CHARM SCIENCES, INC.
ROSA DONQ2 QUANTITATIVE TEST

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GENERAL INFORMATION

ROSA DONQ2 Quantitative Test is an immunoreceptor assay utilizing ROSA (Rapid One Step Assay) lateral flow technology. DON (deoxynivalenol or vomitoxin) is extracted from the samples using water. DON interacts with colored beads in the lateral flow test strip and the color intensity in the test and control zones is measured by the ROSA-M Reader or Charm EZ-M reader and interpreted as parts per million (ppm) DON.

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the Mycotoxin Handbook for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@usda.gov.

Approved Test Kit Information

Test Kit Vendor:	*Charm Sciences, Inc.* 978-687-9200
Test Kit Name:	ROSA DONQ2 Quantitative Test
Product Number:	LF-DONQ2
Effective Date of Instructions:	01/26/2016
Instructions Revision Number	4
Conformance Range:	0.5 – 5 ppm
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	Yes
Approved Commodities:	Corn, wheat, barley, brewer's rice, buckwheat, corn bran, corn germ meal, corn gluten feed, corn gluten meal, distillers dried grain with solubles, hominy, malted barley, milled rice, oats, rapeseed meal, rice bran, rough rice, rye, sorghum, soybean meal, triticale, wheat bran, wheat flour, wheat middlings, wheat red dog, wheat screenings
Extraction method:	Shake vigorously 50 grams ground sample with 250 milliliters (mL) of deionized or distilled water for 1 minute.
Test Format:	Lateral flow strip
Detection Method:	ROSA-M Reader, Model LF-ROSAREADER-M-NB Charm EZ-M, Model LF-ROSA-EZ-M

PREPARATION OF TESTING MATERIALS AND EQUIPMENT

a. Test Strips:

Remove from the container only the number of test strips to be used in 1 day, document time of removal. Keep these test strips at room temperature during daily use for up to 12 hours and unused test strips should be discarded.

b. DONQ2 Dilution Buffer:

(1) Dispense buffer into a clean micro-centrifuge tube and label for each sample to be tested.

(2) Use pre-dispensed buffer tubes and buffer solution at room temperature (18 to 30 °C).

c. Negative Control:

DONQ2 Dilution Buffer is used as a negative control.

d. Positive Control:

Reconstitute the dry positive control (provided with test kit) by adding 6.0 mL DONQ2 Dilution Buffer. Shake well; allow to stand for 10 minutes at room temperature before use, and mix again just before use.

(1) For storing reconstituted positive control see "**STORAGE CONDITIONS AND PRECAUTIONS"** on page 8

e. Reader and Test Strip Performance Testing:

(1) Equipment Setup

(a) **ROSA-M Reader:** Enter performance mode in ROSA-M Reader by selecting DON channel in 3-line mode (DON flashing) and sequentially pressing ESC, 5, ENTER. Follow ROSA-M Reader prompts to test calibration strips (LOWCAL and HIGHCAL) and controls (NEGCONTROL and POSCONTROL). Press ESC to exit.

(b) **Charm EZ-M reader:** Enter performance mode in Charm EZ-M by selecting Perf. Mon. from the Main Menu, followed by Perf. Test. Follow Charm EZ-M prompts to test calibration strips (LO CAL and HI CAL) and controls (NEG CTRL and POS CTRL). Select DONQ2 from the TESTS list if prompted.

(2) Test calibration strips daily to verify ROSA-M Reader or Charm EZ-M performance. Calibration strips must test/perform in the specified ranges.

(3) Test negative control and positive control weekly to verify test strip performance. Valid control ranges are:

(a) Negative Control: less than or equal to 100 ppb (0.1 ppm)

(b) Positive Control: 500 to 1500 ppb (0.5 to 1.5 ppm)

If calibration strips or controls do not perform in specified ranges, discontinue use and contact Charm Sciences for assistance. Notify your monitoring field office or TSD with any documented information for quality control purposes.

f. ROSA Incubator:

ROSA Incubator must be clean and level. The ROSA Incubator temperature must be at 45 ± 1 °C (the temperature indicator should match the incubator temperature).

