

NEOGEN
REVEAL Q+ FOR AFLATOXIN
USING ACCUSCAN III and ACCUSCAN PRO READERS

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GENERAL INFORMATION

The REVEAL Q+ FOR AFLATOXIN test method provided by the Neogen Corporation is a single-step lateral flow immunochromatographic assay based on a competitive immunoassay format. The test provides quantitative analysis for the presence of Aflatoxin. Using an aflatoxin-antibody particle complex coated test strip and the Neogen AccuScan III and AccuScan Pro readers.

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>Neogen Corporation 800/234-5333</i>
Test Kit Name:	REVEAL Q+ FOR AFLATOXIN
Product Number:	8085
Effective Date of Instructions:	2/2/2015
Instructions Revision Number	0
Conformance Range:	5 – 100 ppb
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	Yes
Approved Commodities:	Corn, brewers rice, rough rice, corn flaking grits, corn germ meal, corn gluten meal, corn meal, corn screenings, corn soy blend, corn starch, cracked corn, distillers dried grains with solubles (DDGS) , popcorn, and sorghum.
Extraction method:	<u>Shake only</u> : 50 gram sample with 125 mL of 65% Ethanol / 35% distilled or deionized water (v/v) for 3 minutes.
Test Format:	Lateral Flow Strip
Detection Method:	AccuScan III and AccuScan Pro Readers

PREPARATION OF TESTING MATERIALS

AccuScan III Reader Set-up:

- (1) The AccuScan III reader must have software version 4.21 or newer to analyze the Reveal Q+ tests. Contact Neogen for more information.
 - a. Launch the AccuScan III program on the reader.
 - b. Select the Mycotoxin Q+ category and Q+ Aflatoxin test.
 - c. Enter a sample ID (optional).
 - d. Enter the test kit lot number and A, B, C and D values from the test kit box or select from the history for previously entered lot number details.

Note: Technicians must verify/update test kit lot number in the AccuScan III reader program matches the lot number of the test strips in use before testing.

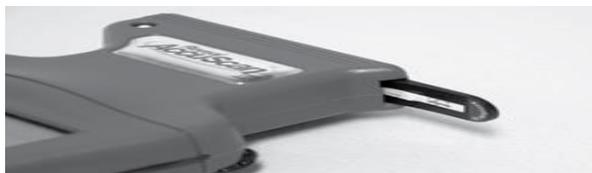
- (2) Reading Test Results with the AccuScan III Reader.

Test strips should be read within 1 minute after completion of the 6 minute incubation period. Refer to Reveal AccuScan III manual for detailed set-up and selection information.

- a. Fully insert the Reveal Q+ test strip into the black cartridge adapter with the sample end first and results facing out.



- b. Insert the cartridge with test strip upside-down into the reader (the test lines will face downward into the reader).



- c. The reader's green light will glow when a cartridge is inserted, and will automatically begin analyzing the cartridge.
- d. The AccuScan III reader will analyze the test strip. Test results will be displayed and stored in the reader.

Note: Refer to the AccuScan III manual for more detailed instructions.

AccuScan Pro Reader Set-up:

The system provides an easy method to objectively read, store, and analyze results from Neogen's line of lateral flow strips.



Note: Please keep and store all packaging materials included in the kit for future storage.

(1) Set-up:



Press the Home icon at any time to return to the main screen.

- a. Plug the outlet of the cord into the power source. Plug the small end of the power cord into the reader.
- b. This will turn the reader on. To turn the reader off, unplug the reader.

Note: To place in the power down mode, press the small power icon in the lower right corner of the Home screen. The screen will turn white and the reader may then be unplugged.

- c. Plug the USB key into the USB port on the side of the reader.



USB, Ethernet, and power cord ports



Start up screen



Home screen

(2) Settings: 

Press the designated icon to set time, date, volume, remote connection, language setting, and allows the user to review current information.



(3) QR Codes: 

The reader is capable of reading information from predefined QR codes. Disposable QR codes containing lot specific information are included in each Reveal Q+ kit.



- a. Whenever a new lot is obtained, and before you run a test, you must verify the QR codes. Simply place the QR code card into the QR code cartridge. From the **Home screen**, select the QR code icon and place the QR code cartridge containing the card in the reader. The specific information automatically will be downloaded into the reader.
- b. The specific QR code cartridges are entered into the reader to identify unique users with a five digit user ID code.

Note: The lot number in the AccuScan Pro reader must match the lot number of test strips being analyzed.

- **Technicians must update or verify reader information (lot number of test strips in use) before official testing.**
- **Refer to the AccuScan Pro manual for more detailed instructions.**

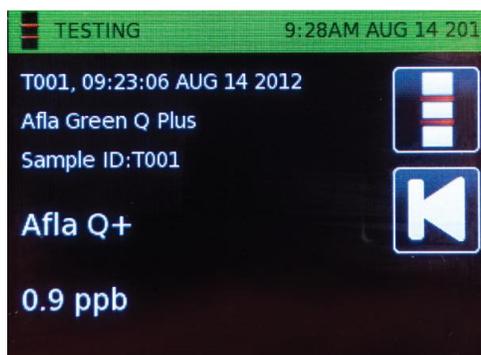
(4) Run test protocol: 

- a. Touch the **Run test** icon, which looks like a test strip, on the **Home screen**.
- b. The test category will display in the first box and the test type will display in the second box. The reader will default to the last test analyzed.

