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DON HANDBOOK
CHAPTER 20
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CHAPTER 20

ROMER AGRAQUANT® DON TEST METHOD

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20.1 GENERAL INFORMATION

The extraction solution and other materials used in the Romer AgraQuant test kit do not necessitate the use of a 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.

20.2 EXTRACTION PROCEDURES

- a. Barley, Malted Barley, Corn, Oats, Wheat, Wheat Flour, and Wheat Midds.
- (1) Place a sheet of filter paper (Whatman #1 folded or S&S 24-cm pleated or equivalent) into a clean funnel mounted over a 25 x 200 mm (diameter x length) test tube or collection beaker.
 - (2) Label the collection container with the sample identification.
 - (3) Thoroughly mix the ground sample and weigh a 50-gram portion.
 - (4) Place the ground 50-gram portion into an 18-ounce Whirlpack bag or similar type of sealable plastic bag.
 - (5) Add 250 ml of distilled or deionized water and shake (by hand or mechanically) vigorously for 3 minutes.
 - (6) Let material stand for 2 minutes to allow the sample to settle before filtering the extract.
 - (7) Filter the extract by pouring at least 15 ml(s) through Whatman # 1 filter or equivalent into a clean container and collect filtrate.
 - (8) Dilute the sample extract by adding 1 ml of sample extract with 3 ml of distilled or deionized water into a clean container.
 - (9) The sample is ready for testing without further preparation.

20.3 PREPARATION OF SOLUTIONS

a. Don Control Standards, Conjugate, Substrate, and Stop Solution.

- (1) Allow all reagents to reach room temperature 18-30°C (64-86°F) before use.
- (2) All reagents are pre-activated, and ready for use.
- (3) Store all reagents in the dark when in use.

b. Wash Solution.

Transfer the contents of Wash Solution Concentrate bottle to a 500 ml(s) plastic squeeze bottle and add 475 ml of distilled/deionized water. Swirl to mix and before to use.

20.4 TEST PROCEDURES

a. Testing Procedures.

Note: It is recommended that an 8-channel pipettor be used to perform the assay.

- (1) Remove the blue/green-bordered Dilution Strips and break off the number of wells needed (five wells for controls, one for each test sample) up to a maximum of:
 - (a) 16 wells (2 strips) when using a single channel pipettor.
 - (b) 24 wells (3 strips) when using an 8 channel multi pipettor).
- (2) Mark one end of blue/green dilution strips with a 0 (zero) for the blank and the other end with an S for samples so that you can identify the wells. Place the wells in the microwell holder.
- (3) Remove an equal number of antibody-coated wells. Mark one end of strip with a 0 (zero) for the blank and the other end with an S for samples and place the strip in the well holder with the 0 (zero) marked end on the left.
- (4) Return unused microwell strips to the foil pouch with the desiccant packet and reseal with tape.
- (5) Mix each reagent by swirling the reagent bottle prior to use.

- (6) Measure the required amount of Conjugate from the green-capped bottle (approximately 2 ml/strip) and place in a reagent boat. Using an 8-channel pipettor dispense 200 μ l of Conjugate into each dilution well. Discard the tips.
- (7) Remove the cap from the 0 ppm control bottle. Firmly place a new pipette tip on the 100 μ l pipettor and add 100 μ l from the 0 ppm control bottle to the first (labeled 0 (zero)) dilution well. Discard the tip and replace the cap on the control bottle.
- (8) Remove the cap from the 0.25 ppm control bottle. Firmly place a new pipette tip on the 100 μ l pipettor and add 100 μ l from the 0.25 ppm control bottle to the second mixing well. Discard the tip and replace the cap on the control bottle.
- (9) Repeat step (7) with the remaining control standards placing 100 μ l amounts of these standards in the third, fourth, and fifth wells, respectively. A new pipette tip should be used for each standard solution.
- (10) Firmly place a new pipette tip on the 100 μ l pipette and add 100 μ l from the sample collection tube of the first sample to the sixth well. Discard the tip.
- (11) Repeat step (9) for each sample, placing 100 μ l of extract from each sample in a different well. Use a new pipette tip for each sample solution.
- (12) Using an 8-channel pipettor with new tips, mix the wells by pipetting the liquid up and down in the tips three times. Transfer 100 μ l to the antibody wells.
- (13) **Do not agitate microwell holder (slide back and forth to mix) as it may cause well to well contamination.**
- (14) Set timer and incubate for **15 minutes**. Discard the dilution wells.
- (15) The initial reaction is now completed. Shake out the contents of antibody-coated wells.
- (16) Using prepared wash solution, fill each antibody-coated well and shake out contents into a waste container. Repeat four (4) more times for a total of five (5) washes.

