

MYCOTOXIN

H A N D B O O K



Aflatoxin • *Deoxynivalenol (DON)*

Fumonisin • *Ochratoxin*

T-2/HT-2 Toxins • *Zearalenone*

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WHAT ARE MYCOTOXINS?

Mycotoxins are toxins produced by organisms categorized as fungi, including mushrooms, yeasts and molds. Fungi of one species or another, or their spores, can be found virtually everywhere. When the growth conditions are right for specific fungi, they will grow very rapidly into colonies, and produce toxins specific to that fungus as a by-product. Growth conditions, which include temperature, humidity, and available organic food sources, can not only affect whether or not a specific fungus will grow, but also the characteristics of the mycotoxin that it may produce.

Mycotoxins can be produced wherever fungi growth conditions exist, for example, in grains preharvest in the field and postharvest in storage. In either case, damage from insects, mishandling and environmental stress can enable the fungi to invade the grains' seeds.

ARE MYCOTOXINS HARMFUL?

As their name implies, mycotoxins are generally considered toxic, although not all mycotoxins are demonstrably toxic to every animal that may ingest them. Some mycotoxins, such as aflatoxin, have been shown to be dangerous to both humans and animals, others dangerous to only specific animal species, and still others, such as penicillin, only lethal to other fungi and bacteria.

Extensive mold growth in grains can have other obvious negative effects, such as producing changes in the grains' color, consistency, and smell—which may make the grains undesirable to livestock and as a human food. Mold growth can also rob grains of their fat, protein, and vitamin content, and lead to nutritional deficiencies in livestock.

HOW MANY MYCOTOXINS ARE THERE?

Researchers have identified thousands of mycotoxins thus far, and continually identify new mycotoxins. Subtypes of numerous mycotoxins have also been identified. Within the identified mycotoxins and their subtypes, a relative few have been determined to pose a significant threat to the health of humans and animals. Those that have been proven to threaten health include aflatoxin, deoxynivalenol (a.k.a., DON or vomitoxin), fumonisin, ochratoxin, T-2/HT-2 toxins, and zearalenone.

CAN MYCOTOXINS BE “KILLED” OR OTHERWISE NEUTRALIZED?

Unlike the fungi that produces them, mycotoxins are chemical substances that are not alive, and cannot be “killed”. The only known treatment to reduce aflatoxin levels, for example, is ammoniation, which leaves the kernels black and smelling like ammonia. There are no proven treatments to both neutralize a mycotoxin and preserve the integrity of the contaminated commodity.

Likewise, extreme heat and freezing do not destroy mycotoxins. Mycotoxins have also been shown to be resistant to breakdown in an animal's digestive system—meaning that they can be passed along in meat and dairy products.

Commodities known to contain a harmful level of a certain mycotoxin are diverted away from use in products destined to be consumed by animals known to be especially sensitive to that mycotoxin. For example, corn products known to contain harmful amounts of fumonisin, a mycotoxin of special concern to horses and rabbits, would be diverted away from use in horse and rabbit feed.

WHAT IS A PPM?

“One part per million” is a lot to think about. Here are some facts that put 1 ppm into perspective.

- There are approximately 13,960 kernels of wheat in 1 pound. One kernel in 71 pounds is equal to 1 ppm.
- There are approximately 3,500,000–4,000,000 grains of sand per pound. If you take 4 grains out of the pound you have removed 1 ppm.
- There are 2,678,400 seconds in August. In the time it took to read this, approximately 10 ppm has gone by.

“One part per billion” is 1,000 times smaller than 1 ppm. For example, one second in 32 years is 1 ppb.

MAJOR MYCOTOXINS

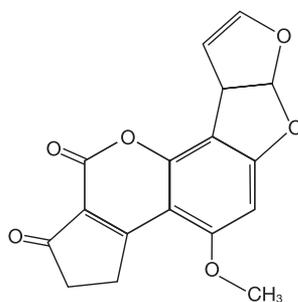
The amount of mycotoxin required to produce adverse effects in humans and animals varies by the mycotoxin, and can even vary from animal to animal of the same species. The amount of risk posed by mycotoxins is a combination of the level of contamination of a given commodity, and the total amount of mycotoxins ingested by a specific animal.

Aflatoxin

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: B₁, B₂, G₁ and G₂ in grains. Aflatoxin B₁ 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 in animals of 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.

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



Aflatoxin B₁

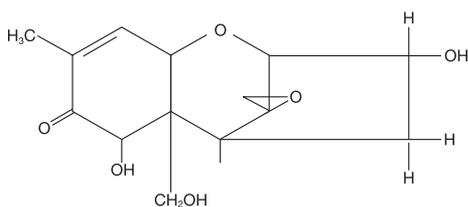
For	Level	Commodities
Humans	20 ppb	All foods except milk
All animal species	20 ppb	All feed (exceptions below)
Exceptions:		
Breeding cattle, breeding swine, mature poultry	100 ppb	Corn
Finishing swine (>100 lbs.)	200 ppb	Corn
Finishing beef cattle	300 ppb	Corn
Finishing beef cattle, swine, poultry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

European Union regulations for aflatoxin (Total B₁+B₂+G₁+G₂) as follows:

Foodstuffs	Maximum Levels
Groundnuts subject to sorting and treatment before human consumption	15.0 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	10.0 ppb
Groundnuts and nuts for direct human consumption Dried fruit for direct human consumption Cereals and products derived from cereals unless otherwise listed	4.0 ppb
Baby foods and cereals intended for infants	0.1 ppb

DON

Deoxynivalenol (DON) is most commonly produced by the pink mold *Fusarium graminearum*. DON, a member of the trichothecene family, is produced by fungi living on cereal commodities such as wheat, corn, barley and ensilages. The toxicological effects attributed to DON include: nausea (vomiting), feed refusal, gastroenteritis, diarrhea, immunosuppression and blood disorders.



