CHARM SCIENCES, INC.
ROSA OCHRATOXIN QUANTITATIVE TEST

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GENERAL INFORMATION

ROSA Ochratoxin Quantitative Test is an immunoreceptor assay utilizing ROSA (Rapid One Step Assay) lateral flow technology. Ochratoxin A is extracted from the sample using 70% methanol in water. Ochratoxin A 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 displayed as parts per billion (ppb) ochratoxin A.

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 Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division 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 Ochratoxin Quantitative Test
Product Number:	LF-OCHRAQ-G
Effective Date of Instructions:	11/19/2015
Instructions Revision Number	1
Conformance Range:	5 – 100 ppb
Number of Analyses to Cover Conformance Range:	2
Type of Service:	Quantitative
Supplemental Analysis:	No
Approved Commodities:	Wheat, barley, buckwheat, corn, corn gluten meal, malted barley, oats, rye, sorghum, soybean meal, and soybeans
Extraction method:	Shake vigorously 50 gram sample with 100 milliliters (mL) 70% methanol/30% distilled or deionized water (v/v) for 1 minute by hand.
Test Format:	Lateral flow strip
Detection Method:	ROSA-M Reader, Model LF-ROSAREADER-M-NB Charm EZ-M reader, 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; discard the unused test strips after the 12 hour period.

b. OCHRAQ 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 °C to 30 °C).

c. Preparation of Extraction Solvent [70% Methanol/30% Water (v/v)]:

The extraction solvent used in the method is a methanol/water mixture consisting of 70% methanol (reagent grade or better) and 30% distilled or deionized water (v/v).

(1) Using a 1000 mL graduated cylinder, measure 700 mL methanol and place it into a clean carboy with spigot.

(2) Using a 500 mL graduated cylinder, measure 300 mL distilled or deionized water and add to the methanol and shake until it is completely mixed.

(3) Label the container stating the mixture 70% methanol/30% water (v/v), date of preparation, and initials of technician who prepared the solution.

(4) Store this solution at room temperature in a tightly closed container until needed. Mix again before use.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts distilled or deionized water.

d. Negative Control:

Prepare negative control by adding 100 microliters (µL) extraction solvent to 1.0 mL OCHRAQ Dilution Buffer in a clean micro-centrifuge tube, cap, mix, and label.

e. Positive Control:

Reconstitute the dry positive control by adding 300 µL extraction solvent followed by 3.0 mL OCHRAQ Dilution Buffer. Shake well and allow to stand at room temperature for 10 minutes. Mix again before use.

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).

g. <u>Reader and Test Strip Performance Testing:</u>

(1) Equipment Setup

(a) **ROSA-M Reader:**

1. For calibration strips, enter performance mode in ROSA-M Reader by selecting **PAT** channel in 3-line mode (**PAT** flashing) and sequentially pressing ESC, 5, ENTER. Follow ROSA-M Reader prompts to test calibration strips (LOWCAL and HIGHCAL). Press ESC to exit.

2. For controls, enter performance mode in ROSA-M Reader by selecting **OCHRA** channel in 3-line mode (**OCHRA** flashing) and sequentially pressing ESC, 5, ENTER. Follow ROSA-M Reader prompts to controls (NEGCONTROL and POSCONTROL). Press ESC to exit.

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

(2) Test calibration strips daily to verify performance of ROSA-M Reader and Charm EZ-M reader. 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 1 ppb

(b) Positive Control: 4 to 10 ppb

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.

EXTRACTION PROCEDURES

<u>Procedure for wheat, barley, buckwheat, corn, corn gluten meal, malted barley, oats, rye, sorghum, soybean meal, and soybeans:</u>

(1) Weigh 50.0 ± 0.2 grams ground samples into a clean extraction container (centrifuge within 30 minutes of extraction).

(2) Add 100 mL extraction solvent.

(3) Shake vigorously for 1 minute by hand.

(4) Transfer 1 to 1.5 mL extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (can be used only for next 2 hours).

(5) Repeat for additional samples (up to four samples for each quad ROSA Incubator).

SAMPLE PREPARATION FOR QUANTITATION

This test kit uses different testing sensitivity ranges (Diluted Extract and Second Diluted Extract) for reporting ochratoxin A measurements for grain and commodities.

a. <u>Sample Preparation of Diluted Extract for 5 to 30 ppb quantitation:</u>

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

(2) Pipet 100 µL centrifuged extract to micro-centrifuge tube containing 1.0 mL OCHRAQ Dilution Buffer, cap, mix (shake vigorously for 5 seconds), and label. This tube contains the Diluted Extract.

