

CHAPTER 10

r-BIOPHARM RIDASCREEN®FAST DON SC TEST KIT

PART NUMBER R5905

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## 10.1 GENERAL INFORMATION

The extraction solution and other materials used in the r-Biopharm RIDASCREEN®FAST DON SC test kit (part number R5905) does 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 the handbook to ensure a safe and efficient work environment.

## 10.2 EXTRACTION PROCEDURES

### a. Standard Extraction Procedure:

- (1) Place 50-grams of ground sample into a suitable container (e.g. sealable plastic bag, or quart size sample container w/top).
- (2) Add 250 ml of distilled/deionized water and seal/close container securely to prevent spillage.
- (3) Shake vigorously (by hand or mechanically) for three minutes.
- (4) Let the extract sit for 2-3 minutes to allow for settling of the sample slurry.
- (5) Filter the extract through Whatman #1 filters or filter syringe (or equivalent) into a clean container that is labeled with sample ID number.
- (6) Dilute the filtered extract with one part sample extract to 3 parts distilled/deionized water. (e.g., 1 ml sample extract plus 3 ml water).
- (7) Use 50 µl of the diluted filtrate per well for sample testing.

### b. Optional (Filter Bag) Extraction Procedure:

- (1) Place 50 grams of ground sample and 250 ml of distilled or deionized water in the front portion (side with the white strip for writing sample number) of the filter bag by pulling the center filter material towards the back side.

- (2) Close the filter bag by grabbing the wire tabs on the top and “whirling” the filter bag with a spinning motion until it can no longer be spun. Fold the wire tabs up and securely twist to lock in place.

**Note: Failure to properly “whirl” the filter bag shut may result in some leaking of extraction solution.**

- (3) Shake by hand for 3 minutes.
- (4) Untwist the tabs and completely open the top of the filter bag.
- (5) Peel the center material away from the back side of the filter bag and towards the front side (where the sample was initially inserted).
- (6) Let sample settle sitting upright for at least 1 minute.
- (7) Pipette 1 ml of filtered liquid from the back portion of the bag into a clean test tube.
- (8) Add 3ml of distilled or deionized water to the test tube with 1ml of filtered extract and thoroughly mix.
- (9) The sample is prepared and ready to be analyzed.

### **10.3 PREPARATION OF SOLUTIONS**

- a. To prepare the Wash Solution, dissolve the contents of the packet containing the buffer salt in 1 liter of distilled water. Document the technician’s name, preparation date, and expiration date on wash solution bottle. A removable gum-label affixed to bottle for documentation is recommended.
- b. Swirl to mix and before use. When stored properly (at 36 - 46° F) the solution has a shelf life of four weeks.
- c. Alternative Preparation of Wash Solution:
  - (1) Dissolve the contents of the packet in only 100 ml(s) of distilled water to obtain a 10 fold concentrated washing buffer. This solution expires after approximately 8 – 12 weeks. Store at room temperature (68 - 77° F).
  - (2) Use 1 part of the concentrate and dissolve with 9 parts of distilled water to obtain the ready to use washing buffer.

**10.4 TEST PROCEDURES**

a. Analysis Procedure.

- (1) Allow reagents and antibody wells to reach room temperature (68 - 77° F) prior to running the test.
- (2) Only 1 control standard (zero standard) is included in the test kit. The standard curve (B/Bo) is provided with the certificate of the test kit.
- (3) Insert a sufficient number and wells into the microwell holder for control standard and samples to be tested. (For example: to test 15 samples use 16 wells - 1 for the control standard and 15 for the test samples).

Test Strip #1

Well #	1	2	3	4	5	6	7	8
Sample	C 0	S1	S2	S3	S4	S5	S6	S7

Test Strip #2

Well #	1	2	3	4	5	6	7	8
Sample	S8	S9	S10	S11	S12	S13	S14	S15

Where C 0 is the zero control, S1 is sample 1, S2 is sample 2, S3 is sample 3, etc.

**NOTE: Do not run more than 3 strips (23 samples) per control standard when using an 8 channel multi pipettor.**

- (4) Using a new pipette tip for zero (0) control standard and each test sample, pipette 50 µl of standard and prepared sample(s) to separate wells.

