

Program Notice

FGIS-PN-10-06

12/28/09

R-BIOPHARM, RIDASCREEN FAST, DON TEST METHOD

1. PURPOSE

The purpose of this program notice is to provide official instructions for the DON Handbook Chapter 22, Ridascreen Fast DON (vomitoxin) test method. The R Biopharm Inc., Ridascreen Fast DON Test Method product number R5901/R5902 has been reinstated/approved by the Grain Inspection, Packers and Stockyards Administration (GIPSA), Technical Services Division (TSD) for quantitative test analysis, DON test kit conformance limits 0.5 – 5 parts per million (ppm).

2. BACKGROUND

The Policies, Procedures, and Market Analysis Branch (PPMAB) and TSD have an ongoing evaluation of new and previously approved (re-certification) mycotoxin test methods. In an effort to offer TSD approved test methods in a timely manner, this program notice is issued prior to the release of the revised DON Handbook. The following test procedures are approved for use by GIPSA and official service providers.

3. TEST INSTRUCTIONS

a. General Information.

The R-Biopharm Ridascreen FAST DON test method (product numbers R5901/R5902), provides quantitative measurements between **0.5 – 5 ppm**. Accurate DON measurements above **5.4 ppm** can be obtained by performing a supplemental analysis. “See Section f of this program notice for specific procedures.”

This test method can also be used to provide qualitative testing (equal to or less than a specified threshold) for DON per applicant request.

The extraction solution, and other materials used with this test method do not necessitate the use of a separate FGIS-approved laboratory space. FGIS personnel may perform the testing in a FGIS approved laboratory or in an 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 DON Handbook to ensure a safe and efficient work environment.

Approved Test Kit Information						
Test Kit Vendor	R-Biopharm Inc. 1-877-789-3033					
Test Kit Name	Ridascreen FAST DON Test Method					
Product Number	Conformance Limit		Type of Service		Extraction Solution	Supplemental Analysis
	Min	Max	Quan	Qual		
R5901 (96 well kit) R5902 (48 well kit)	0.5 ppm	5 ppm	X		Distilled/ Deionized Water	Yes
Grain/Commodities Approved for						
Wheat, Barley, Malted Barley, Oats, and Corn.						

b. Extraction Procedures.

- (1) Place 50-grams of ground sample into a suitable container (e.g., plastic bag).
- (2) Add 250 milliliters (ml) of distilled/deionized water and seal/close container securely to prevent spillage.
- (3) Shake vigorously (by hand or mechanically) for 3 minutes.
- (4) Let the extract sit for 2-3 minutes to allow some settling of the slurry.
- (5) Filter the extract through Whatman #1 filter (or equivalent) into a clean container that is labeled with the sample ID.
- (6) Dilute the filtered extract with one part sample extract to three parts distilled/deionized water (e.g., 1 ml sample extract plus 3 ml water).
- (7) Use 50 microliters (μ l) of the diluted filtrate as test sample for each test.

c. Preparation of Wash Solution.

- (1) To prepare the Wash Solution, dissolve the contents of the one packet containing the buffer salt into 1 liter of distilled/deionized water.
 - (a) Swirl to mix.
 - (b) Place date prepared, and technicians initials on the 1 liter bottle.

Note: When stored properly (36° – 46°F) this solution has a shelf life of four weeks.

d. Test Procedures.

(1) Analysis Procedure.

- (a) Allow reagents and antibody wells to reach room temperature (64° - 86°F) prior to running any test sample.
- (b) Insert a sufficient number of test wells into the microwell holder for all standards and samples to be tested. (For example: to test 11 samples use 16 wells - 5 for all controls and 11 for the test samples).

Test Strip #1

Well #	1	2	3	4	5	6	7	8
Sample	C 0	C .222	C .666	C 2.0	C 6.0	S1	S2	S3

Test Strip #2

Well #	1	2	3	4	5	6	7	8
Sample	S4	S5	S6	S7	S8	S9	S10	S11

Where C 0 is the zero control, C .222 is the 0.222 ppm control, C .666 is the 0.666 ppm control, C 2.0 is the 2.0 ppm control, and C6 is the 6.0 ppm control. S1 is sample 1, S2 is sample 2, S3 is sample 3, etc.

NOTE: Do not run more than 3 strips (19 samples) per set of control standards.

- (c) Using a new pipette tip for each control standards and test sample(s). Pipette (add) 50 µl of standards, and prepared sample to designated separate well locations.
- (d) Add 50 µl of enzyme conjugate (red capped bottle) into each well.
- (e) Add 50 µl of deoxynivalenol antibody (black capped bottle) into each well.

