Read instructions carefully before starting test

NeoColumn™ for DON WB

Quantitative Test

Refrigerate at 2–8°C (35–46°F) • Do not freeze

THE TOXIN

Deoxynivalenol (DON) most commonly is 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.

Pigs have been shown to be highly sensitive to DON. They will refuse to eat feeds when DON levels of ≥1 ppm are present. DON and its analogs cause toxic effects in other species as well, with varying degrees of sensitivity. DON has been implicated in causing problems in processed food, including off-flavor in ready-to-eat cereals and adverse effects on dough quality. Accurate determination of the presence of the toxin is of major importance to those monitoring the quality of feed and food in which DON may occur. Testing these commodities for the toxin requires careful sampling, extraction, sanitation and quantitative analysis.

The U.S. Food and Drug Administration 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

The European Union has regulations for DON as follows:

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

INTENDED USE

NeoColumn™ for DON WB (wide bore) is intended for the quantitative analysis of DON in commodities such as wheat, durum wheat, corn and animal feed.

INTENDED USER

This test kit is designed for use by quality control personnel and others familiar with food and feed possibly contaminated by DON. 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 DON WB is an immunoaffinity column that contains antibodies specific to the toxin. DON 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 DON present. Then, the column is washed to remove any unbound materials. Bound DON is eluted using 100% methanol and is detected by HPLC or Veratox® enzyme-linked immunosorbent assay (ELISA) methods.

NeoColumn for DON is highly specific for DON. NeoColumn for DON can detect DON contamination below levels set in (EC) No. 1126/2007. The test can recover DON from samples at or above levels set in (EC) No. 401/2006. For foodstuffs and animal feed, the equivalent of 0.25 g or (for the determination of low levels of DON) 0.625 g of test sample is run down each column. This should be accounted for when interpreting HPLC results. The range of NeoColumn for DON is 0.1 ppm to 4 ppm. To quantify higher concentrations, a 1:5 dilution of sample is recommended prior to addition of the sample into the immunoaffinity column.

STORAGE REQUIREMENTS

The columns can be stored at 2–8°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 DON WB (Neogen item 8043)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Scale, electronic (digital) (Neogen item 9427)
2. Glassware/plasticware
3. Laboratory blender (Neogen item 9493)
4. Centrifuge or filter paper/filter syringe (Neogen item 9429, 9420)
5. Extraction solvents (HPLC grade)
6. Distilled or deionized water (HPLC grade)
7. Pipettes and tips (Neogen items 9463, 9464 and 9487)
8. Clamp stand (Neogen item 9358)
9. Column rack (Neogen item 9359)
10. Glass or resin – treated (Sigmacote or equivalent) plastic syringe barrel (10 mL and pump unit) (Neogen item 9365)
11. Certified DON standards (see page 5)
12. pH meter (for beer analysis)
13. Fume hood
14. Hot plate capable of reaching 60°C
15. Mini-Vap evaporator (Sigma 22971)
16. Nitrogen
17. Syringe adapter for NeoColumn (Neogen item 9371)
18. Sodium hypochlorite solution/tablets
19. Methanol (HPLC grade needed for elution)
20. Whatman no. 4 filters (Neogen item 9429)
21. Whatman 2V and GF/A filters (Neogen items 9358)
22. Acentonitrile (For HPLC testing only)

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 DON. Gloves and other protective apparel should be worn at all times.
4. It is important 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 DON. 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.

DECONTAMINATION

It is important all equipment is thoroughly cleaned between analyses as residues from the previous test may contaminate subsequent assays. Decontamination can be achieved by immersion in a 5 percent sodium hypochlorite solution followed by immersion in 5 percent v/v acetone for **30 minutes** each. Normal washing may then be performed.