Deoxynivalenol

The FDA has issued advisory levels for DON as follows:

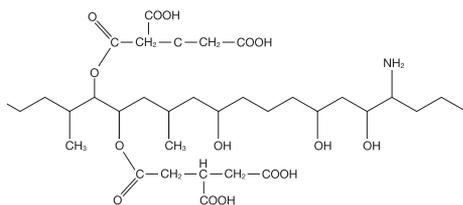
For	Level	Commodities
Humans	1 ppm	Finished wheat products (flour, bran and germ)
Ruminating beef, feedlot cattle, and chickens	10 ppm in <50% of diet (5 ppm total diet)	All grains, grain by-products
Swine	5 ppm in <20% of diet (1 ppm total diet)	All grains, grain by-products
All other animals	5 ppm in <40% of diet (2 ppm total diet)	All grains, grain by-products

European Union regulations for DON as follows:

Foodstuffs	Maximum Levels
Unprocessed cereals other than durum wheat, oats and maize	1250 ppb
Unprocessed durum wheat and oats	1750 ppb
Cereal flour, including maize flour, maize grits and maize meal	750 ppb
Bread, pastries, biscuits, cereal snacks and breakfast cereals	500 ppb
Processed cereal-based foods for infants and young children and baby food	200 ppb

Fumonisin

Discovered in 1989, fumonisins are a family of mycotoxins produced by different species of the mold *Fusarium*. These molds commonly infect corn (in fact, they are considered ubiquitous in corn) and rice, hence the potential for fumonisins to be found in feed and foodstuffs is high. Fumonisin affects various animals differently and have been linked to esophageal cancer in humans. The Environmental Protection Agency classifies fumonisins as Category II-B carcinogens.



Fumonisin B₁

Horses are extremely sensitive to low amounts of fumonisin, which can cause leukoencephalomalacia (liquefaction of the brain). In swine, research has shown fumonisin attacks the cardiopulmonary system causing pulmonary edema, as well as liver and pancreatic lesions.

The FDA has issued a final guidance for total fumonisins (FB₁+FB₂+FB₃) in food and animal feeds:

Human foods	Total fumonisins
Degermed dry milled corn products	2 ppm
Whole/partially dry milled corn products, dry milled corn bran, cleaned corn for masa production	4 ppm
Cleaned corn for popcorn	3 ppm

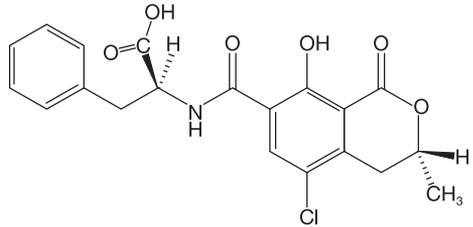
Animal feeds (corn/corn by-products)	Total fumonisins
Equids and rabbits	5 ppm, ≤20% of diet
Swine and catfish	20 ppm, ≤50% of diet
Breeding ruminants, breeding poultry and breeding mink	30 ppm, ≤50% of diet
Ruminants ≥3 months old being raised for slaughter and mink being raised for pelt production	60 ppm, ≤50% of diet
Poultry being raised for slaughter	100 ppm, ≤50% of diet
All other species or classes of livestock and pet animals	10 ppm, ≤50% of diet

European Union regulations for fumonisin (Total B₁+B₂) as follows:

Foodstuffs	Maximum Levels
Unprocessed corn	2.0 ppm
Corn flour, meal, grits, germ, and oil	1.0 ppm
Corn based foods intended for direct human consumption unless otherwise listed	0.4 ppm
Corn based baby foods intended for infants	0.2 ppm

Ochratoxin

Ochratoxin, commonly produced by the molds *Aspergillus ochraceus* and *Penicillium viridicatum*, can be found in corn, barley, green coffee and various dried fruits. Ochratoxin may be present in conjunction with aflatoxin, one of the most potent naturally-occurring carcinogens. In fact, ochratoxin is a suspected carcinogen.



Ochratoxin A

Ochratoxin affects kidneys in animals exposed to naturally-occurring levels of this mycotoxin. Turkeys and other poultry exhibited lower productivity levels during field outbreaks of ochratoxicosis. Symptoms included retarded growth and decreased feed conversion. It has also been known to affect egg production in laying hens.

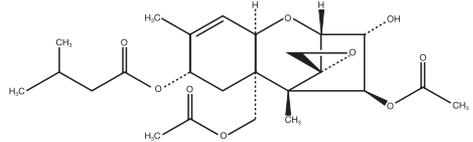
Although there has been no advisory or regulatory level for ochratoxin issued by the Food and Drug Administration, many agree that levels between 10–20 parts per billion (ppb) for commodities destined for human or animal consumption may cause health problems and economic losses. Some international markets have set regulation limits ranging from 5 to 50 ppb.

European Union regulations for ochratoxin as follows:

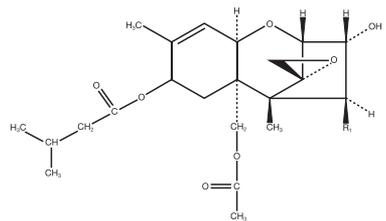
Foodstuffs	Maximum Levels
Dried vine fruits and instant coffee	10.0 ppb
Unprocessed cereals and roasted coffee	5.0 ppb
Processed cereals intended for direct human consumption unless otherwise listed	3.0 ppb
Wine, wine based drinks, and grape juice	2.0 ppb
Baby foods and cereals intended for infants	0.5 ppb

T-2/HT-2 Toxins

T-2/HT-2 toxins are trichothecene mycotoxins produced by several species of *Fusarium* molds. As T-2 toxin is readily metabolized to HT-2 toxin, and the toxins have been shown to produce numerous adverse effects on many animals, these two mycotoxins are frequently evaluated together.



T-2 Toxin



HT-2 Toxin

Animals affected by the toxins include swine, dairy cattle, poultry, dogs, cats and horses. Effects of the toxins include digestive disorders, hemorrhaging, edema, oral lesions, dermatitis, and blood disorders. Damage caused by the toxins to the digestive track is irreversible. In the most severe cases, these toxins will cause death. T-2 toxin is the principal causal toxin in the human disease alimentary toxic aleukia.

Poultry studies have shown T-2 intoxication has led to a reduction in weight gain and other problems such as beak lesions, poor feathering, motor function impairment and increased susceptibility to *Salmonella* spp.

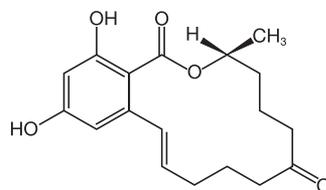
The best protection against these mycotoxins is monitoring for their presence in feeds and foods. That means testing all along the pathway from initial harvest of grains to the finished product.

Zearalenone

Zearalenone is primarily produced by the mold *Fusarium graminearum*, which also commonly produces DON. Hence, there is evidence that if zearalenone is detected, there is a high probability that other fusarial mycotoxins may be present. Zearalenone is classified as an estrogenic mycotoxin because it frequently causes estrogenic responses in animals.