- c. Select the appropriate test category by touching the desired option.
- d. Touch the green check mark to confirm the selection.
- e. Touch the **Test type** icon. Select the desired test by touching the name of the test (e.g., Q+ Aflatoxin). To scroll through the test options, use the white up and down arrows. Touch the green check mark to confirm the selection.
- f. Insert the test strip to begin interpreting the test or enter the sample ID by touching the **Sample ID** button. **NOTE:** Strips may be tested without a sample ID. To do this, insert the test strip cartridge containing the test strip into the reader square end first. The reader automatically will begin analyzing the strip.
- g. If entering a sample ID, type the ID in and press the green check mark to continue.
- h. Insert the test strip into the appropriate strip cartridge. Insert the cartridge containing the strip into the slot on the lower left portion of the reader. The reader automatically will begin analyzing the strip.



- (5) Test Results:
- a. For a standard positive/negative test, the screen will display results as a +POS for a positive sample and – NEG for a negative sample. For a quantitative test, a numerical value will be displayed.
 - b. Touch the screen to view additional test information such as test line and control line ratios.



- c. To print, select the **Print** icon. A report will automatically be sent to the attached printer.
- d. To begin/run a new test, select the “**New test**” icon. This will return the user to the test setup screen.

Reader Notes and Cautions:

- Ensure device is fully inserted into cartridge.
- Removing the cartridge prior to completion can result in invalid readings.
- Reading should be made between 6 and 7 minutes. Reading results after 7 minutes may be inaccurate due to over development of the device.
- The strips must be read using Neogen’s Reveal AccuScan III or AccuScan Pro Readers.

EXTRACTION PROCEDURES

- (1) **Preparation of Extraction Solvent: Ethanol/Water (65/35, v/v):**
 - a. Using a 1000 mL graduated cylinder, measure 650 mL of ethanol and carefully transfer into a clean 1000 mL bottle.
 - b. Using a 500 mL graduated cylinder, measure 350 mL of distilled or deionized water and add into the bottle containing ethanol. Shake until completely mixed.
 - c. Label the container stating the mixture contained, date of preparation, and initial of the analyst who prepared the solvent.
 - d. Store the solvent in a tightly closed container at room temperature until needed.

(2) **Extraction Procedures for: Corn, cracked corn, corn flaking grits, corn germ meal, corn gluten meal, corn meal, corn screenings, corn starch, corn/soy blend, popcorn, brewers rice, and sorghum, rough rice.**

- a. Transfer 50 g \pm 0.2 of ground sample into an extraction mixing jar.
- b. Add 125 mL of extraction solvent.
- c. Cover the extraction jar and shake vigorously by hand or mechanical shaker (with similar hand shaking motion) for 3 minutes.
- d. Allow the sample to settle for 3 minutes. Then filter 3 – 5 mL of the extract with a filter syringe (Neogen item #9420) into a clean sample collection tube labeled with the sample identification.
- e. Dilute the filtered sample 1:1 by adding 1 mL of the filtered sample to 1 mL of extraction solvent in a new test tube. Vortex for 10 seconds. This **diluted filtered extract** is ready for testing.
- f. Proceed to “**Test Procedures**” section.

(3) **Extraction Procedures for: Distillers Dried Grains with Solubles (DDGS).**

- a. Transfer 50 g \pm 0.2 of ground sample into an extraction mixing jar.
- b. Add 75 mL of extraction solvent.
- c. Cover the extraction jar and shake vigorously by hand or mechanical shaker (with similar hand shaking motion) for 3 minutes.
- d. Allow the sample to settle for 3 minutes. Then filter 3 – 5 mL of the extract with a filter syringe (Neogen item #9420) into a clean sample collection tube labeled with the sample identification.
- e. After collecting the filtrate (**filtered sample**), dispose of the filter syringe and ground material according to waste disposal guidelines.
- f. Check the pH of the filtered extract. If pH is not in between 7.0 and 8.0 it needs to be adjusted.

To adjust pH:

- (1) Using a disposable polyethylene transfer pipette, add one drop of 1N NaOH (sodium hydroxide) to the sample extract, vortex or swirl to mix, and check the pH with pH test strip.
- (2) If pH is still below 7.0, add another drop of 1N NaOH, mix, and check pH again. Continue this process until the pH falls between 7.0 and 8.0, and then

proceed to dilution procedure.

- g. Dilute the filtered sample 1:1 by adding 1 mL of the filtered sample to 1 mL of extraction solvent in a new test tube. Vortex for 10 seconds. This **diluted filtered extract** is ready for testing.
- h. Proceed to “**Test Procedures**” section.

