

Romer Labs
AgraStrip Zearalenone Quantitative Test WATEX

TABLE OF CONTENTS	Page
GENERAL INFORMATION.....	1
PREPARATION OF TESTING MATERIALS AND EQUIPMENT.....	2
SAMPLE PREPARATION AND EXTRACTION PROCEDURES.....	2
TEST PROCEDURES.....	3
REPORTING AND CERTIFYING TEST RESULTS.....	4
STORAGE CONDITIONS AND PRECAUTIONS	5
EQUIPMENT AND SUPPLIES.....	5
REVISION HISTORY.....	6
FLOW CHARTS	7

GENERAL INFORMATION

The AgraStrip Zearalenone (ZON) Quantitative Test WATEX is a one-step lateral flow immunochromatographic assay for the quantitative screening of Zearalenone in samples. The test is based on a competition immunoassay format. Antibody-colloidal gold complex (conjugate) lyophilized in a microwell is mixed with sample extract. A Zearalenone strip is placed into the microwell. The mixed content is then wicked onto a membrane of the strip, which contains a test zone and a control zone. The test zone captures free antibody-particle complex (conjugate), allowing color particles to concentrate and form a visible line. The color intensity of the line is inversely proportional to the concentration of Zearalenone in the sample. The line is always visible in the control zone regardless of the presence of Zearalenone. The strips are measured using an AgraVision Reader and the results are determined.

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, reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPBAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@usda.gov.

Approved Test Kit Information

Test Kit Vendor:	<i>Romer Labs, Inc. (636) 583-8600</i>
Test Kit Name:	AgraStrip Zearalenone Quantitative Test WATEX
Product Number:	COKAS5000W
Effective Date of Instructions:	8/24/2016
Instructions Revision Number:	0
Conformance Range:	100 – 1000 ppb
No. of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Approved Commodities:	Corn (including Dent or Field Corn, Corn Meal, Corn Flour, Cracked Corn, Corn Grits or Polenta, and Corn screenings)
Extraction method:	Shake vigorously 50-gram sample with 150 milliliters (mL) of distilled or deionized water and one buffer packet by hand for 2 minutes or shake with similar shaking action (as hand shaking) for 2 minutes using a mechanical shaker.
Test Format:	Lateral Flow Strip
Detection Method:	AgraVision Reader, Model No. EQASR1000

PREPARATION OF TESTING MATERIALS AND EQUIPMENT

All reagents and kit components must be at room temperature 20-24°C (68-75°F) before use.

AgraStrip Incubator

The temperature of AgraStrip Incubator is set at 45°C.

Set the timer on the incubator to three minutes using the arrow keys.

Any time the incubator is open, set the cover in the groove provided on the top of the heating block. This will ensure that the temperature remains constant.

Note: It is recommended that the incubator be turned on in the morning and kept on throughout the whole day. The incubator must be switched on at least 15 minutes before use.

AgraVision Reader

- (1) Turn on the AgraVision reader using the power button located on the back.
- (2) Use the arrow keys on the keypad to highlight “TEST”. Select it using the checkmark key.
- (3) Use the arrow keys on the keypad to highlight “Mycotoxin”. Select it using the check mark key. The barcode scanner will turn on.
- (4) Separate bar codes for corn and wheat as well as the different quantitation ranges are provided in the test kit. Scan the applicable barcode for the commodity to be tested and the range to be used.
- (5) Create a sample ID by using the alphanumeric keys on the keypad. Use the checkmark key to enter.
- (6) Since only one strip is being read, use the pound key to bypass entry for the second sample.
- (7) Enter the operator ID. Press the checkmark key to enter, and press it a second time to move to the next screen.
- (8) The reader is ready to read and will display “start measurement”.

SAMPLE PREPARATION AND EXTRACTION PROCEDURES

Standard Extraction Procedure for corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings)

- (1) Weigh out 50.0 ± 0.2 grams ground sample into one side of a filtering Whirl-Pak bag.
- (2) Place one soluble buffer bag onto the ground sample in the Whirl-Pak bag.