- (5) Add 50  $\mu$ l of enzyme conjugate (red capped bottle) into each well using a repeating pipettor on setting 1.
- (6) Add 50  $\mu$ l of Anti-deoxynivalenol antibody (black capped bottle) into each well using a repeating pipettor on setting 1.
- (7) Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for **10-15 seconds** without spilling reagents.
- (8) Incubate for **5 minutes** ( $\pm$  1 minute) at room temperature.
- (9) Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.
- (10) Using a wash bottle, fill each well with washing buffer solution. Empty the wells again and remove all remaining liquid. Repeat this step 2 times (total of 3 washes).
- (11) Add 100  $\mu$ l of substrate/chromogen (brown cap brown plastic bottle) to each well using a repeating pipettor on setting 2.
- (12) Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for **10-15 seconds** without spilling reagents.
- (13) 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 light sources.
- (14) Add 100  $\mu$ l of stop solution (yellow cap-brown glass bottle) to each well using a repeating pipettor on setting 2.
- (15) Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for **10-15 seconds** without spilling reagents.
- (16) Measure absorbance at 450 nm using the Awareness Technology Stat-Fax Model 303 PLUS (results must be read within 10 minutes).

b. Reading the Results.

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

- (a) Press Menu, the prompt should read: "Select Test" press 1, then ENTER.
- (b) The concentrations and B/BO% should now be printing.
- (c) Display will read: "New B/BO Num Y/N (Yes/No)."  
  
Press "N" if the B/BO matches the QC sheet in the test kit in use.  
  
Press "Y" if the B/BO on the printout does not match the QC sheet in the kit.
- (d) If "Y" was pressed for new B/BO it will now display:  
"Cal 2 B/BO%= \_\_\_\_\_" simply insert the B/BO number from the QC sheet for standard 2 and press ENTER.
- (e) Continue to insert the corresponding B/BO numbers from the QC sheet until they are all updated.
- (f) When completed the reader will print "Test is Updated."  
  
**Note: Please verify new B/BO number entered on the printout match test kit QC sheet.**
- (g) If "N" was pressed for new B/BO, or you just finished updating the B/BO it will now display: "Set Carrier to 1; press ENTER."
- (h) Place the wells in the far right column of the carrier with the zero (0) standard being at the top.
- (i) Align carrier to the far left for column 1. Then press ENTER.
- (j) The reader is now reading the first eight wells. Once complete the display will read: "Plot Curve Y/N Select N."

- (k) Display will now read: "Accept Curve Y/N.

If you are only running one strip, the test is now complete (press the clear button twice). If you have an additional strip to run, select yes. Move the carrier to the right so that the wells are aligned with notch in the center. Now press ENTER.

- (l) The reader is now reading the second set of eight wells.
- (m) Once the last strip is read, press the clear button twice.
- (n) Test is now complete.

## 10.5 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.

## 10.6 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 value 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).



- (4) 1 black-capped bottle of 3 ml(s) anti- deoxynivalenol antibody.
- (5) 1 brown-capped brown plastic bottle of 6 ml (s) substrate/chromogen, stained red.
- (6) 1 yellow-capped brown glass bottle of 6 ml(s) stop solution.
- (7) 1 packet of washing buffer (salt).

b. Materials Required but not Provided.

- (1) Awareness Technology Inc. Stat-Fax Model 303 PLUS with 450-nm filter.
- (2) RIDA™SOFT Win Software.
- (3) 50 µl, 100 µl, and 1000 µl Pipettor and pipette tips.
- (4) Graduated cylinders (plastic or glass): 100 ml, 1 liter.
- (5) Sample shaker (optional).
- (6) Filter funnel.
- (7) Filter bags.
- (8) Whatman #1 filter paper or equivalent.
- (9) Balance.
- (10) Repeating pipettor.
- (11) Paper towels, Kaydry paper or equivalent absorbent material.
- (12) Waste receptacle.
- (13) Timer: 3 channel minimum.
- (14) Waterproof marker, Sharpie or equivalent.
- (15) Wash bottle.
- (16) Deionized or distilled water.

## 10.10 STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions.

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.

b. Precautions.

- (1) Do not interchange individual reagents between kits of different lot numbers.
- (2) Do not use the test kits beyond the noted expiration date.
- (3) The substrate/chromogen solution is light sensitive, therefore, avoid exposure to direct light.