When zearalenone-contaminated feed or grain is eaten by livestock, it can cause a wide variety of reproductive problems. In swine, it causes vulvovaginitis, low birth weights, fetal reabsorption, aborted pregnancies, reduced litter sizes, abnormal estrus and feminization of immature males. The FDA has issued advisory levels for zearalenone at <500 ppb.

European Union regulations for zearalenone as follows:



Zearalenone

Foodstuffs	Maximum Levels
Unprocessed corn and corn for direct human consumption	200.0 ppb
Other unprocessed cereals	100.0 ppb
Other cereals for direct human consumption	75.0 ppb
Bread, pastries, breakfast cereals, and snacks Corn based snacks and corn based breakfast cereals	50.0 ppb
Baby foods and cereals intended for infants	20.0 ppb

Note: Other international bodies may differ in advisory and regulatory levels for each toxin.

Confirmation methods

High performance liquid chromatography (HPLC) is the preferred instrument based confirmation method for mycotoxins. Testing requires a skilled technician, a validated test method, and appropriate equipment. Gas chromatography (GC) and thin layer chromatography (TLC) are also popular confirmation methods. See the *Resources* section on page 22 for qualified mycotoxin laboratories.

DO BLACK LIGHTS WORK TO DETECT AFLATOXIN IN CORN?

Studies have shown that using black light to detect aflatoxin in corn produces unreliable results. The bright yellow-green fluorescence that a black light can produce detects the presence of kojic acid, not aflatoxin. Kojic acid is one of many by-products of *Aspergillus flavus*, one of the two major producers of aflatoxin. But, *Aspergillus flavus* can produce aflatoxin without producing kojic acid, and it can produce kojic acid without producing aflatoxin. In addition, kojic acid can dissipate over time, thus a sample that once “glowed” may not at a later time.

Additionally, the other major producer of aflatoxin, *Aspergillus parasiticus*, does not produce kojic acid at all. So, while a black light procedure can seem to detect corn contaminated with aflatoxin at times, the procedure is an unreliable indicator for the presence of aflatoxin.

HOW DO YOU OBTAIN A REPRESENTATIVE SAMPLE FROM A VERY LARGE QUANTITY, SUCH AS A HOPPER CAR?

Not even the best, most accurate test systems, such as Neogen's, can detect the accurate level of possible mycotoxin contamination in a large load of a commodity if the sample tested is not representative of the entire load. Representative samples for mycotoxin testing are much more difficult to achieve because mycotoxin contamination tends not to be as evenly distributed in a load as other testing targets, such as protein, moisture, and fiber.

Carrier	Probe Length	Probes Per Compartment
Barges	12 feet	1
Hopper car	10–12 feet	1
Boxcar	6 feet	5
Truck	5–6 feet	7
Hopper-bottom truck	6–10 feet	2

Taken from the *The USDA Grain Inspection, Packers & Stockyards Administration (GIPSA) recommended sampling guidelines*. For more information go to <http://www.gipsa.usda.gov/GIPSA/webapp?area=home&subject=lr&topic=hb-hb-1>

HOW FINE MUST A SAMPLE BE GROUND BEFORE TESTING?

The Federal Grain Inspection Service (FGIS) recommends grinding corn so that 75% passes through a 20 mesh screen, which is about the consistency of fine ground coffee. The sample's particle size is extremely important to subsequent test results. One kernel of corn can hold a very high amount of toxin. Unless kernels are ground and distributed evenly throughout the sample to be tested, variable and inaccurate results can occur. Proper cleaning of equipment between sampling is recommended to prevent cross contamination.

DON		
Veratox & Agri-Screen		Reveal Q+
Barley ¹	Oats ¹	Alfalfa
Barley flour	Oat hulls*	Barley
Barley, lightly pearled	Oat fiber	Beet pulp
Beet pulp*	Oat flour	Brewers rice
Canola meal	Pet food*	Brown rice
Corn ¹	Popcorn	Corn
Corn cob*	Potato, white	Corn gluten meal*
Corn bran ¹	Quinoa	Corn gluten feed*
Corn meal	Raw flour	Corn silage*
Corn germ meal ^{1*}	Rice ¹	DDGs*
Corn gluten feed*	Rice gluten	Defatted rice bran
Corn gluten meal*	Rice hulls	Dried blueberries
Corn grits ¹	Rye, raw ¹	Dried peas
Corn screenings	Rye flour ¹	Haylage*
Corn silage ♦	Soy flour	Malted barley
Corn/soy blend	Soy hydrolysate	Millet
Corn starch	Soybean meal	Oats
Corn steep ■	Sunflower meal	Oat groats
DDGs*	Tapioca	Pearled barley
DDGs backset/recycled water*	TMR ♦	Pea protein
DDGs syrup*	Wheat ¹	Rice bran
DDGs wet cake*	Wheat bran ¹	Rice flour
Flaxseed meal	Wheat bran aleurone ¹	Soy flour
Hay ♦	Wheat flour	Soy hulls
Haylage ♦	Wheat flour 2 nd clear ¹	Wheat
Kamut	Wheat middlings ¹	Wheat midds
Malted barley ^{1*}	Wheat midds	Wheat bran
Malted barley flour ¹		Wheat germ
Milo (grain sorghum)		Whole rye

FUMONISIN			
Veratox & Agri-Screen		Veratox 5/5	Reveal Q+
Barley	Pea fiber	Corn	1:4 Extraction
Beet pulp*	Pet food*		Barley #
Corn ¹	Popcorn ¹		Milo #
Corn meal ¹	Potato, white		Oats #
Corn germ meal*	Rice gluten		Rough Rice #
Corn gluten meal*	Rice hulls		Wheat Bran #
Corn/soy blend ¹	Rough rice ¹		Wheat Midds #
Corn steep ■	Rye		Corn Gluten Meal* #
DDGs*	Soy hydrolysate		DDGS* #
DDGs wet cake*	Soybeans		
Milo (grain sorghum)	Soybean meal		1:5 Extraction
Oats	Sunflower meal		Barley #
Oat hulls*	Wheat ¹		Canola Meal
Oats, naked	Wheat bran		Corn ¹
			Corn Gluten Feed*
			Corn Grits
			Corn Meal
			Soybean Meal
			Wheat
			1:3 Extraction
			Brewer's Rice #

¹ = Validated by USDA-GIPSA

* = A pH adjustment step may be necessary

= Contact Neogen for extraction procedure

♦ = Dry sample, grind, pH adjust

‡ = 1:4 extraction in 70% MeOH, 5 minute shake

§ = 1:4 extraction in 70% MeOH, 2 minute blend

■ = Extract, pH adjust, centrifuge 3 minutes at 5,000 rpm

• = Cottonseed samples should be decorticated prior to testing

Note: All commodities have been validated using Neogen's Commodity Validation Protocol. Commodities noted with a ¹ have also been validated by the USDA-GIPSA on their respective GIPSA validated test kits. Contact Neogen for additional details.