EXTRACTION PROCEDURE AND SAMPLE PREPARATION FOR QUANTITATION

a. <u>Procedure for barley, brewer's rice, buckwheat, corn, corn bran, corn gluten feed, corn germ meal, corn gluten meal, hominy, malted barley, milled rice, oats, rapeseed meal, rough rice, rye, sorghum, soybean meal, triticale, wheat, wheat bran, wheat flour, wheat red dog, wheat screenings:</u>

(1) Weigh 50 ± 0.2 grams ground samples into a clean extraction container.

(2) Add 250 mL deionized or distilled water.

(3) Shake vigorously for 1 minute.

(4) Allow sample to settle for 1 minute to obtain settled extract (use within 30 minutes).

(5) Transfer 1 to 1.5 mL settled extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (use within 2 hours).

(6) Pipet 1.0 mL DONQ2 Dilution Buffer into a clean micro-centrifuge tube.

(7) Pipet 50 microliters (µL) centrifuged sample extract to micro-centrifuge tube containing 1.0 mL DONQ2 Dilution Buffer, cap, mix (shake vigorously for 5 s), and label. This sample is the Diluted Extract, and ready for the test (use within 6 hours after preparation).

(8) Repeat steps 1 to 7 for additional samples.

b. <u>Procedure for distillers dried grains with solubles:</u>

(1) Weigh 50 ± 0.2 grams ground samples into a clean extraction container.

(2) Add 250 mL deionized or distilled water.

(3) Shake vigorously for 1 minute.

(4) Allow sample to settle for 1 minute to obtain settled extract (use within 30 minutes).

(5) Transfer 10 to 15 mL settled extract into a clean conical tube and label.

(6) Adjust pH of settled extract by adding 10-30% KOH dropwise until pH is 6.5 to 7.5. Monitor pH with pH strips or pH meter.

(7) Transfer 1 to 1.5 mL pH adjusted extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (use within 2 hours).

(8) Pipet 1.0 mL DONQ2 Dilution Buffer into a clean micro-centrifuge tube.

(9) Pipet 50 µL centrifuged sample extract to micro-centrifuge tube containing 1.0 mL DONQ2 Dilution Buffer, cap, mix (shake vigorously for 5 s), and label. This sample is the Diluted Extract, and ready for the test (use within 6 hours after preparation).

(10) Repeat steps 1 to 9 for additional samples.

c. <u>Procedure for rice bran and wheat middlings:</u>

(1) Weigh 50 ± 0.2 grams ground samples into a clean extraction container.

(2) Add 250 mL deionized or distilled water.

(3) Shake vigorously for 1 minute.

(4) Allow sample to settle for 1 minute to obtain settled extract (use within 30 minutes).

(5) Transfer 1 to 1.5 mL settled extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (use within 2 hours).

(6) Filter each extract by drawing into syringe and passing through GF/CA filter (purchased separately). Collect filtered extract in a clean micro-centrifuge tube and label.

(7) Pipet 1.0 mL DONQ2 Dilution Buffer into a clean micro-centrifuge tube.

(8) Pipet 50 µL filtered sample extract to micro-centrifuge tube containing 1.0 mL DONQ2 Dilution Buffer, cap, mix (shake vigorously for 5 s), and label. This sample is the Diluted Extract, and ready for the test (use within 6 hours after preparation).

(9) Repeat steps 1 to 8 for additional samples.

TEST PROCEDURES

a. <u>Sample Analysis:</u>

(1) Check that the ROSA Incubator temperature is 45 ± 1 °C.

(2) Label test strip(s) to identify sample.

(3) Place test strip in the ROSA Incubator with the flat side facing upward.

(4) Hold the test strip flat in the ROSA Incubator and use tab to expose sample compartment by peeling tape back to “Peel to Here” line.

Avoid lifting the test strip and sponge under tape and bending back the white wick and sponge under the tape.

(5) Hold the pipet vertically and slowly pipet 300 µL test sample (diluted extract or positive and negative control) into the sample compartment at the ROSA Incubator line.

(6) Reseal the tape over the sample pad compartment.

NOTE: When performing multiple tests using a ROSA Incubator:

(a) Peel, pipet, and reseal before starting next strip.

(b) Complete all test strips within 30 seconds.

