Read instructions carefully before starting test

Veratox®

for Ochratoxin Grain

Quantitative Test

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

THE TOXIN

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 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 slowed 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 of at least 10–20 parts per billion (ppb) for commodities destined for human or animal consumption may cause health problems and economic losses. Some foreign markets have set regulation limits ranging from 5 to 50 ppb.

The best protection against 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.

INTENDED USE

Veratox for Ochratoxin Grain is intended for the quantitative analysis of ochratoxin in grain.

INTENDED USER

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

ASSAY PRINCIPLES

Veratox for Ochratoxin Grain is a competitive direct enzyme-linked immunosorbent assay (CD-ELISA) which allows the user to obtain exact concentrations in parts per billion (ppb). Free ochratoxin in the samples and controls is allowed to compete with enzyme-labeled ochratoxin (conjugate) for the antibody binding sites. After a wash step, substrate is added which reacts with the bound conjugate to produce blue color. More blue color means less ochratoxin. The test is read in a microwell reader to yield optical densities. The optical densities of the controls form the standard curve, and the sample optical densities are plotted against the curve to calculate the exact concentration of ochratoxin.

STORAGE REQUIREMENTS

The kit can be used until the expiration date on the label when stored refrigerated at 2–8°C (35–46°F). Do not freeze.

MATERIALS PROVIDED

1. 48 antibody-coated microwells
2. 48 red-marked mixing wells
3. 5 yellow-labeled bottles of 0, 2, 5, 10 and 25 ppb ochratoxin controls (see precautions for handling of methanol solution)
4. 1 blue-labeled bottle of ochratoxin-HRP conjugate solution
5. 1 green-labeled bottle of K-Blue® Substrate solution
6. 1 red-labeled bottle of Red Stop solution
7. Directions for use

MATERIALS REQUIRED BUT NOT PROVIDED

1. Extraction materials (items c through e available in kit form from Neogen, item 8052):
    a. ACS grade methanol
    b. 250 mL graduated cylinder (Neogen item 9368)
    c. Container with 125 mL capacity (Neogen item 9428)
    d. Neogen filter syringes, Whatman no. 1 filter paper, or equivalent (Neogen items 9420, 9430)
    e. Sample collection tubes (Neogen item 9421)
2. High-speed blender (Neogen item 9493, 9477)
3. Agrigrind grinder or equivalent (Neogen items 9401, 9453)
4. Scale capable of weighing 5–50 g (Neogen item 9427)
5. Microwell reader with a 650 nm filter (Neogen item 9303)
6. Pipettor, 12-channel (Neogen item 9273)
7. Pipettor, 100 µL (Neogen items 9272, 9276)
8. Pipette tips for 100 µL and 12-channel pipettors (Neogen items 9410, 9407, 9417)
9. Pipette, 200 µL, fixed (Neogen item 9488)
10. Paper towels or equivalent absorbent material
11. Plastic bucket for use as waste receptacle
12. Microwell holder (Neogen item 9402)
13. Timer (Neogen item 9426)
14. Waterproof marker
15. Wash bottle (Neogen item 9400)
16. 2 reagent boats for 12-channel pipettor (Neogen item 9450)
17. Distilled or deionized water

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. Store test kit between 2–8°C (35–46°F) when not in use. Do not freeze.
3. Do not use kit components beyond expiration date.
4. Do not mix reagents from one kit with reagents from a kit with a different serial number.
5. Do not run more than 24 wells per test.
6. Follow proper pipetting techniques, including priming of tips.
7. Use of incubation times other than those specified may yield inaccurate results.
8. Kits should be brought to room temperature (18–30°C, 64–86°F) prior to use.
9. Avoid prolonged storage of kits at ambient temperatures.
10. Treat all used liquids, including sample extract, and labware as if contaminated with ochratoxin. Gloves and other protective apparel should be worn at all times.
11. To avoid cross-contamination, use clean pipette tips and glassware for each sample, and thoroughly detoxify and wash all glassware between samples.
12. Commodities tested should have a pH of 6–8. Excessively acidic or alkaline samples should be adjusted. For instructions on adjusting pH contact a Neogen representative or Technical Services.