ZEARALENONE

Veratox		Reveal Q+
Barley	Rice	Corn
Corn	Rice, brown	Corn gluten meal*#
DDGs*	Rice flour (white)	DDGs*#
Oats	Rice hulls	Rough rice#
Oat flour	Rye	Soy flour#
Oat hulls*	Soybean meal	Wheat
Oats, naked	Tapioca	
Pet food*	Wheat	
Popcorn	Wheat bran*	
Potato		

OCHRATOXIN

Veratox	
Apricots	Rice
Barley ‡	Rice flour §
Corn	Rice gluten §
Dates	Rice hulls §
Figs	Rye
Green coffee	Soy hydrolysate
Oat ‡	Soybeans
Oat flour	Soybean meal
Pea fiber	Sunflower meal
Popcorn	Tapioca §
Potato, white §	Wheat ‡
Raisins	Wheat bran*

OCHRATOXIN GRAIN

Veratox
Corn
Wheat
Sorghum
Oat
Barley
Rye

T-2/HT-2 TOXINS

Veratox	
Barley	Rye
Corn	Pea fiber
Corn flour	Potato, white
Corn gluten*	Soy
Corn steep ■	Soybean meal
DDGs wet cake*	Tapioca
Oats	Wheat
Oat hulls, whole*	Wheat bran*
Rice, brown	Wheat flour
Rice flour, white	Wheat gluten
Rice gluten	
Rice hulls	

pH ADJUSTMENT PROCEDURE

Commodities to be tested should have a pH of 6.0–8.0. Most raw or unprocessed grains, such as corn or wheat, have a pH between 6.0–8.0 and will not need to be adjusted. To ensure the accuracy of subsequent testing, excessively acidic or alkaline samples should be adjusted using this method:

1. Grind and extract sample per the test kit's written instructions.
2. Filter 5 mL into a clean test tube.
3. Check pH with pH paper or meter.

If acidic (pH is below 6): Adjust the pH with 1N NaOH (sodium hydroxide) to 6.0–8.0. Add one drop of 1N NaOH to the sample extract, vortex or swirl to mix and re-check the pH. If still acidic add another drop and check pH. Continue until the pH is 6.0–8.0

If alkaline (pH is above 8): Adjust the pH with 1N HCl (hydrochloric acid) to 6.0–8.0. Add one drop of 1N HCl to the sample extract, vortex or swirl to mix and re-check the pH. If still alkaline add another drop and check pH. Continue until the pH is 6.0–8.0

4. The sample extract is now ready to test.

SCREENING VS. QUANTIFYING RESULTS

Neogen's rapid tests for the detection of mycotoxins are available in multiple formats. Neogen's Agri-Screen and Reveal tests are the easiest available for those who require only a simple yes/no result, providing screening results in as little as 2 minutes. Neogen's Reveal Q+, Veratox and NeoColumn formats can provide screening results, or results in exact parts per million or billion, in just minutes.

Each requires only a minimal amount of training and equipment.

A. Screening tests

1. **Reveal** – Designed for ease of use, Reveal test kits are extremely easy to use and interpret test strips that screen samples against set thresholds. The AccuScan Pro lateral flow test reader provides an easy method to objectively read, store, and analyze results from Neogen's Reveal product line.
2. **Agri-Screen** – These screening microwell tests compare up to 5 samples at a time against a known level of toxin. The tests provide visible results that clearly show whether a sample contains more or less of a toxin than the control provided.

B. Quantitative tests

1. **Reveal Q+** – These quantitative lateral flow devices provide an easy-to-use rapid test with unparalleled accuracy.
2. **Veratox** – These quantitative tests compare up to 19 samples at a time against test controls. Through the use of a microwell reader, the tests provide accurate sample results in parts per million or billion.
3. **NeoColumn** – These immunoaffinity columns efficiently clean and concentrate the toxins prior to analysis by HPLC, fluorometric reader, or Neogen's Veratox test kits.

Sample Extraction:

Sample extraction is always performed at a specific ratio of solid sample to liquid extraction solution. Sample sizes can vary from the written instruction as long as the sample to extraction solution ratio remains the same.

Example: 1:5 extraction ratio

Ground Sample	Extraction Solution
5 g	25 mL
10 g	50 mL
50 g	250 mL

More representative results are achieved with a greater sample size. For example the USDA-FGIS recommends using a 50 g sample for aflatoxin testing and blending to extract (50 g + 250 mL). However, using a blender to extract is not always feasible and by using a smaller sample, the process can be sped up and simplified by utilizing disposable extraction cups and supplies.

EXAMPLE: Sample Preparation and Extraction for Veratox for Aflatoxin

The sample to be tested should be collected according to accepted sampling techniques. The sample should be ground and thoroughly mixed prior to proceeding with the extraction. When storing samples for an extended period (>1 week), they should be stored refrigerated at 2–8°C (35–46°F) until analyzed to prevent further toxin formation and insect infestation. **Note:** *If you are using Neogen's Mycotoxin Extraction Kit, follow the instructions in that kit for the extraction procedure. If you are preparing your own extraction solution, continue with the instructions that follow.*

1. If not using Neogen's prepared solution, prepare a 70% methanol solution by mixing 7 parts ACS Grade methanol with 3 parts distilled or deionized water for each sample to be tested.
2. Obtain a representative sample. Grind the entire sample so that at least 75% of the ground material passes through a 20 mesh sieve, the particle size of a fine instant coffee.

3. Vigorously shake, using hand or mechanical means, 5 g of ground sample in 25 mL of 70% methanol for 3 minutes. USDA/GIPSA Method: Blend 50 grams of ground sample with 250 mL of 70% methanol for 1 minute in a high-speed blender.
4. Filter the extract by pouring at least 5 mL through a Whatman no. 1 filter (or Neogen filter syringe) and collecting the filtrate as a sample.
5. The sample is now ready for testing.