(7) Close lid on the ROSA Incubator.

(8) Incubate for 2 minutes.

(9) Remove strip(s) from the ROSA Incubator.

Do not squeeze sample compartment. Hold test strip vertically with sample compartment in the down position until interpreted.

(a) Wipe foreign matter (dust, etc.) from the test strip(s).

(b) Inspect and read test strip(s) within 30 seconds of incubation completion. When running multiple test strips in the ROSA Incubator, remove one strip for visual inspection and interpretation at a time.

(c) Lower ROSA Incubator lid; do not re-latch.

b. <u>Visual Inspection:</u>

(1) The test strip is **INVALID** if any of the following are observed:

(a) C (Control) line is missing.

(b) T1, T2 (Test) or C line is smeared or uneven.

(c) T1, T2, or C line is obscured by diluted extract or control.

(d) Beads do not flow past T1, T2 or C lines.

(2) Do not put INVALID test strips in the ROSA-M Reader or Charm EZ-M.

(3) If test strip is INVALID, re-test the diluted extract or control.

c. <u>Interpretation:</u>

(1) ROSA-M Reader

(a) Insert a clean and valid test strip into the ROSA-M Reader. Slide the strip into the slot with the sample compartment in the up position until it stops.

(b) Read results on DON channel in 3-line mode (DON flashing) using the appropriate MATRIX. If desired, enter Sample and/or Operator. Press ENTER to read.

- **MATRIX 00:** Diluted Extract for 0.5 to 5 ppm quantitation.
- **MATRIX 02:** Supplemental Diluted Extract for 5 to 60 ppm quantitation.

Note: For controls, see Reader and Test Strip Performance Testing in PREPARATION OF TESTING MATERIALS AND EQUIPMENT section.

(c) **READING:** The number displayed is the concentration of DON (ppb) in the sample. A reading in ppb must be converted to ppm by dividing the ppb concentration by 1000 (e.g., 500 ppb = 0.5 ppm).

A Diluted Extract READING greater than 5 ppm (5.4 ppm if reporting results to the tenths) indicates that the concentration of the sample is greater than the sensitivity range of the sample dilution.

An applicant can request a supplemental analysis option to report test results above the Diluted Extract sensitivity range of 5 ppm (5.4 ppm if reporting results to the tenths). See SUPPLEMENTAL ANALYSIS section for more information.

(2) Charm EZ-M reader (Read only mode)

(a) Insert a clean and valid test strip into the Charm EZ-M. Slide the strip into the slot with the sample compartment in the down position until it stops.

(b) Read results on DONQ2 from the TESTS list with COMMODITY and DILUTION selected for sample. If desired, enter OPERATOR ID, SAMPLE ID, and/or LOT NUMBER. Close door to read.

- **DE:** Diluted Extract for 0.5 to 5 ppm quantitation.
- **SUPP DE:** Supplemental Diluted Extract for 5 to 60 ppm quantitation.

Note: For controls, see Reader and Test Strip Performance Testing in PREPARATION OF TESTING MATERIALS AND EQUIPMENT section.

(c) **READING:** The number displayed is the concentration of DON in the sample. A reading in ppb must be converted to ppm by dividing the ppb concentration by 1000 (e.g., 500 ppb = 0.5 ppm).

A Diluted Extract READING greater than 5 ppm (5.4 ppm if reporting results to the tenths) indicates that the concentration of the sample is greater than the sensitivity range of the sample dilution.

An applicant can request a supplemental analysis option to report test results above the Diluted Extract sensitivity range of 5 ppm (5.4 ppm if reporting results to the tenths). See SUPPLEMENTAL ANALYSIS section for more information.

SUPPLEMENTAL ANALYSIS

Supplemental analysis is a procedure followed when a result is observed above the upper limit of the concentration range used in GIPSA's test kit performance evaluation.

The range for performance evaluation of quantitative DON test kits is 0.5 to 5 ppm. Therefore, supplemental analysis would be performed for a result above 5 ppm (5.4 ppm if reporting results to the tenths). In supplemental analysis, the extract is diluted so the resulting concentration is between the lower and upper limits of the test kit evaluation range, and a correction for dilution is applied to derive at the final result. For this test kit, the appropriate calibration setting is selected for automatic correction for the supplemental dilution performed.

