrigerator (36°- 45°F) and bring to room temperature for use.

Use all reagents listed below carefully to prevent contamination:

- (1) AFLA Antibody Solution
- (2) AFLA Kit Control
- (3) AFLA-FP-Tracer

b. Consumables.

Use clean glassware for each sample to avoid contamination. Do not use scratched or defective test tubes.

Do not handle the lower portion of the glass test tube. Fingerprints can distort the FP value.

c. Sentry™ 100 Sample Reader Startup.

- (1) Ensure that the reader is calibrated.
- (2) Turn on the Sentry™100 reader and wait for the screen to display 'Run Assay'.
- (3) Press the 'Selec' button.
- (4) Press the 'Down' arrow once.
- (5) Press the 'Forward' arrow once.
- (6) Advance to the next screen by pressing the 'Down' arrow repeatedly until 'Run Assay' is highlighted.



- (7) Press the 'Select' button to advance to the 'Run Assay Batch' screen.
- (8) Keep reading chamber lid closed unless inserting or removing a tube.

6.5 EXTRACTION PROCEDURES

- a. Transfer 50 grams of ground sample into a clean extraction container.
- b. Add 250 ml of the 70 percent methanol / 30 percent distilled or deionized water.
- c. Blend for 1 minute or shake for 2 minutes, and allow sample to settle 1 minute to obtain clarified sample extract. Particulates may distort the FP value.

NOTE: If particles are present after settling, filter or centrifuge to clarify sample extract. **To Filter:** filter the extract through Whatman 2V (or equivalent) filter paper into a labeled collection container. **To Centrifuge:** transfer 1.0-1.5 ml of sample extract to a labeled micro-centrifuge tube and centrifuge for 10 seconds. Clarified extract is now ready for testing.

6.6 TEST PROCEDURES

a. Sample Preparation.

- (1) Pipette 1 ml (1000 μ l) of Antibody Solution into a clean 10 x75 mm glass borosilicate tube.
- (2) Add 100 μ l of Control solution to the test tube, mark as "Control" and vortex.

NOTE: To vortex the sample: Place the test tube on a vortex mixer to mix for 5 – 10 seconds. Use caution to prevent spills or contamination.

- (3) Pipette 1 ml (1000 μ l) of Antibody Solution into a second clean 10 x75 mm glass borosilicate tube.
- (4) Add 100 μ l of Control solution to the second test tube, mark as "Control" and vortex.
- (5) Similarly prepare a sample tube for each sample by adding 1 ml of Antibody Solution into each individual test tube and 100 μ l of the sample extract to each test tube. Label each tube as needed and vortex.

b. Sample Analysis.

- (1) While in the 'Run Assay Batch' screen, open the reader chamber lid and insert the 1st Control tube into the instrument. Make sure the test tube is inserted to the bottom stop of the reading chamber and close the chamber lid.
- (2) Press the Down arrow once to select the 'Blank Int' line and then press the "Read" button.
- (3) After the instrument reading is complete remove the tube. The reader will automatically advance to the next blank intensity reading.
- (4) Continue by reading the 2nd Control tube.
- (5) Continue by reading the sample tubes one by one similar to the 1st Control tube.
- (6) After reading 'Blank Int' for all samples, add 100 µl of AFLA-FP Tracer to the first tube (Control). Vortex thoroughly and incubate for 2 minutes.
- (7) Go to the first reading by entering the number "1" on the reader screen. Place the Control tube back in the reader.
- (8) Press the 'Down' arrow to display 'Sample Int.' and press the 'Read' button. The instrument will display the mP value briefly and it can be retrieved after all the sample tubes have been read. Add 100 µl of AFLA-FP Tracer to the 2nd Control tube, vortex and incubate for 2 minutes.
- (9) Insert the tube back into the reader and press 'READ'.
- (10) Continue with each sample tube using the same steps as the control tubes until all samples have been completed.
- (11) To view the results again press the number "1" and then press the 'Forward' arrow to advance to the next sample. View each sample's results in the order run by pressing the Forward arrow key.
- (12) Take an average mP value of the two Control values (first two tubes) to set the cutoff value.

- (13) Record the mP value on the work record. For Aflatoxin testing, the cutoff mP value is the Control mP average value + 20. (Example: If the control mP average equals 150 then the cutoff equals 170). Therefore, all results that exceed the cutoff value are considered Negative and all results less than or equal to cutoff are considered Positive (Example: If the cutoff is 170 and the sample result is 230 then the sample is **Negative**. Sample results 170 or less would be certified as **Positive**).

6.7 QUALITY CONTROL PROCEDURES

Run the control and calculate the cutoff at the start of each day. Rerun the control and recalculate the cutoff every 100 samples or if the temperature in the lab changes by five degrees (5° F) or more.

6.8 REPORTING AND CERTIFYING TEST RESULTS

- a. Report Negative results on the pan ticket and inspection log as being equal to or less than 20 ppb (≤ 20 ppb). Report Positive results on the pan ticket and inspection log as being greater than 20 ppb (> 20 ppb).
- b. Certify Negative results as being equal to or less than 20 ppb (≤ 20 ppb). Certify Positive results on the pan ticket and inspection log as being greater than 20 ppb (> 20 ppb).
- c. Refer to the Certification section of the handbook for more detailed certification procedures.

6.9 CLEANING LABWARE

- a. Negative Tests (≤ 20 ppb).

- (1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, and then rinse with clean water before reusing.

- (2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, funnels, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, and then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour the liquid down the drain and place the solid material in a garbage bag and discard.

6.10 WASTE DISPOSAL

a. Negative Results (\leq 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of any remaining liquid filtrate in the chemical waste container.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the ground portion remaining in the filter paper must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, filter approximately 50 ml of bleach through the filter containing the ground portion and allow to drain. Discard the filter paper and its contents (ground portion) into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

6.11 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits. (Product # 61545).

- (1) AFLA-FP-Tracer
- (2) AFLA Kit Control
- (3) AFLA Antibody Solution

b. Materials required but not provided.

- (1) Sentry™ 100 Reader.
- (2) Sample grinder.
- (3) Balance.
- (4) Methanol.
- (5) Distilled/deionized water.
- (6) Sample extraction containers.
- (7) 1.0 ml pipettor and pipette tips.
- (8) 100 µl pipettor and pipette tips.
- (9) 500 ml graduated cylinder.
- (10) 10 x 75 mm test tubes.

c. Optional Equipment and Supplies.

- (1) Mini-centrifuge.
- (2) Whatman 2V filter paper or equivalent.
- (3) Filter funnel.
- (4) Vortex mixer.
- (5) 1.5 ml micro centrifuge tubes.

6.12 STORAGE CONDITIONS

a. Storage Conditions.

Test kits should be refrigerated between 36°- 45°F. Test kits can be stored refrigerated for up to one year. Check dates before use.

b. Precautions.

- (1) Do not use the test kits beyond the noted expiration date.
- (2) Prolonged exposure to high temperatures may adversely affect the test results. Keep all reagents away from heat or flames.