- (3) Add 150 mL of distilled or deionized water to the side of the bag with the sample and buffer bag, and securely close Whirl-Pak bag.
- (4) Shake the closed Whirl-Pak bag vigorously by hand for 2 minutes or shake with similar shaking action (as hand shaking) for 2 minutes using a mechanical shaker.
- (5) Allow sample to settle for 2 minutes to obtain the filtered extract (take extract from the opposite side of the filter from the sample). The extract must be used (diluted) within **5 minutes**.
- (6) Dilute the filtered extract 1:21 with dilution buffer. (For example, use a blue pipette tip to add 1000 μ L of dilution buffer to a dilution tube. Then use a yellow or white pipette tip to add 50 μ L of extract into the dilution tube, and mix well).
- (7) Close the cap of the tube, mix well by inverting a few times and the sample is ready for assay. The diluted extract must be used within **1 hour**.

TEST PROCEDURES

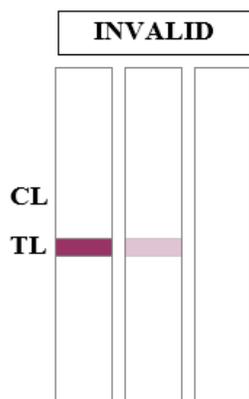
a. Analysis Procedure

IMPORTANT: Analyze only one sample at a time. Make sure the cover is seated on the top of the heater block any time the incubator is uncovered.

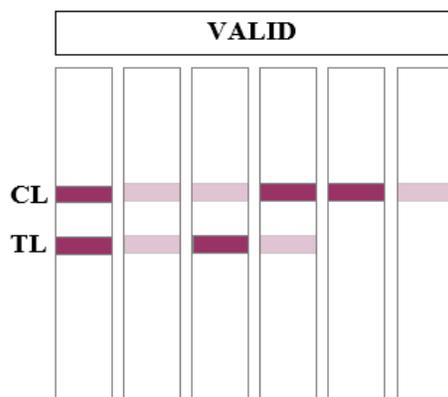
- (1) Break off one conjugate coated microwell and remove its sealing cap. Place the conjugate microwell inside the heat block in one of the holes, ensuring that it is fully seated in the heat block.
- (2) Add 100 μ L of **diluted sample extract** to the conjugate microwell.
- (3) Mix the content in microwell by **pipetting it up and down 10 times**.
- (4) Put one test strip into the microwell. Place the cover back into the heat block over the microwell and the test strip.
- (5) Allow the test strip to develop color for 3 minutes. Lift the heat block cover and remove the test strip (Remember to place the cover on top of the heater block while the incubator is open).
- (6) Wipe the end of the strip test onto an absorbent paper and insert the strip into the strip holder-tray for reading.
IMPORTANT: The test strip must be read immediately (within 1 minute) after incubation is finished.
- (7) Insert the tray into the reader. Press the checkmark key to read.
- (8) After completion of reading, press the checkmark key to save the result in the AgraVision Reader's memory or the pound key to print the result.
Note: after the test, the used microwell can be removed with tweezers.

b. Interpretation of Results

- (1) A color line always appears in the upper section of the test strip to indicate that the test strip is working properly. This line is the Control Line (CL). A line in the lower section of the test strip indicates the test result. This line is the Test Line (TL).
- (2) **Invalid results:** If there is no control line in the control zone, the test is invalid and the sample should be re-tested by using a new test strip. The AgraVision Reader will also indicate “invalid” if the strip is invalid.



- (3) **Valid results:** 2 lines are visible. The intensity of the line in the test zone is dependent on Zearalenone concentration in the sample and must be measured with an AgraVision Reader.