SAMPLING PROCEDURE

NeoColumn immunoaffinity columns efficiently extract DON from a variety of matrices. However, to accurately determine the level of DON 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

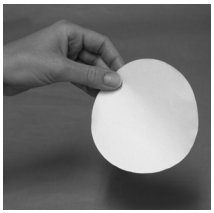
The extraction and test procedures for NeoColumn for DON WB requires two gravity filtration steps—one with fluted filter paper and one with a 12.5 µm microfiber filter. Each filter should be prepared for use prior to starting the extraction and test procedures.

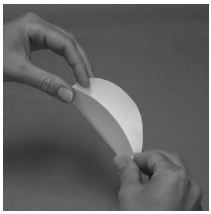
Fluted filtering

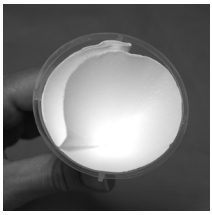
1. Place a funnel into a clean container or graduated cylinder.
2. Place a fluted filter into the funnel. To speed filtration, ensure that the folds in the filter do not become flattened in the funnel.
3. Pour the sample through the filter. Proceed to microfiber filter step below.

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.

PREPARATION OF REAGENTS

Elution buffer

The elution buffer is 100% HPLC grade methanol.

DON STANDARDS

Certified DON standard solutions available from Fluka (Sigma – Aldrich), (catalog number: IRMM315).

Prepare a standard curve of DON as outlined below:

1. In a clean glass vial, prepare a working stock of 5 ppm in 1 mL from the master stock. Aliquot 400 µL of working stock to a glass vial and evaporate over a stream of nitrogen on a 60°C hot plate. Resuspend in 1 mL mobile phase to make a 2 ppm standard. Allow to stand for **5 minutes** prior to mixing.
2. After each dilution, tightly stopper the vials and vortex mix the DON solution.

Required standard (equivalent to µg/mL)	Volume of DON stock (µL)	Volume of diluent (µL)	Dilution
2 ppm	400 µL working stock	1000 µL	A
1 ppm	500 µL A	500 µL	B
0.5 ppm	500 µL B	500 µL	C
0.25 ppm	500 µL C	500 µL	D
0.125 ppm	500 µL D	500 µL	E
0.0625 ppm	500 µL E	500 µL	F

ADJUSTING FOR LOAD VOLUME AND RECOVERY

For commodities of unknown quantity or > 0.2 ppm and ≤ 4 ppm

Running a 2 mL sample through NeoColumn for DON is equivalent to running 0.25 g of sample. NeoColumn for DON can recover, on average, 85% of total toxin in a matrix. Therefore, raw data must be corrected accordingly to achieve optimum results.

Follow the simple calculation for final result.

X = 1/0.25 x 100/85 x Raw data value

Where X is the final result,1 = 1 g, 0.25 = 0.25 g equivalent 100 = 100% recovery and 85 = 85% recovery.

For commodities ≤ 0.2 ppm

Running a 5 mL sample through NeoColumn for DON is equivalent to running 0.625 g of sample. NeoColumn for DON can recover, on average, 85% of total toxin in a matrix. Therefore, raw data must be corrected accordingly to achieve optimum results.
Follow the simple calculation for final result.

X = 1/0.625 x 100/85 x Raw data value

Where X is the final result,1 = 1 g, 0.625 = 0.625 g equivalent 100 = 100% recovery and 85 = 85% recovery.

For highly contaminated commodities and feed > 4 ppm

Running a 5 mL sample through NeoColumn for DON is equivalent to running 0.05 g of sample. NeoColumn for DON can recover, on average, 85% of total toxin in a matrix. Therefore, raw data must be corrected accordingly to achieve optimum results.

Follow the simple calculation for final result.

X = 1/0.05 x 100/85 x Raw data value

Where X is the final result,1 = 1 g, 0.05 = 0.05 g equivalent 100 = 100% recovery and 85 = 85% recovery.