- (17) After the fifth wash expel as much residual water as possible. Turn the wells upside down on a flat surface and vigorously tap microwell strip on several layers of paper towel. Dry the bottom of the microwells with a dry cloth or towel.
- (18) Measure the required amount of Substrate (approximately 1 ml/strip) into the reagent boat, and with new tips on the 8-channel pipettor, pipette 100 μ l of substrate into the microwell strips. Incubate for **5 minutes** at room temperature. Discard the tips.
- (19) Discard the remaining substrate and rinse the reagent boat with water.
- (20) Measure the required amount of Stop Solution (approximately 1 ml/strip) into the reagent boat. Using an 8-channel pipettor with new tips, transfer 100 μ l of Stop Solution to each microwell. The color should change from blue to yellow. Discard the tips.

b. Reading the Results using the StatFax Reader.

- (1) Turn on the StatFax Reader.
- (2) To print out a listing of all preprogrammed methods:
 - (a) Select Menu.
 - (b) The StatFax will prompt "Select Test"; key in 99 and press enter.
- (3) To recall a preprogrammed method:
 - (a) Select Menu.
 - (b) Enter corresponding method number from the list.
 - (c) Press Enter.
- (4) When prompted "Plot Curve Y/N". Select N.
- (5) When prompted "Stored Curve Y/N". Select N.
- (6) The StatFax is then ready for testing. The screen will prompt "Set Carrier to 1. Then press Enter."
- (7) When you are ready to read a microwell strip; set the carrier to 1 (which is the far left position on carrier) and press enter.

- (8) Upon completion of strip reading the StatFax reader will prompt "Plot Curve Y/N. This is optional, if you would like the curve to be plotted on the instrument printout Select Y; if not Select N.
- (9) The StatFax will prompt "Accept Curve Y/N"; Select Y. If additional microwell strips are to be read at this time.
- (10) Set carrier to 1, (move the strip carrier to the right for new strip) then press "Enter."
- (11) To end test and run a new set of microwells and new control standards; "Press the "Clear" button twice. This will exit out of the existing method and back to the "Main Menu" screen.
- (12) When ready to read a new set of microwell strips proceed with steps 2-10.

20.5 REPORTING AND CERTIFYING TEST RESULTS

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.

20.6 SUPPLEMENTAL ANALYSIS

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:

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

$$\begin{aligned} \text{In this example:} \quad \text{True DON Value} &= (15 \div 5) \times 3.1 \text{ ppm} \\ &= 3 \times 3.1 \text{ ppm} = 9.3 \text{ ppm} \end{aligned}$$

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

20.7 CLEANING LABWARE

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

20.8 WASTE DISPOSAL

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

20.9 EQUIPMENT AND SUPPLIES

a. Materials Provided in Test Kits:

- (1) 96 antibody-coated microwells (12 eight-well strips) in a microwell holder (sealed in a foil pouch).
- (2) 96 non-coated microwells (12 eight-well strips marked blue/green).
- (3) 5 vials of 1.5 each DON control standards (0, 0.25, 1.0, 2.0, and 5.0 ppm).
- (4) 1 bottle of 25 ml of DON conjugate solution (green-capped bottle).
- (5) 1 bottle of 15 ml of substrate solution (blue-capped bottle).
- (6) 1 bottle of 15 ml of stop solution (red-capped bottle).
- (7) 1 bottle of 25 ml of 20X wash solution concentrate (blue-capped bottle).

b. Materials Required but not Provided:

- (1) Mixing Bags - Whirlpack bags or similar type of sealable plastic bag.
- (2) Filter funnels.
- (3) Reagents boats.
- (4) Graduated Cylinder - 250 ml capacity.
- (5) Disposable plastic cups.
- (6) Filter paper - Whatman No. 1, or equivalent.
- (7) Timer - 15-minute capacity.
- (8) Markers - Sharpie or equal (permanent ink that will not wash off).
- (9) Absorbent material - Kim wipes or paper towels.
- (10) Wash Bottle 500 ml plastic squeeze bottle.

- (11) StatFax 303 Plus Microwell Strip Reader.
- (12) 8-Channel Pipettor.
- (13) Pipettor and Pipette Tips (100 µl)
- (14) Pipettor and Pipette Tips (1ml).
- (15) Deionized or distilled water.
- (16) Balance.
- (17) Sample Grinder.

20.10 STORAGE CONDITIONS AND PREC AUTIONS

a. Storage Conditions.

Test kits should be refrigerated between (36°- 46°F) when not in use.

b. Precautions.

- (1) Do not use the test kits beyond the noted expiration date.
- (2) Adhere to incubation times stated in the test procedures. Use of incubation times other than those specified may give inaccurate results.