Supplemental analysis is performed only at the request of the applicant.

Preparation and Assay of Supplemental Diluted Extract for 5 to 60 ppm DON.

(1) Prepare Diluted Extract according to Sample Preparation and Extraction Procedures.

(2) Prepare Supplemental Diluted Extract from the Diluted Extract.

(a) Pipet 1.0 mL DONQ2 Dilution Buffer into a clean micro-centrifuge tube.

(b) Pipet 100 µL Diluted Extract to micro-centrifuge tube containing 1.0 mL DONQ2 Dilution Buffer, cap, mix (shake vigorously for 5 s), and label. This sample is the Supplemental Diluted Extract.

(3) Repeat steps 1 to 2 for additional samples.

(4) Use Supplemental Diluted Extract as test sample in Sample Analysis found in TEST PROCEDURES section.

(5) Inspect and interpret the test strip as directed in TEST PROCEDURES section.

Valid Supplemental Diluted Extract final result must be within 4 to 60 ppm detection range of the sample dilution.

A final result less than 3.5 ppm is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the procedure for the Diluted Extract (non-supplemental analysis) and only perform the supplemental analysis again if the value is greater than 5 ppm (5.4 ppm if reporting results to the tenths).

A Supplemental Diluted Extract READING of "+60 ppm" indicates that the concentration of the sample is greater than the sensitivity range of the sample dilution. Report test results as greater than 60 ppm on the work record and certify "DON exceeds 60 ppm".

REPORTING AND CERTIFYING TEST RESULTS

Refer to the Mycotoxin Handbook for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@usda.gov).

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions:

(1) Store test strips refrigerated in tightly closed supplied container.

(2) Store dilution buffer bottle and pre-dispensed micro-centrifuge tubes refrigerated.

(3) Store reconstituted positive control refrigerated (0 to 7 °C) for up to 1 week or aliquot (at least 0.5 mL) to clean micro-centrifuge tubes, label, and freeze within 6 hours of reconstitution (-15 °C or below) for up to 2 months. Thaw slowly (overnight in refrigerator or with cool water) and shake well before use. Store thawed positive control refrigerated and use within 24 hours of thawing; DO NOT REFREEZE.

b. Precautions:

(1) Test Strips

(a) To open test strip canister, remove and save plastic lid with foil lined foam insert to reseal container. Lift foil tab and peel foil seal off container. Discard foil seal.

(b) In high humidity, limit condensation by opening container after it has warmed to room temperature, estimated between 20 to 30 minutes from the time the container was removed from the refrigerator.

(c) Inspect/verify desiccant indicator. Beads inside desiccant packets should be blue. Do not use test strips if the blue beads have turned purple or pink

(2) Use DONQ2 Dilution Buffer supplied with each test kit only.

(3) Do not use the test kits beyond the noted expiration date.

(4) Debris on test strips may alter the ROSA-M Reader or Charm EZ-M optics. Keep equipment clean. Wipe dust and liquid off test strips before inserting into reader.

(5) ROSA Incubator must be clean and level. ROSA Incubator temperature must be 45 ± 1 °C. The temperature indicator should match the ROSA Incubator temperature. A daily thermometer check is recommended. Keep ROSA Incubator lid lowered, but not latched unless performing test procedure. ROSA Incubator may take 10 minutes to reach proper temperature depending on ambient temperature.

EQUIPMENT AND SUPPLIES

a. <u>Test Strips</u>

(1) LF-DONQ2-20K

(a) 1 container of 20 DONQ2 test strips

(b) 1 1000 ppb DON Positive Control

(c) 1 DONQ2 Dilution Buffer

(2) LF-DONQ2-100K

(a) 1 container of 100 DONQ2 test strips

(b) 1 1000 ppb DON Positive Control

(c) 1 DONQ2 Dilution Buffer

(3) LF-DONQ2-500K

(a) 5 containers of 100 DONQ2 test strips

(b) 5 1000 ppb DON Positive Controls

(c) 5 DONQ2 Dilution Buffers

b. <u>Materials required but not provided</u>

(1) 50 µL pipet and pipet tips

(2) 100 µL pipet and pipet tips

(3) 300 µL pipet and pipet tips

(4) 100 to 1000 µL variable volume pipet or 1.0 mL pipet and pipet tips

(5) 250 mL graduated cylinder

(6) Balance

(7) Deionized or distilled water

(8) Micro-centrifuge tubes

(9) Mini-centrifuge

(10) ROSA-M Reader or Charm EZ-M reader

(11) Printer for ROSA-M Reader or Charm EZ-M (optional)