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 kits at 2-8°C (35-47°F) when not in use, and do not use beyond the expiration date. Do not freeze. Do not leave it in direct sunlight.
- (2) Test strips must be kept inside their original tubes.
- (3) Conjugate microwells must be kept inside their original tubes.

b. Precautions

- (1) All reagents must be at room temperature before the assay is running.
- (2) Adhere to the instructions of test procedures.
- (3) Do not re-use test strips and conjugate wells.
- (4) Consider all materials, containers and devices that are exposed to the sample to be contaminated with toxin. Wear protective gloves and safety glasses when using the kit.
- (5) The components in this test kit have been quality control tested as a standard batch unit. Do not mix components from different lot numbers.

EQUIPMENT AND SUPPLIES

a. Materials Supplied with the Kit

- (1) 1 tube containing 24 AgraStrip Zearalenone test strips
- (2) 1 tube containing 24 AgraStrip Zearalenone WATEX Conjugate wells with lyophilized antibody particle complex (conjugate)
- (3) 1 bag containing 24 AgraStrip WATEX Extraction Buffer Bags
- (4) 1 bottle of 30 mL AgraStrip Zearalenone WATEX Dilution Buffer
- (5) 1 bag of 48 yellow or white pipette tips
- (6) 1 bag of 24 micro centrifuge tubes (dilution tubes)
- (7) 24 Filter Whirl-Pak bags

b. Materials Required but not Provided with Kit

- (1) Romer Series II Mill or equivalent, EQOLE1010
- (2) Balance, 400 grams
- (3) EQOLE1050: 250 mL graduated cylinder
- (4) Distilled or deionized water

c. Assay Procedure

- (1) Single channel pipette capable of pipetting up to 100 μ L
- (2) Single channel pipette capable of pipetting up to 1000 μ L
- (3) EQOLE1300: Timer
- (4) EQASR1003: AgraVision Reader without printer or EQASR1000: AgraVision Reader with printer
- (5) EQOEV2060: AgraStrip Incubator with timer or EQASR1005: AgraStrip heat block with cover

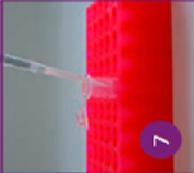
REVISION HISTORY

Revision 0 (8/24/2016)

FLOW CHART

AgraStrip® Mycotoxin WATEX Quick Guide

Procedure of AgraStrip® Zearalenone Quantitative Test WATEX

 <p>1 Preheat AgraStrip® Incubator at least 15 minutes until 45°C is reached.</p>	 <p>2 Weigh-in 50 g of ground sample into one side of a Filter Whirl-Pak® Bag. Add one Extraction Buffer Bag.</p>	 <p>3 Add 150 ml of distilled water to the Filter Whirl-Pak® Bag.</p>	 <p>4 Shake vigorously for 2 minutes.</p>	 <p>5 Let sample settle for 2 minutes.</p>	<p>Result Interpretation A color line always appears in the upper section of the test strip to indicate that the test strip is working properly. This line is the control line.</p> <p>Invalid results: If there is no control line in the control zone, the test is invalid and the sample should be re-tested by using a valid test strip.</p> <p>Valid results: 2 lines are visible. The intensity of the line in the test zone is indirectly proportional to the mycotoxin concentration and has to be measured with the AgraVision™.</p>
 <p>6 Add dilution buffer into a dilution tube at a ratio of 1:21, e.g. 1000 µl dilution buffer.</p>	 <p>7 Take 50 µl of sample extract and transfer into the dilution tube containing 1000 µl dilution buffer. Close tube and mix well.</p>	 <p>8 Put microwell into the AgraStrip® Incubator and add 100 µl diluted sample extract. Mix the content by pipetting it up and down 10 times.</p>	 <p>9 Put a test strip into the well, close the AgraStrip® Incubator lid and allow the strip to develop color for 3 minutes.</p>	 <p>10 Wipe strip onto an absorbent paper and immediately read with AgraVision™ reader.</p>	