Product 8047

Read instructions carefully before starting test

NeoColumn™ for Aflatoxin DR

Quantitative Test

Refrigerate at 4–30°C (39–86°F). • Do not freeze.

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE
LICENSE NUMBER 081002

THE TOXIN

Aflatoxin is a toxic and carcinogenic substance produced by certain strains of the molds *Aspergillus flavus* and *A. parasiticus*. There are four principle types of aflatoxin: B_1, B_2, G_1 and G_2. Aflatoxin B_1 is the most frequently encountered of the group and the most toxic. The commodities most affected by aflatoxin are corn, peanuts, cottonseed, milo, and the majority of tree nuts.

The effects on animals ingesting excessive amounts of the toxin range from chronic health and performance problems to death. Aflatoxin has been shown to cause liver damage or cancer, decreased milk and egg production, immune suppression, and interference with reproductive efficiency.

Many countries have set maximum allowable levels of aflatoxin in food and feed. Therefore, accurate determination of the toxin's presence is of major importance to those monitoring the quality of food and feed in which aflatoxin may occur. Testing these commodities for the toxin requires careful sampling, chemical extraction, sanitation, and quantitative analysis.

The U.S. Food and Drug Administration (FDA) has issued regulatory levels for aflatoxin as follows:

For	Level	Commodities
Humans	20 ppb	All food except milk
All animal species	20 ppb	All feed (exceptions below)
Exceptions:		
Breeding cattle, breeding swine, mature poultry	100 ppb	Corn
Finishing swine (> 100 lbs.)	200 ppb	Corn
Finishing beef cattle	300 ppb	Corn
Finishing beef cattle, swine, poultry	300 ppb	Cottonseed meal

The European Union has issued regulations for aflatoxin (total $B_1+B_2+G_1+G_2$) as follows:

Foodstuffs	Levels
Groundnuts subject to sorting and treatment before human consumption	15 ppb
Dried fruit subject to sorting and treatment before human consumption Spices Corn subject to sorting and treatment before human consumption Nuts subject to sorting and treatment before human consumption	10 ppb
Groundnuts and nuts for direct human consumption Dried fruit for direct human consumption Cereals and products derived from cereals unless otherwise listed	4 ppb
Baby foods and cereals intended for infants	0.1 ppb

INTENDED USE

NeoColumn™ for Aflatoxin DR (direct read) is intended for the quantitative analysis of aflatoxin in commodities such as corn, cornmeal, rice, almonds and peanuts.

INTENDED USER

This test kit is designed for use by quality control personnel and others familiar with food and feed possibly contaminated by aflatoxin. Since technique is very important, operators should be trained by a Neogen representative or someone who has successfully completed Neogen training.

ASSAY PRINCIPLES

NeoColumn for Aflatoxin DR is an affinity column immunoassay. Aflatoxin is extracted from a ground sample by blending and filtering. Extracted toxin in the filtrate is sampled and diluted with water. The diluted extract is filtered and applied to the column. Positive pressure is used to induce flow through the column allowing the antibody to capture any aflatoxin present. Then, the column is washed to remove any non-bound materials. Bound aflatoxin is eluted using 100% methanol and collected in a test tube. For direct read application, aflatoxin fluorescence is enhanced by the addition of a developer (bromine solution) and read in a calibrated fluorometer, which displays the concentration of aflatoxin. For HPLC application, purified water is added and injected onto HPLC.

STORAGE REQUIREMENTS

The columns can be stored at 4–30°C (refrigerated or room temperature). Do not freeze.

Prior to use, check that the column has not dried out and there is buffer above the gel bed. The performance of the column could be adversely affected by extreme pH and temperature changes. See column box for expiry date.

MATERIALS PROVIDED

1. NeoColumn for Aflatoxin DR (Neogen item 8047)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Laboratory scale (Neogen item 9427)
2. Amber glass bottle, 50 mL (Neogen item 9496)
3. Amber glass bottle, 500 mL (Neogen item 9497)
4. Dispenser, 0–3 mL, for 50 mL glass bottle (Neogen item 9355)
5. Dispenser, 0–3 mL, for 500 mL glass bottle (Neogen item 9356)
6. Vortex (Neogen item 9494)
7. Glassware/plasticware
8. Laboratory blender (Neogen item 9493)
9. Blender jar, stainless steel, 1 L (Neogen item 9495)
10. Fluted filter paper, 24 cm (Neogen item 9351)
11. Microfiber filters, 12.5 µm, 11 cm (Neogen item 9352)
12. Filter funnels (Neogen item 9353, 9354)