- (f) Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for 10-15 seconds without spilling reagents.
- (g) Incubate for 5 minutes (\pm 1 minute) at room temperature (64° - 86° F).
- (h) Dump out the contents of the wells. Turn the wells upside down and tap on a paper towel until all the remaining liquid has been removed.
- (i) Using a wash bottle, fill each well with wash solution.
- (j) Empty the wells again and remove all remaining liquid. Repeat this step two times (total of three washes).
- (k) Add 100 μ l of substrate/chromogen (brown cap brown plastic bottle) to each well.
- (l) Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for 10-15 seconds without spilling reagents.
- (m) Incubate for 3 minutes (\pm 0.5 minutes) at room temperature (64° – 86° F). Cover the wells with a paper towel to protect them from any light source.
- (n) Add 100 μ l of stop solution (yellow cap-brown glass bottle) to each well.
- (o) Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for 10-15 seconds without spilling reagents.
- (p) Measure absorbance at 450 nm Stat-Fax Model 303 PLUS.

(Results must be read within 10 minutes)

(2) Stat-Fax Model 303 PLUS Microwell Reader.

- (a) To begin from the "Ready" prompt, press menu, key in the test number, and then press "Enter".
- (b) The screen will read, "Set carrier to A, press "Enter". Place the wells all the way to the right in the carrier. Push the carrier all the way to the left to line up the notch with the wells, and then press "Enter". The carrier will advance into the reader, and it should start to print.

- (c) When the reader is finished reading the strip, the screen will read, "Plot Curve Y/N?"

Press "Yes" (1/A) to print the graph.

Press "No" (0) to skip this feature.

- (d) The screen will read, "Accept Curve Y/N?"

Press "Yes" (1/A) to accept the curve and proceed to read another strip. When finished reading the second strip, press "Clear" twice and the results strip will print "Test Ended".

Press "No" (0) to end the test.

NOTE: Do not certify any result with an Optical Density value of less than 0.6 absorbance value for the zero (0) control standard.

- e. 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.

Applicants may request certification to the nearest tenth ppm. Refer to the Certification section of the handbook for more detailed certification procedures.

- f. Supplemental Analysis.

If quantitative results are above the test method's conformance limit (5.4 ppm), test results are reported as exceeding the 5 ppm conformance 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.

- (5) Peroxidase conjugated deoxynivalenol solution.
- (6) One black-capped bottle of 6 ml (R5901) and 3 ml (R5902) anti-deoxynivalenol antibody.
- (7) One brown-capped brown plastic bottle of 10 ml.
- (8) Substrate/chromogen, stained red.
- (9) One yellow-capped brown glass bottle of 14 ml stop reagent. Caution contains 1 N sulfuric acid.
- (10) One or two packets of washing buffer (salt).

(b) Materials Required but not Provided.

- (1) Stat-Fax Model 303 PLUS.
- (2) RIDATMSOFT Win Software.
- (3) 50 μ l, 100 μ l, and 1000 μ l (1ml) Pipettor and pipette tips.
- (4) Graduated cylinders (plastic or glass): 100 ml, 1 liter.
- (5) Sample shaker (optional).
- (6) Filter funnel.
- (7) Whatman #1 filter paper or equivalent.
- (8) Balance.
- (9) Multi-channel pipettor.
- (10) Paper towels, Kaydry paper or equivalent absorbent material.
- (11) Waste receptacle.
- (12) Timer: 3 channel minimum.
- (13) Waterproof marker, sharpie or equivalent.
- (14) Wash bottle.
- (15) Deionized or distilled water.

j. Storage Conditions and Precautions.

(a) Storage Conditions.

- (1) The reagents supplied with each test kit can be used until the expiration date on the kit label.
- (2) Store refrigerated at temperatures between 36° - 46°F. When stored properly (36° – 46 °F) the Wash Solution has a shelf life of four weeks.

(b) Precautions (Indication of test kit instability or deterioration of reagents).

- (1) Do not certify any test results if the Optical Density value of the zero control standard is less than 0.6 absorbance.
- (2) Do not use the substrate or chromogen if it does not have a reddish color, or is turning bluish before use.

4. FILING

Retain a copy of this program notice with the DON Handbook until the handbook is revised to include the test method stated herein.

5. QUESTIONS

Direct any questions concerning this program notice to Carl Jackson, PPMAB, at (202) 720-8286 or Robert Lijewski, PPMAB, at (202) 720-0224.

/s/ Robert Lijewski

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