

CHAPTER 14

DIACHEMIX® DON FPA TEST KIT

<u>Section Number</u>	<u>Section</u>	<u>Page Number</u>
14.1	GENERAL INFORMATION	14-1
14.2	TESTING AREA	14-1
14.3	SENTRY® 100 READER CALIBRATION	14-1
14.4	PREPARATION OF TESTING MATERIALS	14-5
14.5	EXTRACTION PROCEDURES.....	14-6
14.6	TEST PROCEDURES.....	14-7
14.7	QUALITY CONTROL PROCEDURES	14-8
14.8	REPORTING AND CERTIFYING TEST RESULTS	14-8
14.9	CLEANING LABWARE	14-9
14.10	WASTE DISPOSAL	14-9
14.11	EQUIPMENT AND SUPPLIES	14-9
14.12	STORAGE CONDITIONS.....	14-10

14.1 GENERAL INFORMATION

The DIACHEMIX® DON FPA test kit uses fluorescence polarization assay technology that provides qualitative (equal to or less than 1 ppm) results in wheat. Fluorescence polarization assays were first commercialized in the 1970s, and are 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 mP value. (The mP value refers to the polarization measured by the reader and is pronounced “millipee”)

14.2 TESTING AREA

The extraction solution and other materials used in the DIACHEMIX® DON FPA test kit do not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this handbook to ensure a safe and efficient work environment.

14.3 SENTRY® 100 SAMPLE READER CALIBRATION

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

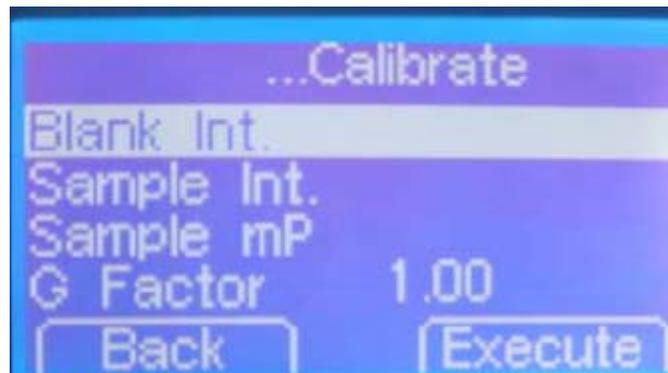
- a. Turn on the SENTRY®.

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) Pour at least 1 ml of the Blank Standard sample (buffer) into a clean 10x75 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) Pour at least 1 ml of Low Polarization Standard into a clean 10x75 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.

- (11) 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.
- (12) 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').
- (13) Press the button associated with the 'Done' command on the screen to accept the edited G Factor.
- (14) 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) Pour at least 1 ml of High Polarization Standard into a clean 10x75 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, 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.

14.4 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) DON Antibody Solution
- (2) DON Kit Control
- (3) DON-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 **Select** 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 the '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.

14.5 EXTRACTION PROCEDURES

- a. Transfer 50 grams of ground sample into a clean extraction container.
- b. Add 500 ml of the distilled water.
- c. Blend for 1 minute or shake for 2 minutes. 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.

14.6 TEST PROCEDURES

a. Sample Preparation.

- (1) Pipet 1 ml (1000 μ l) of Antibody Solution into a clean 10x75 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) 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 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 Read.
- (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 sample tubes one by one similar to the Control tube.
- (5) After reading ‘Blank Int’ for all samples, add 100 μ l of DON-FP Tracer to the first tube (Control). Vortex thoroughly and incubate for 2 minutes.

- (6) Go to the first reading by entering the number “1” on the reader screen. Place the Control tube back in the reader.
- (7) 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 DON-FP Tracer to the next tube, vortex and incubate for 2 minutes.
- (8) Insert the tube back into the reader and press READ.
- (9) Continue with next tube using the same steps as the control tube until all samples have been completed.
- (10) 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.
- (11) Record the mP value on the work record. For DON testing, the cutoff mP value is the Control mP value + 35. (Example: If the control mP equals 150 then the cutoff equals 185). 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 185 and the sample result is 230 then the sample is Negative).

14.7 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 or more.

14.8 REPORTING AND CERTIFYING TEST RESULTS

- a. Report Negative results on the pan ticket and inspection log as being equal to or less than 1.0 ppm (≤ 1.0 ppm). Report Positive results on the pan ticket and inspection log as being greater than 1.0 ppm (>1.0 ppm).
- b. Certify Negative results as being equal to or less than 1.0 ppm (≤ 1.0 ppm). Certify Positive results on the pan ticket and inspection log as being greater than 1.0 ppm (>1.0 ppm).
- c. Refer to the Certification section of the handbook for more detailed certification procedures.

14.9 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

14.10 WASTE DISPOSAL

After the test has been completed, the remaining sample extracts and sample solutions may be poured down the drain. Discard solid material in the trash can for routine disposal.

14.11 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits. (Product # 61530).

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



b. Materials Required but not Provided.

- (1) Sentry® 100 Reader.
- (2) Sample grinder.
- (3) Balance.
- (4) Distilled water.
- (5) Sample extraction containers.
- (6) 1.0 ml pipettor and pipette tips.
- (7) 100 µl pipettor and pipette tips.

- (8) 500 ml graduated cylinder.
- (9) 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.

14.12 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.