U.S. DEPARTMENT OF AGRICULTURE
GRAIN INSPECTION, PACKERS AND STOCKYARDS ADMINISTRATION
FEDERAL GRAIN INSPECTION SERVICE
STOP 3630
WASHINGTON, D.C. 20090-3630

CHAPTER 9

DIAGNOSTIX - EZ-QUANT DON PLATE TEST KITS

9.1 GENERAL INFORMATION

FGIS has approved two separate Diagnositx test kits for quantitative DON testing. The EZ-Quant DON Plate test kit (part number 600312) is used for testing Wheat, Barley, Malted Barley, and Corn from 0.5 to 5 ppm. The EZ-Quant 0.5 PPM DON Plate test kit (part number 600313) is used for testing barley and malted barley from 0.5 to 2.5 ppm.

9.2 TESTING AREA

The extraction solution and other materials used in the Diagnostix EZ-Quant DON Plate test kits (part numbers 600312 and 600313) 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 the handbook to ensure a safe and efficient work environment.

9.3 EXTRACTION PROCEDURES

The extraction procedures listed below are applicable for the EZ-Quant DON test kit (part number 600312) and the EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

a. Place 50 grams of ground sample into a clean plastic bag.

b. Add 250 ml of distilled or deionzed water and seal/close the bag securely to prevent spillage.

c. Shake vigorously (by hand or mechanically) for three minutes.

d. Let the extract sit for 2-3 minutes to allow for some settling of the slurry.

e. Filter a minimum of 15 ml of the extract through Whatman #4 filters (or equivalent) into a clean container that is labeled with sample ID number.

9.4 PREPARATION OF SOLUTIONS

The procedures listed below are applicable for the EZ-Quant DON test kit (part number 600312) and the EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

a. To prepare the Wash Solution, transfer the contents of the Wash Concentrate vial to a 500-ml container and add 475 ml of distilled or deionized water.

b. Swirl to mix.

9.5 TEST PROCEDURES

a. EZ-Quant DON Plate Test Kit (Part Number 600312) - Testing Wheat, Barley, Malted Barley, and Corn from 0.5 to 5 ppm.

(1) Allow reagents, antibody-coated wells, mixing wells, and sample extracts to reach room temperature prior to running the test (approximately one-hour).

(2) Place the appropriate number of red mixing wells and clear test wells into a microwell holder. Do not run more than two strips at a time. Be sure to re-seal unused wells in the zip-lock bag with desiccant.

NOTE: The maximum number of test samples that can be run at one time is 19. Using two strips of 12 wells, designate 5 wells for the calibrators and the remainder of the wells for test samples.

(3) Dispense 100 µl of Enzyme Conjugate into each red mixing well.

(4) Dispense 100 µl of the appropriate calibrators and samples into the appropriate red mixing wells as illustrated below.

NOTE: Use a clean pipette for each addition.

Wells	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
First Strip	**0**	**0.5**	**1.0**	**2.0**	**6.0**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
Second Strip	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**

Key : S= Sample, 0= 0 ppm calibrator, 0.5= 0.5 ppm calibrator, etc.

(5) Using a 12-channel pipette, mix the contents of the wells by repeatedly filling and emptying the tips 5 times in the mixing wells.

(6) Using a 12-channel pipette, transfer 100 µl of the reaction mixture into the corresponding clear test wells and tap the holder several times to mix. Discard the red mixing wells into an appropriate waste container.

(7) Incubate the clear test wells for 10 minutes.

(8) Dump the contents of the wells into an appropriate waste container and carefully shake out any residue solution.

(9) Using a wash bottle filled with wash solution, fill each well to overflowing then dump the contents and shake out any residue solution. Repeat four times for a total of 5 washes.

(10) After the final wash, tap the strips repeatedly onto absorbent paper to remove excess wash. After tapping, check for large bubbles, which should be burst with a clean pipette tip and tapped out again.

(11) Using a 12-channel pipette, dispense 100 µl of Substrate into each well and tap the holder several times to mix the contents.