13. Methanol (HPLC or ACS grade)
14. Disposable cuvettes (Neogen item 9361)
15. Cuvette rack (Neogen item 9440)
16. Plastic cups with lids (100) (Neogen item 9428)
17. Distilled water or deionized (DI) water
18. 5 mL pipettor and tips
19. Glass syringe barrel (10 mL) (Neogen item 9365)
20. Pump stand (Neogen item 9358, 9359)
21. Laboratory-grade salt (NaCl) (Neogen item 7725G)
22. 50 mL graduated cylinder (Neogen item 9367)
23. 250 mL graduated cylinder (Neogen item 9368)
24. 500 mL wash bottle (Neogen item 9366)
25. DRF 2100 Fluorometer (or equivalent) (Neogen item 9360)
26. NeoColumn for Aflatoxin DR developer (Neogen item 8048)
27. NeoColumn for Aflatoxin DR calibrators (Neogen item 8049)
28. NeoColumn for Aflatoxin calibrator for 0.167 g method (Neogen item 8051)

PRECAUTIONS

1. Methanol solution is highly flammable. Keep container tightly closed, and keep away from heat, sparks, open flame and those smoking. It is toxic if swallowed, or if vapor is inhaled. Avoid contact with skin.
2. Do not use kit components beyond expiration date.
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. It is important that all equipment is thoroughly cleaned between analyses as residues from the previous test may contaminate subsequent assays. This can be done by washing with a mild detergent solution and rinsing thoroughly with deionized or distilled water.
5. To eliminate background fluorescence, ensure that reagents and cuvettes are not fluorescing, and thus contributing to the fluorescence measured by the fluorometer.
6. Do not use blender jar lids with waxed cardboard liners. These are not resistant to methanol solutions, and can become contaminated and cause background fluorescence.
7. Do not wash and reuse glass cuvettes. These are designed for one-time use and should be discarded.
8. Treat sample extracts and labware as if containing aflatoxin. To avoid contamination of test samples and laboratory equipment, soak all used labware, pipette tips and kit components in a 10% solution of household bleach before discarding.
9. Columns stores refrigerated should be warmed to room temperature (18–30°C, 64–86°F) prior to use.

SAMPLING PROCEDURE

NeoColumn immunoaffinity columns efficiently extract aflatoxin from a variety of matrices. However, to accurately determine the level of aflatoxin contamination in an entire product or commodity lot, the samples tested must be representative of the entire lot. Recommended sampling procedures should be followed, and the use of at least 1 kg of sample is advised. Also, the testing of several samples from the entire lot will improve accuracy. (See GIPSA handbook for approved sampling procedures.)

FILTRATION PREPARATION

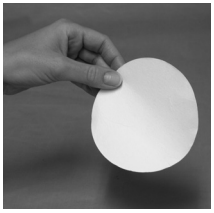
The extraction and test procedures for NeoColumn for Aflatoxin DR requires two gravity filtration steps—one with fluted filter paper and one with a 12.5 µm microfiber filter. Each filter should be prepared for use prior to starting the extraction and test procedures.

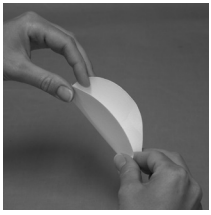
Fluted filtering

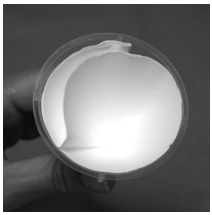
1. Place a funnel into a clean container or graduated cylinder.
2. Place a fluted/folded Whatman no. 4 filter into the funnel. To speed filtration, ensure that the folds in the filter do not become flattened in the funnel.
3. Pour the sample through the filter. Proceed to microfiber filter step below.

Folding microfiber filter paper

Using single microfiber filter, fold filter in half and then in half again. Place folded filter in funnel, then place funnel in cup.





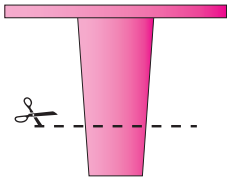


Microfiber filtering

1. Place a funnel into a clean container or graduated cylinder as illustrated above.
2. Fold the filter loosely and place in the funnel.
3. To ensure complete filtration, do not use a microfiber filter that is torn, punctured, or otherwise compromised.
4. Pour the filtrate through the microfiber filter.

