

ROMER LABS
AgraStrip Total Aflatoxin Qualitative Test
(20 ppb Cutoff)

TABLE OF CONTENTS	PAGE
GENERAL INFORMATION	1
PREPARATION OF TESTING MATERIALS.....	2
SAMPLE PREPARATION AND EXTRACTION PROCEDURES	2
TEST PROCEDURES	3
SUPPLEMENTAL ANALYSIS.....	4
REPORTING AND CERTIFYING TEST RESULTS	4
STORAGE CONDITIONS AND PRECAUTIONS.....	4
EQUIPMENT AND SUPPLIES	5
REVISION HISTORY	5

GENERAL INFORMATION

The AgraStrip Total Aflatoxin Test is a one-step lateral flow immunochromatographic assay with a detection threshold of 20ppb aflatoxins based on an inhibition immunoassay format. Antibody-particle complex is dissolved in assay diluent and mixed with sample extract. The mixed content is then wicked onto a membrane, which contains a test zone and a control zone. The test zone captures free antibody particle complex, allowing color particles to concentrate and form a visible line. A positive sample with aflatoxin above the detection threshold will result in no visual line in the test zone. Alternatively, a negative sample with aflatoxin below the detection threshold will form a visible line in the test zone. The line will always be visible in the control zone regardless of the presence of aflatoxins.

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 current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division 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:	<i>Romer Labs, Inc. (636) 583-8600</i>
Test Kit Name:	AgraStrip Total Aflatoxin Qualitative Test (20 ppb Cutoff)
Product Number:	COKAS1000U
Effective Date of Instructions:	07/07/2015
Instructions Revision Number:	0
Detection Threshold:	20 ppb
Type of Service:	Qualitative
Supplemental Analysis:	No
Approved Commodities:	Corn
Extraction Method:	Shake 50-gram sample for 1 minute with 100 milliliters (mL) 70% methanol/30% distilled or deionized water (v/v) or shake 50-gram sample for 1 minute with 100 milliliters (mL) 50% ethanol/50% deionized water (v/v).
Test Format:	Lateral Flow Strip
Detection Method:	Visual

PREPARATION OF TESTING MATERIALS

**a. Preparation of 70% Methanol/30% water (v/v) Extraction Solvent:
70% Methanol/ 30% water may be purchased pre-mixed.**

- (1) Alternatively a 70% methanol/30% deionized or distilled water solution (v/v) can be made as follows.
- (2) Using a graduated cylinder, measure 700 mL of methanol (ACS reagent grade or better) and place it into a glass bottle or carboy.
- (2) Add 300 mL deionized or distilled water to the methanol and shake vigorously until completely mixed.
- (3) Label the container stating the mixture (70% methanol/30% water), date of preparation and initials of technician that prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container.
- (5) To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

b. Preparation of 50%Ethanol/50% water (v/v) Extraction Solvent:

- 2) Using a graduated cylinder, measure 500 mL of ethanol (ACS reagent grade or better) and place it into a glass bottle or carboy.
- (2) Add 500 mL deionized or distilled water to the ethanol and shake vigorously until completely mixed.
- (3) Label the container stating the mixture (50% ethanol/50% water), date of preparation and initials of technician that prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container.
- (5) To prepare smaller or larger amounts of solution use the ratio of 5 parts ethanol to 5 parts of deionized or distilled water.

SAMPLE PREPARATION AND EXTRACTION PROCEDURES

Extraction Procedure for Corn:

- (1) Weigh out 50 grams (± 0.2) of ground sample into a Whirl-Pak bag.
- (2) Add 100 mL of 70% methanol/30% water extraction solvent or 100 mL of 50% ethanol/50% water extraction solvent and securely close the Whirl-Pak bag.

- (3) Shake vigorously the securely closed Whirl-Pak bag for 1 minute by hand or by mechanical shaker with similar shaking action.
- (4) Allow sample to settle for 3 minutes. The clear upper layer of supernatant should be used for the test.