(12) Using a paper towel to block out the light reflectance, cover the wells and incubate for 5 minutes.

(13) Using a 12-channel pipette, dispense 100 µl of Stop Solution into each well.

(14) Within 10 minutes, read and record the absorbance of each well at 450 nm using the Bio-Tek EL 301™ Microwell Strip Reader equipped with a 450 nm filter.

b. <u>EZ-Quant 0.5 PPM DON Plate Test Kit (Part Number 600313) - Testing Barley and Malted Barley from 0.5 to 2.5 ppm</u>.

(1) Allow reagents, antibody-coated wells, mixing wells, and sample extracts to reach room temperature prior to running the test (approximately one-hour).

(2) Place the appropriate number of red mixing wells and clear test wells into a microwell holder. Do not run more than two strips at a time. Be sure to re-seal unused wells in the zip-lock bag with desiccant.

NOTE: The maximum number of test samples that can be run at one time is 19. Using two strips of 12 wells, designate 5 wells for the calibrators and the remainder of the wells for test samples.

(3) Dispense 100 µl of Enzyme Conjugate into each red mixing well.

(4) Dispense 100 µl of the appropriate calibrators and samples into the appropriate red mixing wells as illustrated below.

NOTE: Use a clean pipette for each addition.

Wells	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
First Strip	**0**	**0.2**	**0.5**	**1.0**	**2.5**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
Second Strip	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**

Key : S= Sample, 0= 0 ppm calibrator, 0.5= 0.5 ppm calibrator, etc.

(5) Using a 12-channel pipette, mix the contents of the wells by repeatedly filling and emptying the tips 5 times in the mixing wells.

(6) Using a 12-channel pipette, transfer 100 µl of the reaction mixture into the corresponding clear test wells and tap the holder several times to mix. Discard the red mixing wells into an appropriate waste container.

(7) Incubate the clear test wells for 10 minutes.

(8) Dump the contents of the wells into an appropriate waste container and carefully shake out any residue solution.

(9) Using a wash bottle filled with wash solution, fill each well to overflowing then dump the contents and shake out any residue solution. Repeat four times for a total of 5 washes.

(10) After the final wash, tap the strips repeatedly onto absorbent paper to remove excess wash. After tapping, check for large bubbles, which should be burst with a clean pipette tip and tapped out again.

(11) Using a 12-channel pipette, dispense 100 µl of Substrate into each well and tap the holder several times to mix the contents.

(12) Using a paper towel to block out the light reflectance, cover the wells and incubate for 5 minutes.

(13) Using a 12-channel pipette, dispense 100 µl of Stop Solution into each well.

(14) Within 10 minutes, read and record the absorbance of each well at 450 nm using the Bio-Tek EL 301™ Microwell Strip Reader equipped with a 450 nm filter.

c. Reading the Results.

The procedures listed below are applicable for the EZ-Quant DON test kit (part number 600312) and the EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

(1) Allow the EL 301 Microwell reader to warm up for a minimum of 15 minutes before using.

(2) Remove the sample carriage and hit "Enter."

(3) Insert W2 filter (405 nm) and hit "Enter."

(4) Insert W1 filter (450nm) and hit "Enter."

(5) Hit "Clear"and "Blank." This will cause the instrument to read air as the blank sample.

(6) Load the anti-body coated wells into the carriage so that the control labeled 0 (zero) is in position A1.

(7) Load the sample carriage into the well reader so that position A1 is under the reader.

(8) Hit "Read" and record the absorbance value obtained for A1 in the screen of the Microwell reader.

(9) Slide the carriage to position A2 and hit "Read." Record the absorbance value obtained for A2.

(10) Repeat step (9) until absorbance values have been obtained for all controls and samples.