PUMP STAND AND COLUMN PREPARATION

The pump stand serves both as a reservoir for the reagents and sample extract that are pushed through the column, and to provide the air pressure necessary to push the liquids through the column at a controlled pace (dropwise). The necessary air pressure can be achieved with an aquarium-type air pump that can be adjusted to achieve the correct flow of liquid through the column. **NOTE**: A hand syringe barrel also may be utilized to create the appropriate air pressure.



1. Connect the pump tube to the aquarium-type air pump.
2. Take top cap from a NeoColumn for Aflatoxin DR column and snip or slice approximately 1⁄8 of an inch off the bottom of the cap. This will open up the cap and allow liquid to flow through the cap and into the column. Attach this to the glass syringe barrel base.
3. Attach the column to the syringe barrel. The syringe barrel serves as the reservoir for sample extract and reagents.

PREPARATION OF REAGENTS

1. Extraction solution
   a. Either HPLC or ACS grade methanol may be used as the extraction solution.
   b. Methanol solution is highly flammable. Keep container tightly closed, and keep away from heat, sparks, open flame and those smoking. It is toxic if swallowed, or if vapor is inhaled. Avoid contact with skin.
   c. The examples below will prepare 1 L of solution. Solution volumes may be increased or decreased as needed provided the proper ratio is used. Prepared extraction solution may be used and stored for up to 2 weeks.

% Solution (methanol: water)	Methanol	DI or distilled water	Total volume
80% (80:20)	800 mL	200 mL	1 L
70% (70:30)	700 mL	300 mL	1 L
60% (60:40)	600 mL	400 mL	1 L

2. NeoColumn developer solution
   a. Pipette 5 mL NeoColumn developer and place in a 50 mL amber glass bottle with dispenser.
   b. Add 45 mL distilled or deionized water, and mix.
   c. Attach dispenser tightly and prime tip before use.
   d. Developer should be made fresh every **8 hours**. Open bottles of developer concentrate should be discarded after **30 days**.

REAGENT AND CUVETTE CHECKS

1. Reagents and cuvettes should be checked to ensure they are not fluorescing, and thus contributing to the fluorescence measured by the fluorometer. This check should be performed daily, or when new reagents are prepared or cuvettes used.
2. Calibrate the fluorometer per the standard method.
3. Each reagent should read a 0 (zero).
4. Solutions that do not read 0 should be retested using a new cuvette. If the solution still is not reporting a 0, the solution should be discarded, made fresh and tested again.

Reagents to check:

a. **HPLC grade methanol**: Add 2 mL HPLC grade methanol into a clean cuvette and read.
b. **Water**: Add 2 mL DI or distilled water into a clean cuvette and read.
c. **Developer**: Add 1 mL methanol and 1 mL prepared developer solution into a clean cuvette, mix, and read.

EQUIPMENT CLEANING NOTES

As noted earlier in the reagent and cuvette checking section, the accuracy of the NeoColumn for Aflatoxin DR test depends on the absence of background fluorescence from unexpected sources. Care must also be taken to eliminate the possibility that test equipment is not contaminated with materials that may cause background fluorescence.

Cleaning notes:

1. Before use, equipment to be used in the test, or used to hold samples or sample extracts should be washed with a mild detergent and thoroughly rinsed with purified water. Pipettors for dispensing methanol need only be rinsed with methanol prior to use.
2. After each assay, equipment used in the test or to hold samples or sample extracts, should be washed with a mild detergent and thoroughly rinsed with purified water.
3. **DO NOT REUSE CUVETTES**. The cuvettes are intended to be disposed of after a single use.

FLUOROMETER CALIBRATION VALUES

	DRF 2100	Vicam Series IV	DRF 2100	Vicam Series IV
	Aflatoxin 1 g calibration		Aflatoxin 0.5 g calibration	
Range	1–50	1–50	1–100	1–100
Red	18	22	36	44
Green	-1	-1	-1	-1
Yellow	9	11	18	22
+/- value	1	1	2	2
Delay	60	60	60	60

	DRF 2100	Vicam Series IV
	Aflatoxin 0.167 g calibration	
Range	1–320	1–320
Red	140	140
Green	-2	-2
Yellow	69	69
+/- value	+5	+5
Delay	60	60

