

NEOGEN
REVEAL Q+ FOR AFLATOXIN GREEN
WITH ACCUSCAN PRO READER

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GENERAL INFORMATION

The REVEAL Q+ FOR AFLATOXIN GREEN 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 non-hazardous, water-based extraction solution along with an aflatoxin-antibody particle complex coated test strip and the Neogen AccuScan Pro reader.

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 (PPMB) 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 PPMB by phone at 816-891-8403 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 GREEN
Product Number:	8086
Effective Date of Instructions:	11/07/2014
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, corn bran, corn flour, corn grits, corn meal, flaking corn grits, hominy, hominy feed, and soybeans
Extraction method:	<u>Blend only:</u> Blend 50 gram sample with 250 mL Green Extraction Solution for 30 seconds.
Test Format:	Lateral Flow Strip
Detection Method:	AccuScan Pro Reader Only

PREPARATION OF TESTING MATERIALS

Extraction Solution:

The extraction solution used in the Reveal Q+ for Aflatoxin Green test method is a water-based, non-hazardous consisting of Neogen Green Extraction Step 1, Step 2, and distilled water.

- (1) Prepare the extraction solution by adding 1 bottle of Green Extraction **Step 1** followed by 1 bottle of Green Extraction **Step 2** (*Shake Green Extraction step 2 before adding to other solutions*) into 1 liter of distilled water. Rinse the green extraction Step 2 bottle twice with extraction mixture to make sure everything is taken into the solution.
- (2) Shake the solution to mix. Ensure all components are added together before mixing.
- (3) Label the container stating the mixture (Green Extraction Solution), date of preparation, and initials of the technician who prepared the solution.

Prepared Green Extraction solution is stable for 5 months when stored at ambient temperature in a tightly closed container until needed. One liter of Green Extraction is enough to run approximately 4 tests. Contact Neogen to order additional Green Extraction components if needed.

Note: See Storage and Precautions section for more information.

AccuScan Pro Reader Set-up:

- (1) Enter the lot-specific QR code by selecting the QR code icon on the reader.
- (2) Place the QR code into the cartridge and insert the cartridge into the reader.
- (3) Confirm the number on the screen matches the one provided in the Q+ Aflatoxin Green box or on the CoA.

Note: For instructions on manually entering sample IDs, calibration/normalization see the AccuScan Pro user manual, and if needed contact Neogen

- (4) Return to the home screen and select the test strip icon.
- (5) Touch the mycotoxin category.
- (6) Select the Q+ Aflatoxin Green test type.
- (7) Ensure that the correct lot number appears on the screen for the lot that is being used.

SAMPLE PREPERATION AND EXTRACTION PROCEDURES

Standard Extraction Procedure for corn, corn bran, corn flour, corn grits, corn meal, flaking corn grits, hominy, hominy feed, and soybeans:

- (1) Weigh 50 ± 0.2 grams ground samples into a blender cup.
- (2) Add 250 mL of Green Extraction Solution and close the blender securely to prevent spillage.
- (3) Blend for 30 seconds.
- (4) Filter by pouring off some of the extract into a separate cup.
- (5) Fold a Whatman #1 filter into quarters to form a funnel and place the folded filter inside the cup to allow the extract to filter to the inside of the Whatman #1 filter. The sample for testing is taken from the filtered extract inside the Whatman #1 filter. This is called reverse filtration and analyst should be trained before performing the test.

Note: Only for Corn Bran, check the pH of the filtered extract. Ensure that there is enough filtered extract for pH adjustment by allowing the extract to filter for 10 minutes. Using a pipette, transfer the sample into a 15 mL tube. Ideally a pH of 7.0 is preferred; however the tolerable range is between 6.0 and 8.0. To adjust the pH between 6.0-8.0, determine if your sample is either acidic (pH 0.0-6.0) or basic (8.0-14.0)

- a. If acidic: Add 1N NaOH drop-wise to the sample while constantly stirring with swirling motion or vortex action. Check the pH again and keep adjusting until the optimum pH range is achieved.
- b. If basic: Add 1N HCl drop-wise to the sample while constantly stirring with a swirling motion or vortex action. Check the pH again and keep adjusting until the optimum pH range is achieved.
- c. If you have adjusted your sample too far do not adjust with the opposite solvent. Either add more filtered extract to the overly-adjusted sample or begin with a newly filtered sample.

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 Green 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 Reveal AccuScan Pro Reader.

b. Reading the Results

- (1) The strips must be immediately read using Neogen's 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 black cartridge adapter with the sample end first and results facing out.
- (4) Insert the cartridge with test strip side up into the AccuScan.
- (5) The reader will automatically begin analyzing the cartridge.
- (6) The AccuScan Pro reader will analyze the test strip and test results will be displayed and stored in the reader.

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 Green Extraction solution.
- (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 extractions solvent, the dilution factor is two (2).

AccuScan Pro 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 (816-891-8403 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) Mix Green Extraction thoroughly before each use. Green extraction solution normally appears turbid.

EQUIPMENT AND SUPPLIES

a. Materials provided in test kits.

- (1) 25 Reveal Q+ for Aflatoxin Green test strips.
- (2) 25 red sample dilution cups.
- (3) 25 clear sample cups.
- (4) 1 bottle of sample diluent.
- (5) 2 bottles of Green Extraction Step 1.
- (6) 2 bottles of Green Extraction Step 2.

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 Pro Reader (Neogen item #9565)
- (7) Disposable polyethylene transfer pipettes.
- (8) Distilled Water.
- (9) Dispensing pump or graduated cylinder. (Neogen item #9448, #9447)
- (10) Pipette tips, 200, & 1000 μ L.
- (11) Sample grinder.
- (12) Scale capable of weighing 5 – 50 grams.
- (13) Bottle, 1 Liter. (Neogen item #9472)
- (14) Green Extraction kit. (Neogen item # 8087)

REVISION HISTORY

Revision 1 (11/07/2014)

- Eight additional commodities were approved (corn bran, corn flour, corn grits, corn meal, flaking corn grits, hominy, hominy feed, and soybeans) for REVEAL Q+ FOR AFLATOXIN GREEN test. Test procedure for these additional commodities has been incorporated in this revision.
- Removed Carl Jackson as a contact.
- Added Dr. Ajit Ghosh as contact for the Technology and Science Division.