EXAMPLE: Sample Preparation and Extraction for Veratox for DON

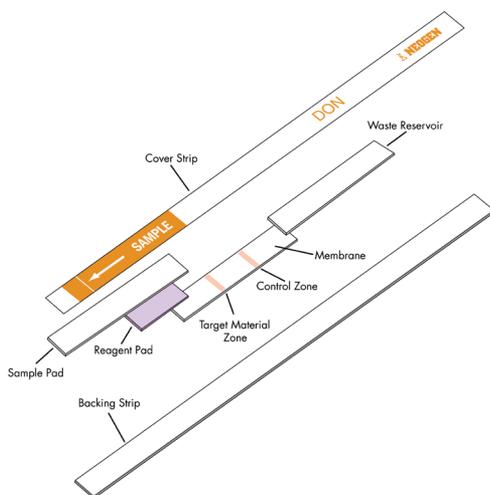
The sample to be tested should be collected according to accepted sampling techniques. The sample should be ground and thoroughly mixed prior to proceeding with the extraction. When storing samples for an extended period (>1 week), they should be refrigerated at 2–8°C (35–46°F) until analyzed to prevent further toxin formation and insect infestation.

1. Obtain a representative sample. Grind the entire sample so that at least 75% of the ground material passes through a 20 mesh sieve, the particle size of a fine instant coffee.
2. Using hand or mechanical means, vigorously shake 10 g of ground sample in 100 mL of distilled or deionized water for 3 minutes.
3. Let material set for 2–3 minutes to enable some of the sample to settle before filtering extract.
4. Filter the extract by pouring at least 5 mL through a Whatman no. 1 filter (or Neogen filter syringe) and collecting the filtrate as a sample.
5. The sample is ready for testing.

HOW DO NEOGEN'S MYCOTOXIN TESTS WORK?

A. Reveal and Reveal Q+

Neogen's Reveal tests for the detection of mycotoxins are single-step lateral flow assays based on a competitive immunoassay format. The extract is wicked through a reagent zone, which contains antibodies specific for the target mycotoxin conjugated to colloidal gold particles. If the target mycotoxin is present, it will be captured by the particle-antibody complex. The mycotoxin-labeled antibody complex is then wicked onto a membrane, which contains a zone of mycotoxin. This zone captures any unbound mycotoxin antibody, allowing the particles to concentrate and form a visible line. As the level of mycotoxin in a sample increases, free mycotoxin will bind with the antibody-gold particles. This allows less antibody-gold to be captured in the test zone. Therefore, as the concentration of target mycotoxin in the sample increases, the test line density decreases. The membrane also contains a control line which will always form regardless of the presence of mycotoxin, ensuring the strip is functioning properly.



For the Reveal Q+ tests, the AccuScan Pro reader is utilized to convert the line densities into a quantitative result displayed in ppm or ppb.

Example: Reveal Q+ for DON Test Procedure



1. Prepare by entering the QR code into the AccuScan Pro reader.



2. Obtain a representative sample. Grind and weigh out a 10 g sample.



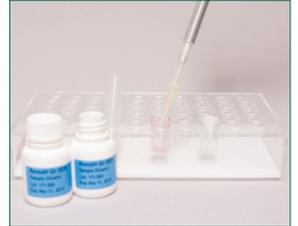
3. Add 100 mL of distilled or deionized water to sample.



4. Shake vigorously for 3 minutes, or blend for 1 minute.



5. Settle, then filter.



6. Add 1000 μ L of sample diluent to red dilution cup.



7. Add 100 μ L sample extract to red dilution cup and mix up and down 5 times.



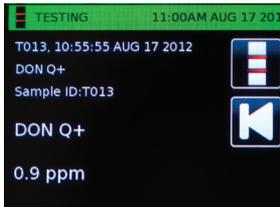
8. Transfer 100 μ L to sample cup.



9. Place a new Reveal Q+ DON strip into the sample cup. Set a timer for 3 minutes.



10. Remove promptly at 3 minutes and interpret results using the AccuScan Pro reader.



Note: There are subtle differences in sample preparation from what is illustrated above for the various Reveal Q+ tests.

Reveal and Reveal Q+ Quick Reference Guide

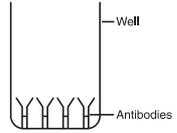
Product Number	Test	Extraction	Ratio	Incubations (minutes)	Other
8015	Reveal for Aflatoxin	10 g sample, 20 mL of 70% MeOH. Shake vigorously or blend for 1 minute.	1:2	3	Dilute 200 µL of sample diluent and 200 µL of sample extract.
8085	Reveal Q+ for Aflatoxin	10 g sample, 50 mL of 65% ethanol. Shake vigorously for 3 minutes or blend for 1 minute.	1:5	6	Dilute 500 µL of sample diluent and 100 µL of sample extract.
8086	Reveal Q+ for Aflatoxin Green	10 g sample, 50 mL Green Extraction Solution. Shake vigorously for 3 minutes or blend for 1 minute.	1:5	6	Dilute 500 µL of sample diluent and 100 µL of sample extract.
8385	Reveal Q+ for DON	10 g sample in 100 mL of DI or distilled water. Shake 3 minutes, settle and filter.	1:10	3	Dilute 1 mL (1,000 µL) of sample diluent and 100 µL of sample extract.
8885	Reveal Q+ for Fumonisin	10 g sample in 50 mL of 65% ethanol. Shake 3 minutes, settle and filter.	1:5	6	Dilute 200 µL of sample diluent and 100 µL of sample extract.
8685	Reveal Q+ for Ochratoxin	10 g sample in 40mL of 70% methanol. Shake 3 minutes, settle and filter.	1:5	9	Dilute 200 µL of sample diluent and 100 µL of sample extract.
8285	Reveal Q+ for T-2/HT-2	10 g sample in 100 mL of distilled water. Shake 3 minutes, settle and filter.	1:10	6	Dilute 500 µL of sample diluent and 100 µL of sample extract.
8185	Reveal Q+ for Zearalenone	Corn: 10 g in 30 mL of 65% ethanol. Wheat: 10 g in 50 mL of 65% ethanol. Shake vigorously for 3 minutes or blend for 1 minute.	Corn 1:3 Wheat 1:5	6	Dilute 200 µL of sample diluent and 100 µL of sample extract.