(3) Draw Diluted Extract into 1 mL syringe, pass through syringe filter and collect in a clean micro-centrifuge tube and label.

(a) Minisart RC15 syringe filter is used for wheat, barley, corn, corn gluten meal, malted barley, oats, rice, rye, sorghum, soybean meal, and soybeans.

(b) GF/CA syringe filter is used for **buckwheat**.

(4) Repeat for additional samples.

(5) Use filtered Diluted Extract (within 6 hours after preparation) as your test sample in Sample Analysis found in TEST PROCEDURES section.

b. <u>Sample Preparation of Second Diluted Extract for 20 to 100 ppb quantitation:</u>

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

(2) Pipet 300 µL filtered Diluted Extract to micro-centrifuge tube containing 1.0 mL OCHRAQ Dilution Buffer, cap, mix (shake vigorously for 5 seconds), and label. This tube contains the Second Diluted Extract.

(3) Repeat for additional samples.

(4) Use Second Diluted Extract (within 6 hours after preparation) as your test sample in Sample Analysis found in TEST PROCEDURES section.

TEST PROCEDURES

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

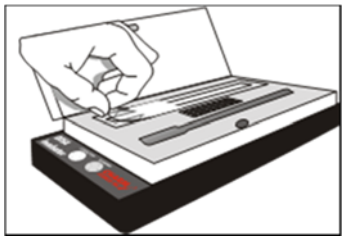
(1) Check that the ROSA Incubator temperature is 45 ± 1 °C.

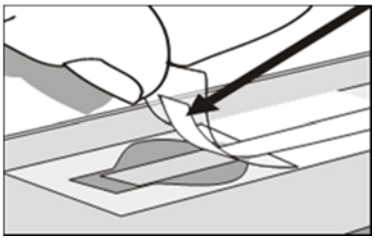
(2) Label test strip(s) to identify sample.

(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 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 1 minute.

(7) Close lid on the ROSA Incubator.

(8) Incubate for 10 minutes.

(9) Remove strip from the ROSA Incubator.

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

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

(b) Inspect and interpret test strip.

When running multiple test strips in the ROSA Incubator, remove one strip for visual inspection and interpretation at a time and complete procedure for all test strips within 1 minute of incubation completion.

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

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

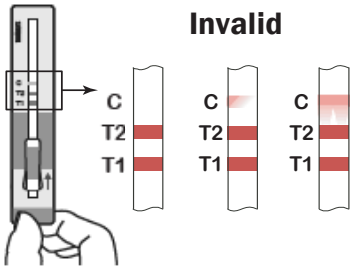
(1) The test strip is **INVALID** if any of the following are observed:

(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 reader.

(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 **OCHRA** channel in 3-line mode (**OCHRA** flashing) using the appropriate MATRIX. If desired, enter Sample and/or Operator. Press ENTER to read.

- **MATRIX 00:** Assay of Diluted Extract for 5 to 30 ppb quantitation.
- **MATRIX 01:** Assay of Second Diluted Extract for 20 to 30 ppb 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 ochratoxin A (ppb) in the sample.

A "+" sign on a READING value indicates that the concentration of the sample is greater than the Sensitivity range. For example, a filtered Diluted Extract READING of "+30 ppb" indicates a value greater than 30 ppb. For quantitation of 20 to 100 ppb ochratoxin A, prepare the Second Diluted Extract and use with another test strip.

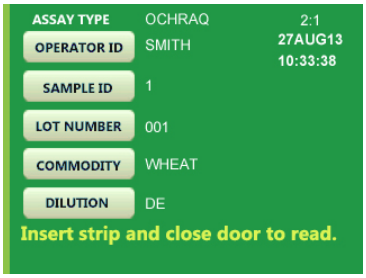
A Second Diluted Extract READING less than 20 ppb indicates a value below the detection range. Re-test filtered Diluted Extract on another test strip for quantitation from 5 to 30 ppb ochratoxin A.

A Second Diluted Extract READING greater than 100 ppb indicates that the concentration of the sample is greater than the sensitivity range of the sample dilution. Report test result as greater than 100 ppb on the work record and certify "Ochratoxin A exceeds 100 ppb".

Note: Applicants may request qualitative certification in lieu of retesting of results outside of the filtered Diluted Extract or Second Diluted Extract test sample sensitivity ranges/concentrations.

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

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



(b) Read results on **OCHRAQ** 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 5 to 30 ppb quantitation.
- **2ND DE:** Second Diluted Extract for 20 to 100 ppb 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 ochratoxin A (ppb) in the sample.