PROCEDURAL NOTES

1. **Substrate**: K-Blue Substrate is ready for use. The substrate should be clear to a very light blue—discard if it has turned dark blue. Only pour the needed volume of substrate into a reagent boat. **Do not return unused substrate to bottle.** Cover the reagent boat to keep the substrate protected from light until it is needed.
2. **Antibody wells**: Keep wells sealed in the foil pouch until needed. Remove wells from the foil pouch only after samples are extracted and the test procedure is set to begin.
3. **Extraction**: In order to obtain optimum recovery of ochratoxin using the Veratox system, samples should be extracted using a 50% methanol/water solution.

SAMPLE PREPARATION AND EXTRACTION

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. Store samples at 2–8°C (35–46°F) until analyzed. If using Neogen's Mycotoxin Extraction Kit, follow the instructions in that kit for the extraction procedure. If preparing the extraction solution in house, continue with the following instructions.

1. Prepare a 50% methanol solution by mixing 1 part ACS grade methanol with 1 part 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. Blend 25 g of ground sample with 100 mL of 50% methanol/water solution for **3 minutes** in a high-speed blender.
   ALTERNATIVE: Add 10 g of ground sample to 40 mL of 50% methanol/water and shake vigorously for **3 minutes**.
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. After filtration, dilute the sample extract 1:1 with 50% methanol (1 mL extract with 1 mL 50% methanol).

TEST PROCEDURE

Allow all reagents to warm to room temperature (18–30°C, 64–86°F) before use.

1. Remove 1 red-marked mixing well for each sample to be tested plus 5 red-marked wells for controls and place in the well holder.
2. Remove an equal number of antibody-coated wells. Return antibody wells that will not be used immediately to the foil pack with desiccant. Reseal the foil pack to protect the antibody. Mark one end of strip with a "1" and place strip in the well holder with the marked end on the left. Do not mark the inside or bottom of the wells.
3. Mix each reagent by swirling the reagent bottle prior to use.
4. Place **200 µL** of conjugate from the blue-labeled bottle in each red-marked mixing well.
5. Using a new pipette tip for each, transfer 100 µL of controls and samples to the red-marked mixing wells as described below.

0	2	5	10	25	S1	S2	S3	S4	S5	S6	S7	Strip 1
S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	Strip 2

6. Using a 12-channel pipettor, mix the liquid in the wells by pipetting it up and down 5 times. Prime and pipette 100 µL of the mixture into the wells. Set the timer for a **10 minute** room temperature (18–30°C, 64–86°F) incubation, mixing the wells for the first **30 seconds** of the incubation by sliding the microwell holder back and forth on a flat surface without splashing reagents from the wells. Discard the red-marked mixing wells.
7. Shake out the contents of the antibody wells. Fill the wells with distilled or deionized water and dump them out. Repeat this step 5 times, then turn the wells upside-down and tap out on a paper towel until the remaining water has been removed.
8. Pour the needed volume of substrate from the green-labeled bottle into the green-labeled reagent boat.
9. Add new tips to a 12-channel pipettor. Prime and pipette 100 µL of the substrate into the wells. Set the timer for a **10 minute** room temperature (18–30°C, 64–86°F) incubation, mixing the wells for the first **30 seconds** of the incubation by sliding the microwell holder back and forth on a flat surface without splashing reagents from the wells. Discard the red-marked mixing wells.
10. Pour Red Stop solution from the red-labeled bottle into the red-labeled reagent boat.
11. Eject the excess substrate from the 12-channel pipettor, prime the tips, and pipette 100 µL of Red Stop to each well. Mix by sliding back and forth on a flat surface. Discard the tips.
12. Wipe the bottom of the microwells with a dry cloth or towel and read in a microwell reader using a 650 nm filter. Air bubbles should be eliminated, as they could affect analytical results. Results should be read within **20 minutes** after the addition of Red Stop.
13. Read and calculate results using Neogen's Awareness StatFax microwell reader, or equivalent. If using an EL301 reader or other strip/plate reader, calculate results using Neogen's Veratox software.

PERFORMANCE CHARACTERISTICS

Limit of detection: 1 ppb (Determined by the mean average of 10 ochratoxin free samples plus 2 standard deviations.)

Limit of quantitation: 2 ppb (Described as the lowest concentration point on the calibration curve that this test can reliably detect ochratoxin.)

Range of quantitation: 2–25 ppb (For quantitating samples above 25 ppb contact Neogen Technical Services for dilution instructions.)

Validated matrices: Corn, wheat, sorghum, oat, barley, rye.
NOTE: Neogen continues to validate new commodities. Please contact a representative for the latest validated commodity list.

RETESTING

If positives occur in commodities not previously tested, confirm with an additional approved method prior to taking action.

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.

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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