EXTRACTION: COMMODITIES OF UNKNOWN QUANTITY OR > 0.2 PPM AND ≤ 4 PPM

1. Grind the sample in a laboratory grinder until it has the consistency of finely ground coffee.
2. Weigh out 25 g of finely ground sample.
3. Place the sample into a laboratory blender with 200 mL of distilled or deionized water.
4. Blend at high speed for **2 minutes**.
5. Filter the liquid supernatant through Whatman 2V or similar fluted filter paper. Alternatively, centrifuge for **10 minutes** at 4,000 rpm and retain supernatant.
6. Briefly mix solution, then filter through a Whatman GF/A or similar.
7. Remove top plug and loosen bottom cap of the column.
8. Attach a 10 mL syringe reservoir to the column, using a column adaptor.
9. Add 2 mL of sample to the reservoir.
10. Remove the bottom cap of the column; allow the sample to flow through the column under gravity. Do not let the column dry out.
11. Add 5 mL of distilled water to the reservoir, and allow the wash to flow through the column under gravity.
12. Ensure all the liquid is removed from the column by forcing air through the column using positive pressure from a syringe or pump.
13. Elute the bound DON. See page 7 for HPLC. See page 8 for ELISA (Veratox).

EXTRACTION: COMMODITIES ≤ 0.2 PPM

1. Grind the sample in a laboratory grinder until it has the consistency of finely ground coffee.
2. Weigh out 25 g of finely ground sample.
3. Place the sample into a laboratory blender with 200 mL of distilled or deionized water.
4. Blend at high speed for **2 minutes**.
5. Filter the liquid supernatant through Whatman 2V or similar fluted filter paper. Alternatively, centrifuge for **10 minutes** at 4,000 rpm and retain supernatant.
6. Briefly mix solution, then filter through a Whatman GF/A or similar.
7. Remove top plug and loosen bottom cap of the column.
8. Attach a 10 mL syringe reservoir to the column, using a column adaptor.
9. Add 5 mL of sample to the reservoir.
10. Remove the bottom cap of the column; allow the sample to flow through the column under gravity. Do not let the column dry out.
11. Add 12.5 mL of distilled water to the reservoir, and allow the wash to flow through the column under gravity.
12. Ensure all the liquid is removed from the column by forcing air through the column using positive pressure from a syringe or pump.
13. Elute the bound DON. See page 7 for HPLC. See page 8 for ELISA (Veratox).

EXTRACTION: HIGHLY CONTAMINATED COMMODITIES FOR FEED >4 PPM

1. Grind the sample in a laboratory grinder until it has the consistency of finely ground coffee.
2. To dilute the sample 1:5, weigh out 5 g of finely ground sample.
3. Place the sample into a laboratory blender with 200 mL of distilled or deionized water.
4. Blend at high speed for **2 minutes**.
5. Filter the liquid supernatant through Whatman 2V or similar fluted filter paper. Alternatively, centrifuge for **10 minutes** at 4,000 rpm and retain supernatant.
6. Briefly mix solution, then filter through a Whatman GF/A or similar.
7. Remove top plug and loosen bottom cap of the column.
8. Attach a 10 mL syringe reservoir to the column, using a column adaptor.
9. Add 2 mL of sample to the reservoir.
10. Remove the bottom cap of the column; allow the sample to flow through the column under gravity. Do not let the column dry out.
11. Add 5 mL of distilled water to the reservoir, and allow the wash to flow through the column under gravity.
12. Ensure all the liquid is removed from the column by forcing air through the column using positive pressure from a syringe or pump.
13. Elute the bound DON. See below for HPLC. See page 8 for ELISA (Veratox).

EXTRACTION: BEER

1. De-gas the sample by filtering in excess of 100 mL of beer through a glass microfiber filter paper (Whatman GF/A or similar).
2. Measure out 100 mL of de-gassed beer.
3. Adjust the sample pH to 7.2.
4. Run sample through column dropwise – note that the flow rate should be controlled by other means than gravity.
5. Add 5 mL of distilled water to the reservoir, and allow the wash to flow through the column under gravity.
6. Ensure all the liquid is removed from the column by forcing air through the column using positive pressure from a syringe or pump.
7. Elute the bound DON. See below for HPLC. See page 8 for ELISA (Veratox).