A "+" sign on a READING value indicates that the concentration of the sample is greater than the Sensitivity range. For example, a filtered Diluted Extract READING of "+30 ppb" indicates a value greater than 30 ppb. For quantitation of 20 to 100 ppb ochratoxin A, prepare the Second Diluted Extract and use with another test strip.

A Second Diluted Extract READING less than 20 ppb indicates a value below the detection range. Re-test filtered Diluted Extract on another test strip for quantitation from 5 to 30 ppb ochratoxin A.

A Second Diluted Extract READING greater than 100 ppb indicates that the concentration of the sample is greater than the sensitivity range of the sample dilution. Report test result as greater than 100 ppb on the work record and certify "Ochratoxin A exceeds 100 ppb".

Note: Applicants may request qualitative certification in lieu of retesting of results outside of the filtered Diluted Extract or Second Diluted Extract test sample sensitivity ranges/concentrations.

SUPPLEMENTAL ANALYSIS

There is no Supplemental Analysis for this test kit.

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 °C to 7 °C) for up to 1 week or aliquot (at least 1.5 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 25 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.

(d) Re-shape dented sample compartments to fit into ROSA Incubator.

(2) Use OCHRAQ 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 reader 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-OCHRAQ-G-20K

(a) 1 container of 20 OCHRAQ test strips

(b) 1 OCHRAQ Grain Positive Control

(c) 1 OCHRAQ Dilution Buffer

(2) LF-OCHRAQ-G-100K

(a) 1 container of 100 OCHRAQ test strips

(b) 1 OCHRAQ Grain Positive Control

(c) 1 OCHRAQ Dilution Buffer

(3) LF-OCHRAQ-G-500K

(a) 5 containers of 100 OCHRAQ test strips

(b) 5 OCHRAQ Grain Positive Controls

(c) 5 OCHRAQ Dilution Buffers

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

(1) 100 µL pipet and pipet tips

(2) 300 µL pipet and pipet tips

(3) 1000 µL fixed volume pipet or 100 to 1000 µL variable volume pipet and pipet tips

(4) 100 and 1000 mL graduated cylinders

(5) Balance

(6) Deionized or distilled water

(7) Methanol (reagent grade or better)

(8) Micro-centrifuge tubes and rack

(9) Mini-centrifuge

(10) ROSA Incubator

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

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

(13) Sample extraction containers or Whirl-pak bags

(14) Sample grinder

(15) Storage bottle

(16) Syringes

(17) Syringe filters

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

(b) Minisart RC15 syringe filters (Sartorius Minisart RC 15, Part No. 17762)

(18) Transfer pipets

REVISION HISTORY

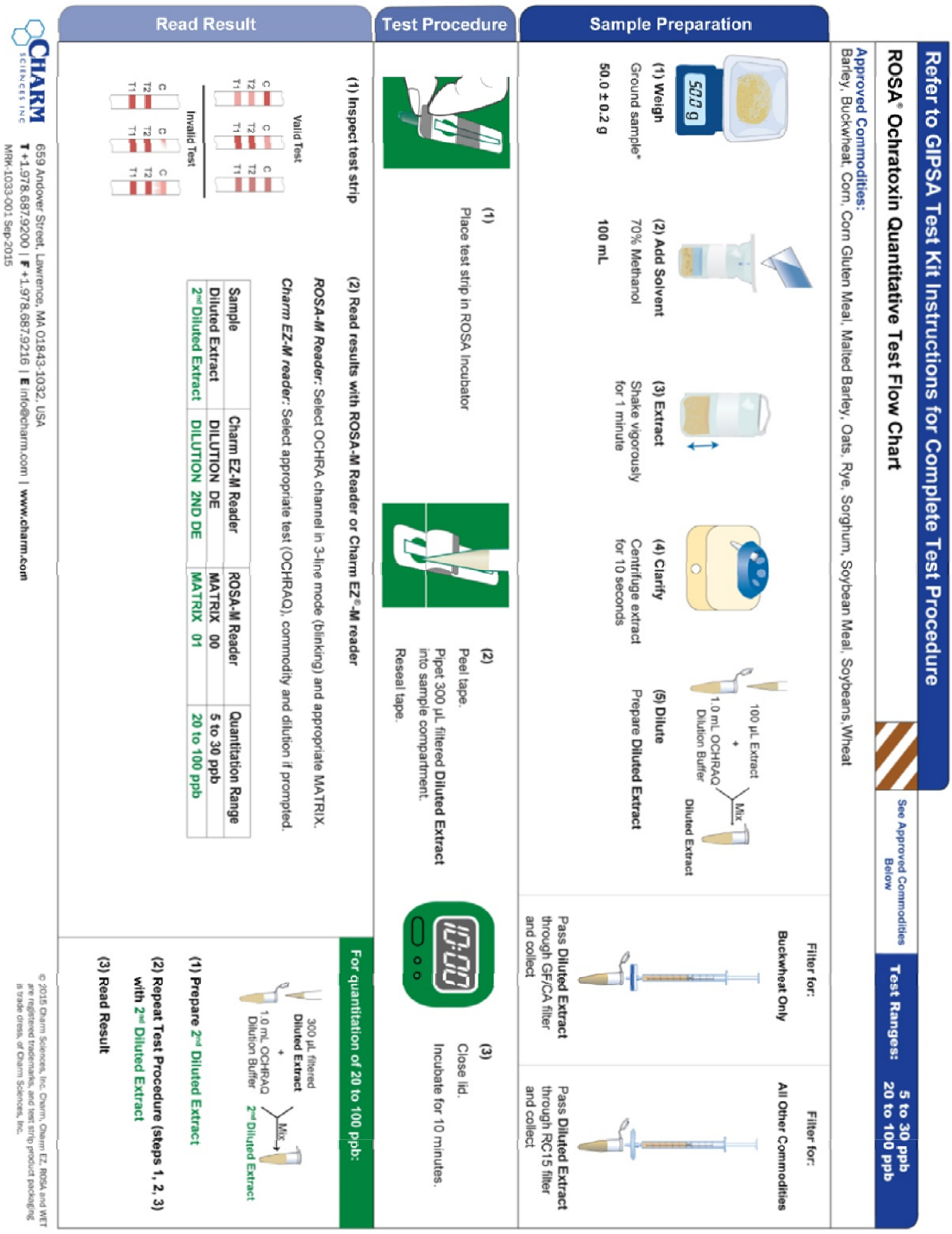
Revision 0 (03/30/2015)