(11) Use the Data Reduction software provided by Diagnostix to convert the absorbance values into concentration values.

d. Interpretation of Results - (Diagnostix Data Reduction Worksheet Program)

The procedures listed below are applicable for the EZ-Quant DON test kit (part number 600312) and the EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

To generate a calibration curve and convert sample absorbances into DON concentrations, use the Diagnostix Data Reduction Worksheet program provided by Diagnostix. A computer equipped with the Microsoft Excel program is required to run the EZ-Quant DON Plate Kit Data Reduction Worksheet.

(1) Place the diskette provided into the appropriate drive. Open the Microsoft Excel program, access the diskette, and click on **"Data Reduction Worksheet."**

(2) Complete the following information on the worksheet:

(a) Operator (b) Date

(c) Assay ID (d) Kit Lot #

(3) Under the **"Absorbance"** column in **"Section 1 Calibrator Curve"**, input the Standard absorbance values to the appropriate designated rows.

After the absorbance values are added, the worksheet automatically calculates B/B_o, then plots a calibration curve in the graph field (Log (standard concentration) on the abscissa, Logit B/B_o on the ordinate). In addition R^2, Slope and Intercept values are calculated.

(4) Under the **"Abs."** column in **"Section II - Sample Calculations"**, input the absorbance value for each sample tested (the worksheet provides enough space for 19 samples). A **"Sample ID"** column is provided to the left of the "Abs." column to identify each sample.

After the sample absorbance values are added, the worksheet automatically calculates the concentration in parts per million (ppm) and presents them in the "ppm" column.

(5) Repeat steps (2), (3), and (4) for each new set of data.

e. Validation Requirements - EZ-Quant DON Plate Test Kit (Part Number 600312) Testing Wheat, Barley, Malted Barley, and Corn from 0.5 to 5 ppm.

For a given test to be considered valid, the 0 ppm standard should have an absorbance greater than 0.600 and the calibration curve must meet the following two criteria:

(1) The R^2 value should be between -0.992 and -1.000.

(2) The B/B_o values for the standards should fall within the following range.

Standard	Acceptable Range
0.5 ppm	0.67 - 0.80
1.0 ppm	0.53 - 0.65
2.00 ppm	0.38 - 0.49
6.00 ppm	0.18 - 0.28

NOTE: If the R^2 value on the B/B_o values for the standards fall outside the specified range call Diagnostix at 1-800-282-4075 for assistance.

f. Validation Requirements - EZ-Quant DON Plate Test Kit (Part Number 600313) Testing Barley and Malted Barley from 0.5 to 2.5 ppm

For a given test to be considered valid, the 0 ppm standard should have an absorbance value between 0.6 and 2.5 and the calibration curve must meet the following two criteria:

(1) The R^2 value should be between -0.992 and -1.000.

(2) The B/B_o values for the standards should fall within the following range

Standard	Acceptable Range
0.2 ppm	0.70 - 0.74
0.5 ppm	0.47 - 0.55
1.0 ppm	0.35 - 0.40
2.5 ppm	0.17 - 0.26

NOTE: If the R^2 value on the B/B_o values for the standards fall outside the specified range call Diagnostix at 1-800-282-4075 for assistance.

9.6 REPORTING AND CERTIFYING TEST RESULTS

a. EZ-Quant DON Plate Test Kit (Part Number 600312) - Testing Wheat, Barley, Malted Barley, and Corn from 0.5 to 5 ppm.

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

b. EZ-Quant DON Plate Test Kit (Part Number 600313) - Testing Barley and Malted Barley from 0.5 to 2.5 ppm

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 2.5 ppm. Results exceeding 2.5 ppm are reported as > 2.5 ppm

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 2.4 ppm are certified to the nearest whole ppm.

Test results that are equal to the conformance limit (2.5 ppm) are certified as being equal to 2.5ppm.

Test results over 2.5 ppm are certified as exceeding 2.5 ppm.

Refer to the Certification section of the handbook for more detailed certification procedures.

NOTE: A supplemental analysis (diluting the mixture) is not allowed to obtain results above the 2.5 ppm conformance limit. To obtain accurate results above the 2.5 ppm conformance limit the sample must be tested using a test kit with a conformance range between 0.5 - 5 ppm.