NOTE: The value obtained from analysis of the eluate will be the total amount of DON in the concentrated sample. For HPLC, multiply result by 1/100 to achieve corrected value. For ELISA, multiply result by 2.5/1000 (if using Veratox for DON 2/3) or 1.25/500 (if using Veratox for DON HS) to achieve corrected value.

ELUTION OF BOUND DON FOR HPLC

1. Slowly elute the bound DON from the column by passing 1.5 mL of the elution buffer through the column into a clean glass vial dropwise. This will ensure the removal of the bound DON.
2. Evaporate off the methanol under a stream of nitrogen on a 60°C hot plate. Reconstitute the sample by adding 1 mL mobile phase and allow to stand for **5 minutes** prior to mixing.
3. The sample is now ready for analysis. See page 8 for HPLC conditions.

HPLC CONDITIONS

1. Temperature control
   – Maintain analytical and guard columns at 40°C
2. Analytical column
   – C18 5 µm particle size
   – Recommended 4.6 mm by 250 mm
3. Guard column
   – C18 5 µm particle size
4. Mobile phase
   – 450 mL HPLC grade water
   – 25 mL HPLC grade acetonitrile
   – 25 mL HPLC grade methanol
5. HPLC pump
   – To deliver mobile phase dropwise
6. UV detector
   – 218 nm
7. Injection volume
   – 100 µL
8. Integrator/data analyses software
   – Chromeleon

QUANTIFICATION BY HPLC

Inject 100 µL of the each standard solution, and sample into the HPLC system. Inject standards in the order of lowest to highest concentration. Quantify DON concentrations in the sample by comparing sample peak heights/areas of the sample to the standards.

ELUTION OF BOUND DON FOR VERATOX FOR DON 5/5 OR DON 2/3

NOTE: The elution buffer comprises 100% HPLC grade methanol. For each column used, 1.5 mL 100% HPLC grade methanol is required. The buffer should be stored in a tightly closed screw cap bottle.

1. Slowly elute the bound DON from the column by passing 1.5 mL of the elution buffer through the column dropwise into a clean glass vial. This will ensure the removal of the bound DON.
2. Evaporate off the methanol under a stream of nitrogen on a 60°C hot plate. Reconstitute the sample by adding 2.5 mL distilled or deionized water; allow to stand for **5 minutes** then mix.
3. This gives an equivalent recovery of DON from 0.1 g/mL of sample. This is equal to an extracted sample tested with Veratox for DON 5/5 or Veratox for DON 2/3.
4. The sample is now ready for analysis.

ELUTION OF BOUND DON FOR VERATOX FOR DON HS

NOTE: The elution buffer comprises 100% HPLC grade methanol. For each column used, 1.5 mL 100% HPLC grade methanol is required. The buffer should be stored in a tightly closed screw cap bottle.

1. Slowly elute the bound DON from the column by passing 1.5 mL of the elution buffer through the column dropwise into a clean glass vial . This will ensure the removal of the bound DON.
2. Evaporate off the methanol under a stream of nitrogen on a 60°C hot plate. Reconstitute any eluted DON by adding 1.25 mL distilled or deionized water; allow to stand for **5 minutes** then mix.
3. This gives an equivalent recovery of DON from 0.2 g/mL of sample. This is equal to an extracted sample tested in the Veratox for DON HS.
4. The sample is now ready for analysis.

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 test kits, on Neogen's website at www.neogen.com, or by calling Neogen at 800/234-5333 or 517/372-9200.

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 of the 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.

NOTES

NOTES

TESTING 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*, *Salmonella enteritidis*

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

Food allergens
- Almonds, crustacea, eggs, gliadin, hazelnut, lupine, milk, mustard, peanut, sesame, soy, walnut

Genetic modification
- CP4 (Roundup Ready®)

Ruminant by-products
- Meat and bone meal, feed

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