Revision 1 (11/19/2015)

- The ROSA Ochratoxin Quantitative Test kit was recertified and both ROSA-M Reader and Charm EZ-M reader were approved for reading the test strips.

- Buckwheat and soybeans were approved as additional commodities and the test procedure has been incorporated in this revision.

FLOW CHART


Refer to GIPSA Test Kit Instructions for Complete Test Procedure
ROSA® Ochratoxin Quantitative Test Flow Chart
See Approved Commodities Below
Test Ranges: 5 to 30 ppb 20 to 100 ppb
Approved Commodities:
Barley, Buckwheat, Corn, Corn Gluten Meal, Malted Barley, Oats, Rye, Sorghum, Soybean Meal, Soybeans, Wheat
Sample Preparation
50.0 g
(1) Weigh
Ground sample*
50.0 ± 0.2 g
(2) Add Solvent
70% Methanol
100 mL
(3) Extract
Shake vigorously for 1 minute
(4) Clarify
Centrifuge extract for 10 seconds
100 µL Extract
+
1.0 mL OCHRAQ Dilution Buffer
Mix
Diluted Extract
(5) Dilute
Prepare Diluted Extract
Filter for:
Buckwheat Only
Pass Diluted Extract through GF/CA filter and collect
Filter for:
All Other Commodities
Pass Diluted Extract through RC15 filter and collect
Test Procedure
(1)
Place test strip in ROSA Incubator
(2)
Peel tape.
Pipet 300 µL filtered Diluted Extract into sample compartment.
Reseal tape.
10:00
(3)
Close lid.
Incubate for 10 minutes.
For quantitation of 20 to 100 ppb:
300 µL filtered Diluted Extract
+
1.0 mL OCHRAQ Dilution Buffer
Mix
2nd Diluted Extract
(1) Prepare 2nd Diluted Extract
(2) Repeat Test Procedure (steps 1, 2, 3) with 2nd Diluted Extract
(3) Read Result
Read Result
(1) Inspect test strip
Valid Test
C T2 T1
C T2 T1
Invalid Test
C T2 T1
C T2 T1
(2) Read results with ROSA-M Reader or Charm EZ®-M reader
ROSA-M Reader: Select OCHRA channel in 3-line mode (blinking) and appropriate MATRIX.
Charm EZ-M reader: Select appropriate test (OCHRAQ), commodity and dilution if prompted.
Sample | Charm EZ-M Reader | ROSA-M Reader | Quantitation Range
Diluted Extract | DILUTION DE | MATRIX 00 | 5 to 30 ppb
2nd Diluted Extract | DILUTION 2ND DE | MATRIX 01 | 20 to 100 ppb
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