CORN AND PEANUT AOAC TEST PROCEDURE: 1 G EQUIVALENT, 0–50 PPB

Extraction procedure

1. Grind the sample in a laboratory grinder until it has the consistency of finely ground coffee (corn) or briefly grind the sample (**20–30 seconds**) in a laboratory grinder, taking care to avoid clumping of the sample due to release of nut oil resulting from over-grinding (peanut).
2. Weigh out 25 g of ground sample, and to it add 5 g of salt (NaCl).
3. Fill a laboratory blender with 125 mL of 70% methanol, and place the sample/salt mixture into the blender.
4. Cover and blend at high speed for **2 minutes**.
5. Allow the sample to settle, and then filter through fluted filter paper.
6. Dilute by adding 15 mL of the filtered extract to 30 mL DI or distilled water, and mix.
7. Filter through a glass microfiber filter paper (Whatman GF/A or similar).
8. The sample is now ready to test. Proceed to **Test procedure** at the top of page 7.

CORN, ROUGH RICE AND MILLED RICE TEST PROCEDURE: 1 G EQUIVALENT, 0–50 PPB

Extraction procedure

1. Grind the sample in a laboratory grinder until it has the consistency of finely ground coffee.
2. Weigh out 50 g of ground sample, and to it add 5 g of salt (NaCl).
3. Fill a laboratory blender with 100 mL of 80% methanol, and place the sample/salt mixture into the blender.
4. Cover and blend at high speed for **1 minute**.
5. Allow the sample to settle, then filter through fluted filter paper.
6. Add 10 mL of the filtered extract to 40 mL DI or distilled water, and mix.
7. Filter through a glass microfiber filter paper (Whatman GF/A or similar).
8. The sample is now ready to test. Proceed to **Test procedure** at the top of page 7.

Test procedure

1. Loosen the bottom cap of the column, remove the top plug and attach the column to a 10 mL glass syringe reservoir in the pump stand.
2. Place a disposable plastic cup under the column to collect waste.
3. Add 10 mL of prepared sample extract to the reservoir.
4. Remove the bottom cap of the column, and apply pressure to the column to initiate flow dropwise. Ensure no air bubbles form in the column as this will restrict the flow of the sample through the column.
5. Allow the entire sample to pass through the column.
6. Add 10 mL of DI or distilled water to the reservoir, and allow the wash to flow through the column dropwise.
7. Repeat step 6 wash for a total of two washes, or 20 mL.
8. Ensure all the liquid is removed from the column by forcing air through the column by using either the aquarium pump or syringe.
9. Remove the plastic cup used to collect waste, and place a new cuvette under the column.
10. Elute bound aflatoxin by adding 1 mL 100% methanol to the reservoir, pushing through dropwise, and collecting all the sample eluate in the cuvette.
11. Immediately add 1 mL of prepared NeoColumn Developer directly to the cuvette. Mix well by vortexing, wipe clean and place cuvette in a calibrated fluorometer.

CORN, ROUGH RICE AND MILLED RICE TEST PROCEDURE: 0.5 G EQUIVALENT, 0–100 PPB

Extraction procedure

1. Grind the sample in a laboratory grinder until it has the consistency of finely ground coffee.
2. Weigh out 50 g of ground sample, and to it add 5 g of salt (NaCl).
3. Fill a laboratory blender with 100 mL of 80% methanol, and place the sample/salt mixture into the blender.
4. Cover and blend at high speed for **1 minute**.
5. Allow the sample to settle, then filter through fluted filter paper.
6. Add 10 mL of the filtered extract to 40 mL DI or distilled water, and mix.
7. Filter through a glass microfiber filter paper (Whatman GF/A or similar).
8. The sample is now ready to test.

Test procedure

1. Loosen the bottom cap of the column, remove the top plug and attach the column to a 10 mL glass syringe reservoir in the pump stand.
2. Place a disposable plastic cup under the column to collect waste.
3. Add 5 mL of prepared sample extract to the reservoir.
4. Remove the bottom cap of the column, and apply pressure to the column to initiate flow dropwise. Ensure no air bubbles form in the column as this will restrict the flow of the sample through the column.
5. Allow the entire sample to pass through the column.
6. Add 5 mL of DI or distilled water to the reservoir, and allow the wash to flow through the column dropwise.
7. Repeat step 6 wash for a total of two washes, or 10 mL.
8. Ensure all the liquid is removed from the column by forcing air through the column.
9. Remove the plastic cup used to collect waste, and place a new cuvette under the column.
10. Elute bound aflatoxin by adding 1 mL 100% methanol to the reservoir, pushing through dropwise, and collecting all the sample eluate in the cuvette.
11. Immediately add 1 mL of prepared NeoColumn Developer directly to the cuvette. Mix well by vortexing, wipe clean and place cuvette in a calibrated fluorometer.