TEST PROCEDURES

a. Analysis Procedure.

- (1) Place the appropriate number of red sample dilution cups and clear sample cups for each test sample in the sample cup rack. Label cups if necessary.
- (2) Using a single-channel pipettor with a new pipette tip, add 500 microliters (μL) of sample diluent to each red sample dilution cup.
- (3) Using a new pipette tip, add 100 μL of sample extract into each red dilution cup containing 500 μL sample diluents. Mix by swirling with the pipette tip and then by pipetting up and down 5 times.
- (4) Transfer 100 μL of diluted sample extract into a new clear sample cup.
- (5) Place a new Reveal Q+ for Aflatoxin test strip with the sample end down into the sample cup. Start timer and incubate for 6 minutes.
- (6) At the end of the 6 minute incubation/development period, remove the test strip from the sample cup. Read the test strip within one minute using only Neogen’s AccuScan III or AccuScan Pro Reader.

b. Reading the Results

- (1) The strips must be immediately read using Neogen’s AccuScan III or AccuScan Pro Reader to analyze test strip. Test results will be displayed and stored in the reader.
- (2) Reading should be made between 6 and 7 minutes. Reading results after 7 minutes may be inaccurate due to over development of the device and should not be reported.
- (3) Fully inserted the Reveal Q+ test strip into the reader specific black cartridge adapter with the sample end first and results facing out.
- (4) Insert the cartridge with test strip side up into the AccuScan.

SUPPLEMENTAL ANALYSIS

Supplemental analysis (corn only) 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 aflatoxin test kits is 5 – 100 ppb. Therefore, supplemental analysis would be performed for a result above 100 ppb. In supplemental analysis, the extract is diluted so the resulting concentration is between the lower and upper limits of the test kit evaluation range (i.e., 5 – 100 ppb for aflatoxins), and a correction for dilution is applied to derive at the final result. Supplemental analysis is performed only at the request of the applicant.

Supplemental Dilution Procedure:

- (1) Combine 100 µL filtered extract with 100 µL of extraction solvent.
- (2) Mix by swirling or pipetting up and down 5 times. This is the diluted filtered extract used for supplemental analysis.
- (3) Refer to Test Procedures section for analysis.
- (4) Read and record results on the work record, then multiply the analytical results obtained from the AccuScan reader by 2 to obtain the actual Aflatoxin concentration of the original test sample (show all results on the work record).

Example: If the filtered extract is diluted 1:1 (v/v) using extraction solvent, the dilution factor is two (2).

AccuScan reader supplemental analysis results:	90 ppb
Multiplied by the dilution factor	<u>x 2</u>
Sample results - TOTAL:	180 ppb

A final result less than 53 ppb 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 100 ppb.

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

Store the kit components at room temperature (18-30°C, 64-86°F) to ensure full shelf life. Test strips should remain capped in their original tubes until used to ensure optimal performance.

b. Precautions

- (1) Do not use test kit components beyond the expiration date.
- (2) Test strip development times, other than those specified in Test Procedures section, may give inaccurate results.
- (3) Treat all used liquids, including sample extract, and labware as if contaminated with Aflatoxin, gloves and other protective apparel should be worn at all times.
- (4) To avoid cross-contamination, use clean glassware for each sample and thoroughly wash all glassware between samples.
- (5) Ensure the device, lot number and curve details match the lot ID number selected on the reader. Failure to update the lot-specific QR code within the AccuScan reader will cause inaccurate results.

EQUIPMENT AND SUPPLIES

a. Materials provided in test kits.

- (1) 25 Reveal Q+ for Aflatoxin test strips.
- (2) 25 red sample dilution cups.
- (3) 25 clear sample cups.
- (4) 1 bottle of sample diluent.

b. Materials required but not provided.

- (1) Timer. (Neogen item #9426)
- (2) 100 µL pipettor (or equivalent) with pipette tips.
- (3) 500 µL pipettor (or equivalent) with pipette tips.
- (4) Sample collection cups with lids. (Neogen item #9428)
- (5) Reveal sample rack. (Neogen item #9475)

- (6) Reveal AccuScan III Reader (Neogen item #9590)
- (7) Reveal AccuScan Pro Reader (Neogen item #9565)
- (8) Disposable polyethylene transfer pipettes.
- (9) 65% Ethanol, reagent grade or better (Neogen item #8073, #8074)
- (10) Dispensing pump or graduated cylinder. (Neogen item #9448, #9447)
- (11) Sodium Hydroxide (1N NaOH)
- (12) pH test strips (Neogen item #9478)
- (13) Filter syringe (Neogen item #9420)
- (14) Sample grinder.
- (15) Scale capable of weighing 5 – 50 grams.
- (16) Bottle, 1 Liter. (Neogen item #9472)

REVISION HISTORY

- Revision 0 (2/2/2015)