(12) ROSA Incubator

(13) Sample grinder

(14) Sample extraction containers or Whirl-pak Bags

(15) Storage bottle

(16) Transfer pipets (optional)

c. <u>Materials required but not provided for testing distillers dried grain with solubles</u>

(1) 10-30% KOH (w/v) in water

(2) Conical tubes

(3) pH paper or pH meter

d. <u>Materials required but not provided for rice bran or wheat middlings</u>

(1) GF/CA syringe filters (Phenomenex Part No. AF0-8A09-12)

(2) Syringes

REVISION HISTORY

Revision 4 (01/26/2016)

- The aliquot volume of positive control used for freezing has been changed from 1.5 mL to 0.5 mL.

Revision 3 (12/04/2015)

- Procedure for mixing the diluted extract was updated.
- Flow chart was added.

Revision 2 (10/27/2015)

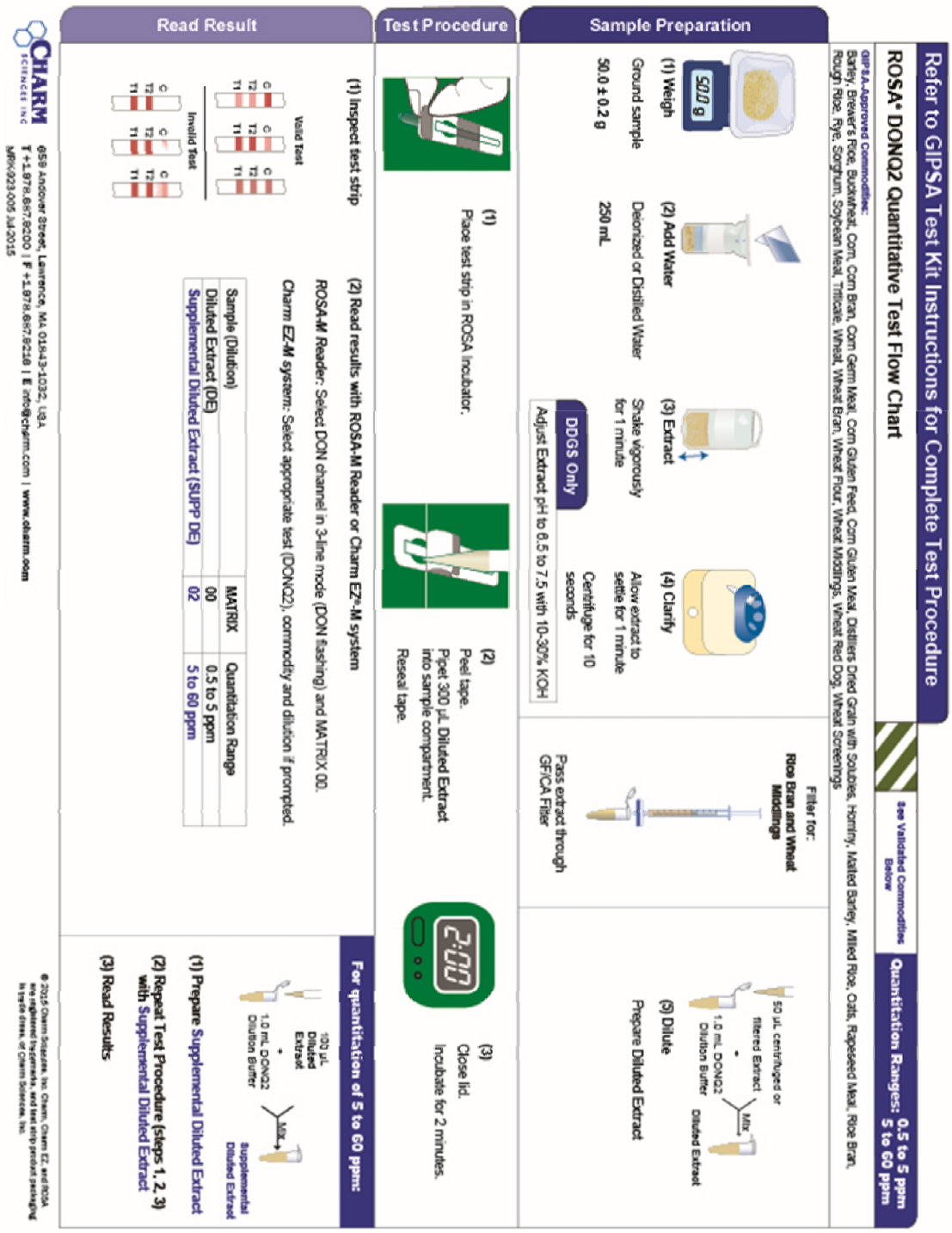
- Quantitation range has been changed from 0.5 to 5.0 ppm to 0.5 to 5 ppm in accordance with whole number reporting.