9.7 SUPPLEMENTAL ANALYSIS

A supplemental analysis is applicable only to the EZ-Quant DON Plate Test Kit (Part Number 600312) - Testing Wheat, Barley, Malted Barley, and Corn from 0.5 to 5 ppm.

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON result at 9.0 ppm and the conformance limit value is 5 ppm, in order to obtain a true value, dilute 5 ml of the original extract with 10 ml of the extraction solution (distilled/ deionized water). The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 3.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

The calculation is as follows:

$$\text{True DON Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result}$$

In this example: True DON Value = (15 ÷ 5) x 3.1 ppm
= 3 x 3.1 ppm = 9.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the controls provided with the kits.

9.8 CLEANING LABWARE

The procedures listed below are applicable for the EZ-Quant DON test kit (part number 600312) and the EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

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

9.9 WASTE DISPOSAL

The procedures listed below are applicable for the EZ-Quant DON test kit (part number 600312) and the EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

After the test has been completed, the remaining sample extract and sample solutions may be poured down the drain followed by copious amounts of water. Discard solid material in the trash can for routine disposal.

9.10 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits: EZ-Quant DON test kit (part number 600312)

(1) 96 antibody-coated clear microtiter wells and 96 red mixing microtiter wells.

(2) 5 vials, each containing 4 ml of DON calibrators corresponding to 0, 0.5, 1.0, 2.0, and 6 µg/ml (ppm) of DON. (Note: because of the 1:5 dilution of the grain sample in the extraction step, the calibrator actually contains 1/5 of the stated value. No further correction back to the concentration in the original grain sample is required).

(3) 1 Vial containing 12 ml of DON-HRP Enzyme Conjugate.

(4) 1 Vial containing 12 ml of Substrate.

(5) 1 Vial containing 12 ml of Stop Solution. (Caution! 1 N HCL Handle with care).

(6) 1 Vial containing 25 ml of 20X Wash Solution.

(7) Strip holder (provided separately by Diagnostix).

(8) Data reduction program (provided separately by Diagnostix).

b. Materials Supplied in Test Kits: EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

(1) 96 antibody-coated clear microtiter wells and 96 red mixing microtiter wells.

(2) 5 vials, each containing 4 ml of DON calibrators corresponding to 0, 0.2, 0.5, 1.0, and 2.5 µg/ml (ppm) of DON. (Note: because of the 1:5 dilution of the grain sample in the extraction step, the calibrator actually contains 1/5 of the stated value. No further correction back to the concentration in the original grain sample is required).

(3) 1 Vial containing 12 ml of DON-HRP Enzyme Conjugate.

(4) 1 Vial containing 12 ml of Substrate.

(5) 1 Vial containing 12 ml of Stop Solution. (Caution! 1 N HCL Handle with care).

(6) 1 Vial containing 25 ml of 20X Wash Solution.

(7) Strip holder (provided separately by Diagnostix).

(8) Data reduction program (provided separately by Diagnostix).

c. Materials Required but not provided in the EZ-Quant DON test kit (part number 600312) and the EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

(1) Laboratory quality distilled or deionized water.

(2) Graduated cylinder, 1000 ml.

(3) Sealable containers for sample extraction and extract collection.

(4) Filter paper, Whatman No. 4 or equivalent.

(5) Pipette capable of dispensing 100 µl.

(6) 12-Channel pipette capable of dispensing 100 µl per channel.

(7) Disposable tips compatible with pipettes.

(8) Paper towels or equivalent absorbent materials.

(9) Bio-Tek EL 301™ Microwell Strip Reader with 450 nm filter.

(10) Timer.

(11) Reagent reservoirs.

(12) Wash bottle.

(13) Balance.

(14) Sample Grinder.

9.11 STORAGE CONDITIONS

The storage conditions listed below are applicable for the EZ-Quant DON test kit (part number 600312) and the EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 36° F and 46° F.

On the day the kit will be used it is acceptable to store the kit at ambient temperature (62° F to 82° F).