B. Agri-Screen and Veratox

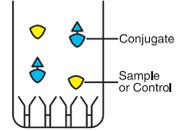
Neogen's microwell mycotoxin tests are competitive direct enzyme-linked immunosorbent assays (CD-ELISAs). Each test kit contains antibody-coated microwells with antibodies specific to the kit's target mycotoxin. First, samples and controls are added to their respective test wells. Next, an enzyme conjugate (the target mycotoxin chemically linked with an enzyme) is added. The samples/controls and conjugate are mixed and transferred to antibody wells where they compete for the antibody binding sites. The more target substance in the sample, the less conjugate that binds in the wells. After an incubation, the wells are washed to remove all unbound materials.

A substrate, which changes color in the presence of the conjugate, is then added to the wells. During an incubation, blue color develops in proportion to the amount of conjugate versus target mycotoxin in the wells. The more conjugate bound, the more blue color that develops, indicating less mycotoxin present. Results are read visually in the screening Agri-Screen format—the less blue color, or more red, the more target substance detected. In the Veratox quantitative format, results are obtained by measuring the wells' color change in a microwell reader and comparing the readings against a standard curve.

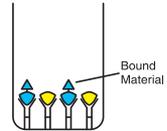
1. Microwells are coated with antibodies specific to the target substance



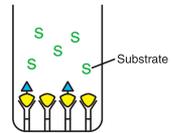
2. Conjugate competes with target substance/controls for antibody binding sites



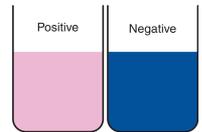
3. Conjugate and target substance/controls remain bound in wells



4. Substrate is added to produce a color change



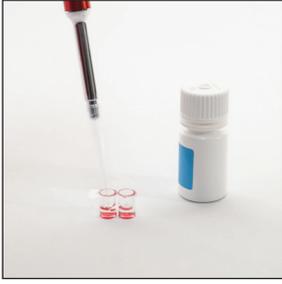
5. Results are read visually or in a reader—the less blue color, or more red, the more target substance detected



Mycotoxin Agri-Screen and Veratox Quick Reference Guide

Product Number	Test	Extraction	Ratio	Controls	Incubations (minutes)	Other
8010	Agri-Screen Aflatoxin	10 g sample, 50 mL of 70% MeOH. Shake vigorously for 3 minutes.	1:5	20 (ppb)	2/3	None.
8030	Veratox Aflatoxin	10 g sample, 50 mL of 70% MeOH. Shake vigorously for 3 minutes.	1:5	0, 5, 15, 50 (ppb)	2/3	None.
8031	Veratox HS Aflatoxin	25 g sample, 125 mL of 70% MeOH. Blend for 2 minutes.	1:5	0, 1, 2, 4, 8 (ppb)	10/10	None.
8310	Agri-Screen DON	10 g sample, 50 mL of DI or distilled water. Shake vigorously for 3 minutes.	1:5	1 (ppm)	5/5	None.
8331	Veratox DON 5/5	10 g sample, 100 mL of DI or distilled water. Shake vigorously for 3 minutes.	1:10	0, 0.5, 1, 2, 6 (ppm)	5/5	None.
8335	Veratox DON 2/3	10 g sample, 100 mL of DI or distilled water. Shake vigorously for 3 minutes.	1:10	0, 0.5, 1, 2, 6 (ppm)	2/3	None.
8810	Agri-Screen Fumonisin	10 g sample, 50 mL of 70% MeOH. Shake vigorously for 3 minutes.	1:5	5 (ppm)	5/10	100 µL of extract into dilution bottle.
8830	Veratox Fumonisin 10/10	10 g sample, 50 mL of 70% MeOH. Shake vigorously for 3 minutes.	1:5	0, 1, 2, 4, 6 (ppm)	10/10	100 µL of extract into dilution bottle.
8835	Veratox Fumonisin 5/10	10 g sample, 50 mL of 70% MeOH. Shake vigorously for 3 minutes.	1:5	0, 0.5, 1, 3, 6 (ppm)	5/10	100 µL of extract into dilution bottle.
8840	Veratox Fumonisin 5/5	10 g sample, 50 mL of DI water. Shake vigorously for 3 minutes.	1:5	0, 0.25, 1, 3, 6 (ppm)	5/5	100 µL of extract into 900 µL of diluent.
8610	Veratox for Ochratoxin	10 g sample, 40 mL of 50% MeOH. Shake for 3 minutes (wheat, barley and rye samples must be extracted in 70% MeOH).	1:4	0, 2, 5, 10, 25 (ppb)	10/10	None.
8630	Veratox for Ochratoxin Grain	10 g sample, 40 mL of 50% MeOH. Shake for 3 minutes.	1:4	0, 2, 5, 10, 25 (ppb)	10/10	None.
8230	Veratox for T-2/HT-2 Toxins	10 g sample, 50 mL of 70% MeOH. Shake vigorously for 3 minutes	1:5	0, 25, 50, 100, 250 (ppb)	5/5	Dilute extract 1:1 in DI or distilled water.
8110	Veratox for Zearalenone	10 g sample, 50 mL of 70% MeOH. Shake vigorously for 3 minutes	1:5	0, 25, 75, 150, 500 (ppb)	5/5	Dilute extract 1:5 in DI or distilled water.

Example: Agri-Screen Test Procedure



1. Add 100 μ L conjugate to each red marked mixing well.



2. Add 100 μ L control and extracted sample to their respective wells. Mix.



3. Transfer 100 μ L to antibody wells. Incubate at room temperature.



4. Dump liquid from antibody wells.



5. Wash wells thoroughly 5 times with DI water.



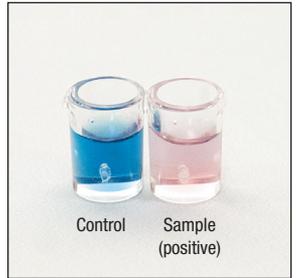
6. Tap out water on absorbent paper towel.



7. Add 100 μ L substrate to wells. Incubate at room temperature.



8. Add 100 μ L Red Stop to wells.



9. Visually read results.

Example: Veratox Test Procedure



1. Add 100 μL conjugate to each red marked mixing well.



2. Add 100 μL controls and extracted samples to their respective wells.



3. Mix. Transfer 100 μL to antibody wells. Incubate at room temperature.



4. Dump liquid from antibody wells.



5. Wash wells thoroughly 5 times with DI water.



6. Tap out water on absorbent paper towel.



7. Transfer 100 μL substrate from reagent boat to antibody wells using 12-channel pipettor. Incubate at room temperature.