PEANUT AND ALMOND TEST PROCEDURE: 1 G EQUIVALENT, 0–50 PPB

Extraction procedure

1. Briefly grind the sample (**20–30 seconds**) in a laboratory grinder, taking care to avoid clumping of the sample due to release of nut oil resulting from over-grinding.
2. Weigh out 25 g of ground sample, and to it add 5 g of salt (NaCl).
3. Fill a laboratory grinder with 125 mL of 60% methanol solution, and place the sample/salt mixture into the blender.
4. Cover and blend at high speed for **1 minute**.
5. Allow the sample to settle, then filter through fluted filter paper.
6. Add 20 mL of the filtered extract to 20 mL DI or distilled water, and mix.
7. Filter through a glass microfiber filter paper (Whatman GF/A or similar).
8. The sample is now ready to test.

Test procedure

1. Loosen the bottom cap of the column, remove the top plug and attach the column to a 10 mL glass syringe reservoir in the pump stand.
2. Place a disposable plastic cup under the column to collect waste.
3. Add 10 mL of prepared extract to the reservoir.
4. Remove the bottom cap of the column, and apply pressure to the column to initiate flow dropwise. Ensure no air bubbles form in the column as this will restrict the flow of the sample through the column.
5. Allow the entire sample to pass through the column.
6. Add 10 mL of DI or distilled water to the reservoir, and allow the wash to flow through the column dropwise.
7. Repeat wash step 6 for a total of two washes, or 20 mL.
8. Ensure all the liquid is removed from the column by forcing air through the column.
9. Remove the plastic cup used to collect waste, and place a new cuvette under the column.
10. Elute bound aflatoxin by adding 1 mL 100% HPLC methanol to the reservoir, pushing through dropwise, and collecting all the sample eluate in the cuvette.
11. Immediately add 1 mL of prepared NeoColumn Developer directly to the cuvette. Mix well by vortexing, wipe clean and place cuvette in a calibrated fluorometer.

PEANUT AND ALMOND TEST PROCEDURE: 0.5 G EQUIVALENT, 0–100 PPB

Extraction procedure

1. Briefly grind the sample (**20–30 seconds**) in a laboratory grinder, taking care to avoid clumping of the sample due to release of nut oil resulting from over-grinding.
2. Weigh out 25 g of ground sample, and to it add 5 g of salt (NaCl).
3. Fill a laboratory grinder with 125 mL of 60% methanol solution, and place the sample/salt mixture into the blender.
4. Cover and blend at high speed for **1 minute**.
5. Allow the sample to settle, then filter through fluted filter paper.
6. Add 20 mL of the filtered extract to 20 mL DI or distilled water, and mix.
7. Filter through a glass microfiber filter paper (Whatman GF/A or similar).
8. The sample is now ready to test.

Test procedure

1. Loosen the bottom cap of the column, remove the top plug and attach the column to a 10 mL glass syringe reservoir in the pump stand.
2. Place a disposable plastic cup under the column to collect waste.
3. Add 5 mL of prepared extract to the reservoir.
4. Remove the bottom cap of the column, and apply pressure to the column to initiate flow dropwise. Ensure no air bubbles form in the column as this will restrict the flow of the sample through the column.
5. Allow the entire sample to pass through the column.
6. Add 5 mL of DI or distilled water to the reservoir, and allow the wash to flow through the column dropwise.
7. Repeat wash step 6 for a total of two washes, or 10 mL.
8. Ensure all the liquid is removed from the column by forcing air through the column.
9. Remove the plastic cup used to collect waste, and place a new cuvette under the column.
10. Elute bound aflatoxin by adding 1 mL 100% HPLC methanol to the reservoir, pushing through dropwise, and collecting all the sample eluate in the cuvette.
11. Immediately add 1 mL of prepared NeoColumn Developer directly to the cuvette. Mix well by vortexing, wipe clean and place cuvette in a calibrated fluorometer.