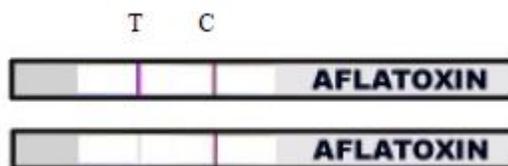
TEST PROCEDURES

a. Analysis Procedure

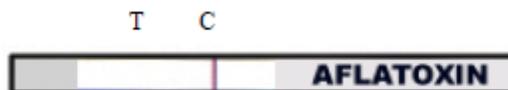
- (1) Remove microwell sealer, and place the appropriate number of microwells in a microwell holder. Re-seal those un-used microwells.
- (2) Using a single channel pipette, add 50 μ L of assay diluent to each microwell. Dissolve the coating conjugate in the microwell by pipetting the content up and down 5 times.
- (3) Add 50 μ L of sample extracts to each microwell, mixing the content in each well by pipetting it up and down 3 times.
- (4) Put one test strip into one well.
- (5) Allow the test strip to develop color for 5 minutes.
- (6) Interpret test results immediately.

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 (C). A line in the lower section of the test strip indicates the test results. This line is the Test Line (T).

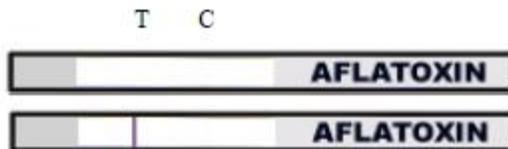


- (2) **Results less than 20ppb:** 2 lines are visible. This indicates the sample contains total aflatoxin less than 20ppb (negative sample). A negative sample may be determined as soon as 2 lines are visible on the strip. (Please see following image)



- (3) **Results greater than 20ppb or equal to 20ppb:** 1 line is visible. This indicates the sample contains total aflatoxin great than or equal to 20 ppb (positive sample). (Please see following image).

- (4) **Invalid results:** If there is no line in control zone, the test is invalid and the sample should be re-tested by using a valid test strip. (Please see following image).



NOTE: For some samples, a third line may appear on the strip at a position of approximately 0.4cm from the bottom portion of the AgraStrip. Please note that if the line at the bottom of the strip appears, it is not a valid line and can be ignored. Only the Test line and the Control line, which correspond to the letters "T" and "C" on the back of strip respectively, are used in interpreting the results. The Control line must always be present for the test to be valid.

SUPPLEMENTAL ANALYSIS

N/A

REPORTING AND CERTIFYING TEST RESULTS

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@udsa.gov).

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions

- (1) Store test kits at 2-25°C (36-77°F) when not in use, and do not use beyond the expiration date.
- (2) Test strips and conjugate coated microwells must be kept inside their original tubes.

b. Precautions

- (1) All reagents must be at room temperature before the assay is run.
- (2) Adhere to the instructions of test procedures.
- (3) Do not re-use test strips and conjugate wells.
- (4) Methanol is flammable. Caution must be taken in its use and storage.
- (5) 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.
- (6) 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 aflatoxin test strips
- (2) 1 tube containing 24 microtiter wells coated with antibody-particle complex (conjugate)
- (3) 1 bottle of 1.8mL assay diluent
- (4) 1 piece of microwell holder
- (5) 1 bag of 48 pieces of yellow pipette tips
- (6) 1 roll of 24 pieces of Whirl-Pak bags for 50 gram sample extraction

b. Materials Required but not Provided with Kit

Extraction Procedure

- (1) EQMMS2010: Romer Series II Mill or equivalent
- (2) EQOLE1025: Blender or a tightly sealing jar with lid
- (3) EQOLE1010: Balance, 400 grams
- (4) EQOLE1050: Graduated cylinder: 100 mL
- (5) Premixed 50% ethanol or Formula 3A ethanol for making 50 % ethanol
- (6) Distilled or de-ionized water. Single channel pipette capable of pipetting up to 50 μ L
- (7) Single channel pipette capable of pipetting up to 50 μ L
- (8) EQOLE1300: Timer

REVISION HISTORY

Revision 0 (07/07/2015)