8. Transfer 100 μL Red Stop from reagent boat to antibody wells.

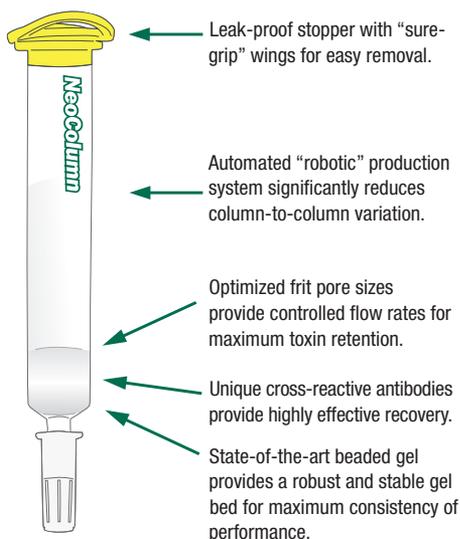


9. Read results using a microwell reader with a 650 nm filter.

C. NeoColumn

The NeoColumn test format is a high performance immunoaffinity column designed for the clean-up and concentration of a sample prior to HPLC, GC-MS, ELISA and other analytical methods. Clean-up columns are available for aflatoxin in both narrow and wide bore columns and in the wide bore column for DON, ochratoxin A and zearalenone. These columns deliver highly accurate results and recoveries on a range of validated matrices.

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. Aflatoxin fluorescence is enhanced by the addition of a developer (bromine solution) and read in a calibrated fluorometer, which displays the concentration of aflatoxin.



NeoColumn Quick Reference Guide

Product Number	Test	Limit of Detection	Recovery	Testing Time
8040 Narrow Bore	NeoColumn for Aflatoxin (for HPLC clean-up)	0.1 ppb	>90% each of B ₁ , B ₂ , G ₁ , G ₂ for cereal grains and nuts; conditions may vary depending on commodity	25 minutes
8043 Wide Bore	NeoColumn for Aflatoxin (for HPLC clean-up)	0.1 ppb	>90% each of B ₁ , B ₂ , G ₁ , G ₂ for cereal grains and nuts; conditions may vary depending on commodity	25 minutes
8047	NeoColumn for Aflatoxin DR (Direct-read & HPLC clean-up)	1 ppb	>90% B ₁ >80% B ₂ , G ₁ , G ₂	5 minutes
8140	NeoColumn for Zearalenone	5 ppb	>90% for maize, wheat, animal feed and breakfast cereals; conditions may vary depending on commodity	20 minutes
8240	NeoColumn for T-2/HT-2 Toxins	125 ppb	≥95%	100 minutes
8340	NeoColumn for DON	0.1 ppm	>85%	25 minutes
8640	NeoColumn for Ochratoxin A	<0.1 ppb	>95% for cereals; conditions may vary depending on commodity	30 minutes

Example: NeoColumn Aflatoxin DR Test Procedure



1. Attach column to glass syringe in pump stand.



2. Add prepared sample extract to syringe reservoir.



3. Remove bottom cap and apply pressure to column (1–2 drops per second).



4. Add DI or distilled water to wash (2 drops per second). Repeat wash.



5. Remove the cup and place a clean cuvette under the column.



6. Add 1 mL of 100% methanol to reservoir and apply air (1 drop per second).



7. Add 1 mL of prepared Developer solution to cuvette. Mix and wipe clean.



8. Place in a calibrated DRF 2100 Fluorometer.

EQUIPMENT LIST

		Reveal	Reveal Q+	Agri-Screen	Veratox
9401	Grinder	•	•	•	•
9453					
9427	Scale	•	•	•	•
9428	Extraction container	•	•	•	•
9447	Graduated cylinder	•	•	•	•
9368					
8055	70% methanol	•		•*	•*
8071	65% ethanol		•*		
N/A	Deionized or distilled water	•	•	•	•
9420	Filter syringe or equivalent	•	•	•	•
9421	Sample collection tube		•	•	•
9475	Reveal sample cup rack	•	•		
9448	Dispenser pump		•		
9402	Well holder			•	•
9400	Wash bottle			•	•
9426	Timer	•	•	•	•
9452					
9278	Pipettor, 100 µL	•	•	•	•
9272					
9337					
9273	Pipettor, 12-channel				•
9463	Pipettor, 100–1000 µL		•		
9488	Pipettor, 200 µL fixed		•		
9336	Pipettor, 500 µL fixed		•		
9407	Pipette tips, 20–200 µL	•	•	•	•
9417					
9410					
9464	Pipette tips, 200–1000 µL		•		
9487					
9565	AccuScan Pro Reader	optional	•		
9303	Neogen 4700 Reader				•

* Not required for Aflatoxin Green, DON and T-2/HT-2 Reveal Q+ tests and Veratox for Fumonisin 5/5.

RESOURCES

Neogen Corporation, 517/372-9200; www.neogen.com (certified reference material, test kits, confidential mycotoxin lab testing)

Canadian Grain Commission; www.grainscanada.gc.ca

North American Miller's Association (NAMA) 202/484-2200; www.namamillers.org

USDA GIPSA; www.gipsa.usda.gov (Grain Inspection, Packers and Stockyards Administration)

FAPAS Central Science Laboratory, Sand Hutton, York, UK; Tel: (+44) 1904 462100; www.fapas.com

North Dakota State University, Veterinary Diagnostic Laboratory; 701/231-8307; www.vdl.ndsu.edu

APPENDIX

Helpful hints and pipetting techniques

1. Swirl, don't shake, all reagents before using. Otherwise, the reagents will foam.
2. Always change pipette tips when there is a change in the reagent.
3. Test kits should be stored at 2–8°C (35–46°F) but allowed to warm to ambient temperature, 18–30°C (64–86°C) before use to ensure optimum performance.
4. Prime pipette tips prior to dispensing all reagents. To prime the tip, draw up the reagent and discharge it back into the same container. Priming the tips coats the inside of the pipette tip so that the volume dispensed will be identical regardless of tip wetting properties.
5. When drawing or dispensing reagents, always drag the pipette tip against the container rim to remove liquid on the outside of the tip.
6. When using the multi-channel pipettor, use the overfill method for transferring liquid as below:
 - a) Depress the pipettor button slightly past the first “stop” and slowly release to draw up more than 100 µL.
 - b) Dispense the liquid by depressing the button only to the first “stop”. This delivers exactly 100 µL into the microwells. After removing the tips from the microwells, release the button slowly so the excess liquid in the tips does not get into the multi-channel pipettor.
7. When dispensing reagents into the microwells, place the tip point against the inside wall of the microwell. This helps draw all of the liquid out of the tip and eliminates drops that form on the end of the pipette tip. In addition, placing the tip against the microwell holds the tip in place as the liquid is dispensed.
8. Always check the fluid levels in your tips prior to dispensing to be sure that the same amount is being collected each time (100 µL). If the proper amount was not collected or bubbles are present, refill the tip.
9. Most pipettors should be lubricated and calibrated at least every 12 months. Please contact your sales representative, or Technical Services for assistance.
10. Within the first 20 seconds (60 seconds for Fumonisin 5/10) of each incubation period, thoroughly mix reagents in the microwells by sliding the wells back and forth across a smooth surface. Be careful not to splash the reagents out of the wells.
11. If a sample result is greater than the kit's stated range of quantitation (many times the kit's highest control), it is not considered an accurate result. For exact results you should dilute and rerun the sample. Please contact your sales representative, or Technical Services if assistance is required.