CORN TEST PROCEDURE: 0.167 GRAM EQUIVALENT, 0–320 PPB

Extraction procedure

1. Grind the sample in a laboratory grinder until it has the consistency of finely ground coffee.
2. Weigh out 50 g of ground sample, and to it add 5 g of salt (NaCl).
3. Fill a laboratory blender with 100 mL of 80% methanol, and place the sample/salt mixture into the blender.
4. Cover and blend at high speed for **1 minute**.
5. Allow the sample to settle, then filter through fluted filter paper.
6. Dilute by adding 10 mL of the filtered extract to 20 mL DI or distilled water, and mix.
7. Filter through a glass microfiber filter paper (Whatman GF/A or similar).
8. The sample is now ready to test.

Test procedure

1. Loosen the bottom cap of the column, remove the top plug and attach the column to a 10 mL glass syringe reservoir in the pump stand.
2. Place a disposable plastic cup under the column to collect waste.
3. Add 1 mL of prepared sample extract to the reservoir.
4. Remove the bottom cap of the column, and apply pressure to the column to initiate flow dropwise. Ensure no air bubbles form in the column as this will restrict the flow of the sample through the column.
5. Allow the entire sample to passed through the column.
6. Add 1 mL of DI or distilled water to the reservoir, and allow the wash to flow through the column dropwise.
7. Repeat step 6 wash for a total of two washes, or 2 mL.
8. Ensure all the liquid is removed from the column by forcing air through the column.
9. Remove the plastic cup used to collect waste, and place a new cuvette under the column.
10. Elute bound aflatoxin by adding 1 mL 100% methanol to the reservoir, pushing through at a rate of 1 drop per second, and collecting all the sample eluate in the cuvette. Force air through the column to ensure the entire 1 mL has been eluted.
11. Immediately add 1 mL of prepared NeoColumn Developer directly to the cuvette. Mix well by vortexing, wipe clean and place cuvette in a calibrated fluorometer.

HPLC CORN AND PEANUT AOAC TEST PROCEDURE: 1 G EQUIVALENT, 0–50 PPB

For use with Kobra cell

1. Temperature control: Maintain analytical and guard columns at 40°C.
2. Analytical column: C18 5 µm particle size; minimum 150 mm by 4.6 mm; recommended 250 mm by 5 mm.
3. Guard column: Similar composition to analytical column 5 µm particle size
4. Mobile phase:
   – 600 mL Distilled water containing 0.119 g potassium bromide (KBr),
   – 61.2 µL Nitric acid (70% solution)
   – 200 mL HPLC grade methanol
   – 200 mL HPLC grade acetonitrile
5. HPLC pump: To deliver mobile phase dropwise
6. Fluorescence detector:
   – Excitation = 360 nm
   – Emission = 440 nm
7. Injection volume: 100 µL
8. Integrator/data analyses software

For use with post-column derivitization with iodine

1. Mobile Phase:
   – 600 mL distilled water.
   – 200 mL HPLC grade methanol.
   – 200 mL HPLC grade acetonitrile.
2. HPLC pump: 2x HPLC pumps to deliver (a) mobile phase dropwise (b) saturated iodine solution dropwise. Mixing T piece to introduce post column derivitization reagent into the column effluent and a reaction coil of 1.5 m x 0.5 mm internal diameter stainless steel tubing, immersed in a water bath at 70°C.
3. Fluorescence detector:
   – Excitation = 360 nm
   – Emission = 440 nm
4. Injection volume: 100 µL
5. Integrator/data analyses software

Extraction procedure

1. Grind the sample in a laboratory grinder until it has the consistency of finely ground coffee (corn) or briefly grind the sample (**20–30 seconds**) in a laboratory grinder, taking care to avoid clumping of the sample due to release of nut oil resulting from over-grinding (peanut).
2. Weigh out 25 g of ground sample, and to it add 5 g of salt (NaCl).
3. Fill a laboratory blender with 125 mL of 70% methanol, and place the sample/salt mixture into the blender.
4. Cover and blend at high speed for **2 minutes**.
5. Allow the sample to settle, then filter through fluted filter paper.
6. Dilute by adding 15 mL of the filtered extract to 30 mL DI or distilled water, and mix.
7. Filter through a glass microfiber filter paper (Whatman GF/A or similar).
8. The sample is now ready to test.

Test procedure

1. Loosen the bottom cap of the column, remove the top plug and attach the column to a 10 mL glass syringe reservoir in the pump stand.
2. Place a disposable plastic cup under the column to collect waste.
3. Add 10 mL of prepared sample extract to the reservoir.
4. Remove the bottom cap of the column, and apply pressure to the column to initiate flow dropwise. Ensure no air bubbles form in the column as this will restrict the flow of the sample through the column.