Revision 1 (01/07/2015)

- Correct Acronym of Policies, Procedure, and Market Analysis Branch (PPMAB) has been used.
- Phone number of Patrick McCluskey (816-659-8403) has been corrected.

Revision 0 (11/14/2014)

FLOW CHART


Refer to GIPSA Test Kit Instructions for Complete Test Procedure
ROSA® DONQ2 Quantitative Test Flow Chart
See Validated Commodities Below
Quantitation Ranges: 0.5 to 5 ppm 5 to 60 ppm
GIPSA-Approved Commodities:
Barley, Brewer's Rice, Buckwheat, Corn, Corn Bran, Corn Germ Meal, Corn Gluten Feed, Corn Gluten Meal, Distillers Dried Grain with Solubles, Hominy, Malted Barley, Milled Rice, Oats, Rapeseed Meal, Rice Bran, Rough Rice, Rye, Sorghum, Soybean Meal, Triticale, Wheat, Wheat Bran, Wheat Flour, Wheat Middlings, Wheat Red Dog, Wheat Screenings
Sample Preparation
50.0 g
(1) Weigh
Ground sample
50.0 ± 0.2 g
(2) Add Water
Deionized or Distilled Water
250 mL
(3) Extract
Shake vigorously for 1 minute
(4) Clarify
Allow extract to settle for 1 minute
Centrifuge for 10 seconds
DDGS Only
Adjust Extract pH to 6.5 to 7.5 with 10-30% KOH
Filter for:
Rice Bran and Wheat Middlings
Pass extract through GF/CA Filter
50 µL centrifuged or filtered Extract
1.0 mL DONQ2 Dilution Buffer
Mix
Diluted Extract
(5) Dilute
Prepare Diluted Extract
Test Procedure
(1)
Place test strip in ROSA Incubator.
(2)
Peel tape.
Pipet 300 µL Diluted Extract into sample compartment.
Reseal tape.
2:00
(3)
Close lid.
Incubate for 2 minutes.
Read Result
(1) Inspect test strip
Valid Test
Invalid Test
C
T2
T1
(2) Read results with ROSA-M Reader or Charm EZ™-M system
ROSA-M Reader: Select DON channel in 3-line mode (DON flashing) and MATRIX 00.
Charm EZ-M system: Select appropriate test (DONQ2), commodity and dilution if prompted.
Sample (Dilution) | MATRIX | Quantitation Range
Diluted Extract (DE) | 00 | 0.5 to 5 ppm
Supplemental Diluted Extract (SUPP DE) | 02 | 5 to 60 ppm
For quantitation of 5 to 60 ppm:
100 µL Diluted Extract
1.0 mL DONQ2 Dilution Buffer
Mix
Supplemental Diluted Extract
(1) Prepare Supplemental Diluted Extract
(2) Repeat Test Procedure (steps 1, 2, 3) with Supplemental Diluted Extract
(3) Read Results
CHARM SCIENCES INC
6659 Andover Street, Lawrence, MA 01843-1032, USA
T +1.978.687.8200 | F +1.978.687.8218 | E info@charm.com | www.charm.com
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