NEOGEN'S MYCOTOXIN TESTS & APPROVALS

GIPSA AOAC

Aflatoxin			
8010	Agri-Screen for Aflatoxin – screens at 20 ppb, up to 18 samples.	✓	✓
8015	Reveal for Aflatoxin – screens at 20 ppb, 25 samples.	✓	
8085	Reveal Q+ for Aflatoxin – range of 2–150 ppb, up to 25 samples.	✓	
8086	Reveal Q+ for Aflatoxin Green – (water-based extraction) range of 2–150 ppb, up to 25 samples.		
8030	Veratox for Aflatoxin – range 5–50 ppb, up to 40 samples.	✓	✓
8031	Veratox for Aflatoxin HS (High Sensitivity) – range of 1–8 ppb, up to 38 samples.		
8043	NeoColumn for Aflatoxin – wide bore, clean-up column, 50 columns.		
8047	NeoColumn for Aflatoxin DR – direct read, clean-up column, 50 columns.		✓

DON (vomitoxin)			
8310	Agri-Screen for DON – screens at 1.0 ppm, up to 20 samples.	✓	
8385	Reveal Q+ for DON – range of 0.3–6 ppm, up to 25 samples.	✓	
8335	Veratox for DON 2/3 – range of 0.5–5.0 ppm, up to 38 samples.	✓	✓
8331	Veratox for DON 5/5 – range of 0.5–5.0 ppm, up to 38 samples.	✓	
8332	Veratox for DON HS – range of 25–250 ppb, up to 38 samples.		
8340	NeoColumn for DON – Wide Bore, Clean-Up Column, 50 columns.		

Fumonisin			
8810	Agri-Screen for Fumonisin – screens at 5 ppm, up to 20 samples.		
8885	Reveal Q+ for Fumonisin – range of 0.3–6 ppm, up to 25 samples.	✓	
8830	Veratox for Fumonisin – range of 1–6 ppm, up to 38 samples.		✓
8835	Veratox for Fumonisin 5/10 – range of 0.5–6 ppm, up to 38 samples.		
8840	Veratox for Fumonisin 5/5 – range of 0.25–6 ppm, up to 38 samples.		
8832	Veratox for Fumonisin HS (High Sensitivity) – range of 50–600 ppb, up to 38 samples.		

Ochratoxin			
8610	Veratox for Ochratoxin – range of 2–25 ppb, up to 38 samples.		
8630	Veratox for Ochratoxin Grain – range of 2–25 ppb, up to 38 samples.		
8640	NeoColumn for Ochratoxin A – wide bore, clean-up column, 50 columns.		
8685	Reveal Q+ for Ochratoxin – range of 2–20 ppb, 25 samples.		

T-2/HT-2 Toxins			
8230	Veratox for T-2/HT-2 Toxins – range of 25–250 ppb, up to 38 samples.		
8285	Reveal Q+ for T-2/HT-2 Toxins – range of 50–600 ppb, up to 25 samples.		

Zearalenone			
8185	Reveal Q+ for Zearalenone – range of 50–1200 ppb, up to 25 samples.	✓	
8110	Veratox for Zearalenone – range of 25–500 ppb, up to 38 samples.		
8140	NeoColumn for Zearalenone – wide bore, clean-up column, 50 columns.		

Validated commodity list available upon request.

Veratox[®]

Neogen's Veratox quantitative kits can test up to 19 samples at a time. Through the use of a microwell reader, the tests provide accurate sample results in parts per million or parts per billion.

Agri-Screen[®]

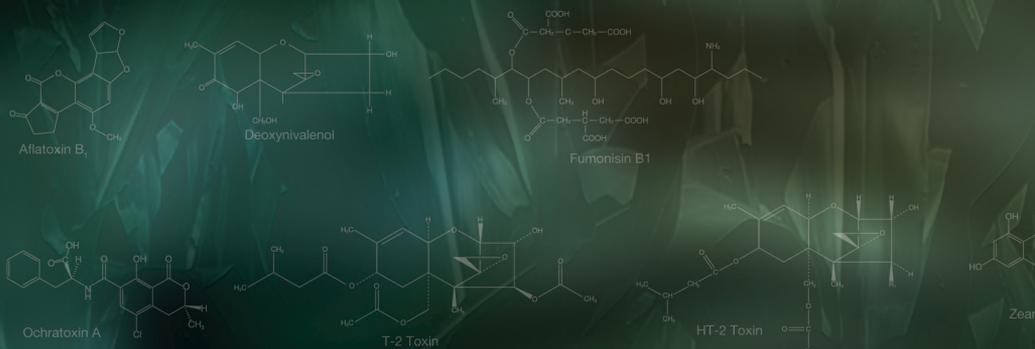
Neogen's Agri-Screen qualitative natural toxin kits can test up to 5 samples at a time against a known level of toxin. The tests provide visual results that clearly show whether a sample contains more or less toxin than the control provided. No reader is required.

Reveal[®] and Reveal Q+[®]

Neogen's new Reveal Q+ products are quantitative lateral flow tests that will provide precise parts per million (ppm) or parts per billion (ppb) levels of toxins in as little as 3 minutes following sample preparation. Reveal Q+ tests utilize the AccuScan Pro reader to interpret and document sample results. Neogen's original Reveal test format is a rapid visual screening test. After an extraction is completed, a sample is interpreted as greater or less than a threshold.

NeoColumn[™]

Neogen's NeoColumn test format is a high performance immunoaffinity column designed for the clean-up and concentration of a sample prior to HPLC, GC-MS, ELISA or direct read fluorometry.



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