5. After a portion of the sample extract has passed through the column (approximately 5 mL), add an additional 5 mL of sample extract to the reservoir to make a total of 15 mL sample extract.
6. Continue flow rate dropwise, allowing the entire sample to pass through the column.
7. Add 10 mL of DI or distilled water to the reservoir, and allow the wash to flow through the column dropwise.
8. Repeat step 6 wash for a total of two washes, or 20 mL.
9. Ensure all the liquid is removed from the column by forcing air through the column.
10. Remove the plastic cup used to collect waste, and place a new cuvette under the column.
11. Elute bound aflatoxin by adding 1 mL 100% methanol to the reservoir, pushing through dropwise, and collecting all the sample eluate in the cuvette. Force air through the column to ensure the entire 1 mL has been eluted.
12. Immediately add 1 mL of purified water to eluate and inject 100 µL into HPLC.

INTERPRETING RESULTS USING THE DRF 2100

1. Select **Run Assay** from the main menu.
2. Select the appropriate test protocol. If a new protocol is necessary, return to the main menu and go to **Manage Protocols**.
3. If the calibration is INVALID, select **Run Calibration** and follow the instructions indicated on the screen.
4. If the calibration is VALID, select **Run Assay** and insert the sample.

PERFORMANCE CHARACTERISTICS

Range of quantitation:	1 g = 0–50 ppb
	0.5 g = 0–100 ppb
	0.167 g = 0–320 ppb
Antibody cross-reactivity:	Total aflatoxins B_1, B_2, G_1, G_2

For additional commodity procedures, contact Neogen Technical Services.

CUSTOMER SERVICE

Neogen Customer Assistance and Technical Services can be reached by using the contact information on the back of this booklet. Training on this product, and all Neogen test kits, is available.

MSDS INFORMATION AVAILABLE

Material safety data sheets (MSDS) are available for this test kit, and all of Neogen's Food Safety test kits, at www.neogen.com, or by calling Neogen at 800/234-5333 or 517/372-9200.

TERMS AND CONDITIONS

For Neogen's full terms and conditions, please visit www.neogen.com/Corporate/termsconditions.html.

WARRANTY

Neogen Corporation makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. If any materials are defective, Neogen will provide a replacement product. Buyer assumes all risk and liability resulting from the use of this product. There is no warranty of merchantability of this product or of the fitness of the product for any purpose. Neogen shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.

TEST KITS AVAILABLE FROM NEOGEN

Natural toxins
- Aflatoxin, DON, ochratoxin, zearalenone, T-2/HT-2 toxins, fumonisin, histamine

Foodborne bacteria
- *E. coli* O157:H7, *Salmonella*, *Listeria*, *Listeria monocytogenes, Campylobacter, Staphylococcus aureus*

Sanitation
- ATP, yeast and mold, total plate count, generic *E. coli* and total coliforms, protein residues

Food allergens
- Almonds, crustaceans, eggs, gliadin, hazelnut, lupine, milk, mustard, peanuts, sesame, soy, walnuts

Genetic modification
- CP4 (Roundup Ready®)

Ruminant by-products
- Meat and bone meal, feed

Species identification
- Raw and cooked meat samples

North America
Neogen Headquarters
620 Lesher Place, Lansing, MI 48912 USA
800/234-5333 (USA/Canada) or 517/372-9200
Fax: 517/372-2006 • foodsafety@neogen.com
www.neogen.com

Europe, Middle East and Africa
Neogen Europe
The Dairy School, Auchincruive, Ayr
KA6 5HW Scotland, UK
+ 44 (0) 1292 525 600
Fax: + 44 (0) 1292 525 601
info_uk@neogeneurope.com
www.neogeneurope.com

Mexico
Neogen Latinoamérica
Darwin No. 83, Col. Anzures, México, 11590 D.F.
(55) 5254-8235, (55) 5203-0111, (55) 5531-2837
Fax: (55) 5531-1647
informacion@neogenlac.com • www.neogen.com

Brazil
Neogen do Brasil
Rua: Alberto Guizo 760, Distrito Industrial João Narezzi, Indaiatuba – SP Brasil, Cep: 13.347-402
Tel: +55 19 3935.3727
info@neogendobrasil.com.br • www.neogen.com

9J8047A NeoColumn_Afla_DR_0717