Detecting the Allergen Aflatoxin via ELISA Using Neogen's Veratox® for Aflatoxin Kit

Neogen Logo [9]

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Note to Reader:

The following document is intended for food laboratory technicians to detect aflatoxins using Neogen's Veratox® Aflatoxin kits.

What are Aflatoxins?

According to the European Food Safety Authority, aflatoxins are mycotoxins (toxic compounds that are naturally produced by certain types of molds) produced by two species of Aspergillus, a fungus found especially in areas with hot and humid climates. [1]

Aflatoxins are known to be genotoxic (the damage of genes caused by mutations from certain chemical agents) and carcinogenic. [5] They can be found in a variation of food products like groundnuts, tree nuts, maize, rice, spices, and cocoa beans. Aflatoxins can arise from fungal contamination before and after harvest. There are ten types of aflatoxins. Aflatoxin B1 is the most common in food and among the most potent. It is produced both by the fungi Aspergillus flavus and Aspergillus parasiticus.

Types of Tests

There are various tests to detect the presence of aflatoxins. Common tests are thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), mass spectroscopy (MS), and enzyme-linked immune-sorbent assay (ELISA). [14]

Enzyme-linked immune sorbent assay or ELISA is an immunological assay used to measure antibodies, antigens, proteins, and glycoproteins in samples. The use of Neogen's Veratox® for Aflatoxin extraction kits are fast and effective in detecting aflatoxins.

Materials

You will need the following items:

- Analytical balance
- Sample extraction bottle(s)
- Lab notebook / worksheet
- Writing utensil (pen/pencil, etc.)
- Goggles / Disposable latex gloves / Lab coat
- Paper towels
- Disposable reagent boats
- Disposable 200 µL pipette tips in a 200 µL pipette rack
- Clean disposable test tubes
- Test tube rack
- Microplate well holder
- Wash bottle with deionized water

Tools/Kits

The following items are needed:

- Neogen's Veratox® for Aflatoxin Extraction Kit
- 100 µL pipettor
- 12 channel 100 µL pipettor
- Microwell reader with a 650nm filter
- Centrifuge
- Timer
- Vortex shaker

Chemicals

The following chemical is needed for this procedure:

- 70% Methanol

Test Setup

Before testing, you will need to setup your workstation. Doing so ensures that all materials, tools, kits, and chemicals are available and ready.

1. Take out the extraction kit from the fridge.
2. Remove and place all extraction reagents from the extraction kit on your workstation. Let it sit for **~45 minutes to 1 hour** to reach room temperature.

Neogen's Veratox® for Aflatoxin Extraction Kit [12]

3. Place a filled bottle of deionized water, empty extraction bottles with lids, a clean microplate well holder with paper towels underneath, and additional paper towels on your workstation.

Sample Preparation

All samples will need to be prepped prior to testing.

1. Label each extraction bottle and test tube of the sample(s) you are testing.
2. Weigh five grams of your sample(s) in an extraction bottle using an analytical balance.

   Note: You may not exactly weigh out 5 grams, weigh as close to it as possible. It is okay to go over 5 grams. Do **not** weigh more than 5.20 grams.

3. Write down the weight of your sample(s) on your lab notebook/ worksheet.
4. **Add** 25 mLs of 70% methanol into the extraction bottle(s).
5. Place a clean lid on the extraction bottle(s), close and tighten it, and use the vortex shaker for each one for 3 minutes.

Extraction Bottle [10]

6. Once finished, allow the extraction bottle(s) to **settle for 1 minute**. You will notice a separation of 70% methanol and the sample(s).
7. Remove the lid and pour up to three-fourths of each sample to each labeled test tube. Place test tube(s) on a test tube rack if needed.
8. Place the test tube(s) in the centrifuge and centrifuge for **10 minutes** at an RPM of ~500.

Centrifuge [13]

9. Wait for the centrifuge to come to a **complete stop**.

> **DANGER: Centrifuges that are still spinning after completion can cause damage to hands when removing test tubes.**

10. Remove test tubes from the centrifuge and place them in the test tube rack.

Test Procedure Part I

Before you begin, make sure you have completed test setup and sample preparation. Refer to those sections if you have not done so already.

1. Remove 1 red-marked mixing well for each sample to be tested plus 4 red-marked wells for controls and place in the microwell holder.

Microwell holder with wells [1]

2. Remove an equal number of clear antibody-coated wells. Return antibody wells that will not be used immediately to the foil pack with desiccant.
3. Reseal the foil pack to protect the antibody.
4. Mark one end of strip with a "1," and place strip in the well holder with the marked end on the left.

   Note: Do not mark the inside or bottom of the wells.

5. **Mix** each reagent by swirling the reagent bottle prior to use.
6. Extract 100 µL of conjugate from the blue-labeled bottle in each red-marked mixing well using a micro pipettor.
7. Using a new pipette tip for each, transfer 100 µL of controls and samples to the red-marked mixing wells.
8. Using a 12-channel pipettor, mix the liquid in the wells by pipetting it up and down **3 times**. Transfer 100 µL to the antibody-coated wells. Discard the red-marked mixing wells.

12-Channel Pipettor [4]

9. Mix the wells for 10–20 seconds by sliding the microwell holder back and forth on a flat surface without splashing reagents from wells. Set and run the timer for **2 minutes**.
10. Shake out the contents of the antibody wells. Fill the wells with the bottle containing the deionized water and dump them out. **Repeat this step 5 times.**
11. Turn the wells upside-down and tap it on a paper towel. Do this until the remaining water is completely gone.

    Note: Carefully pat the well holder as the wells can slip out. If it does slip out, you will have to start all over.

Test Procedure Part II

This is a continuation of the testing procedure. Do **not** perform if you have not done the first part.

12. Pour the needed volume of substrate from the green-labeled bottle into a clean reagent boat.
13. With new tips on the 12-channel pipettor, pipette 100 µL of substrate into the wells.
14. Mix the wells for the first 10–20 seconds by sliding back and forth on a flat surface. Set and run the timer for **3 minutes**.
15. Discard the remaining substrate in the reagent boat.
16. Pour Red Stop solution from the red-labeled bottle into another clean reagent boat.
17. **Discard** the tips used and place new pipette tips. Pipette 100 µL of Red Stop to each well.
18. Mix by sliding back and forth on a flat surface again for 10-20 seconds. Discard the tips.
19. Wipe the bottom of the microwells in the holder with a dry cloth or towel.

    Caution:
    Air bubbles should be eliminated, as they can affect results.

20. Place the holder in the microwell reader and run the device. Results should be read **within 20 minutes** after the addition of Red Stop.
21. Read and calculate results using the microwell reader. If using a strip/plate reader, calculate results using NEOGEN's Veratox software.

Testing products that contain harmful allergens like aflatoxin is crucial in ensuring safety when consumed. Neogen's Veratox® Aflatoxin kit is effective in determining and measuring the amount of these harmful allergens. As such, it has greatly prevented food, pharmaceutical and cosmetic industries from selling items with concentrations that exceed health and safety standards.

Safety Hazard Information

When performing any laboratory test, it is imperative that the reader is aware of the dangers and risks of the chemicals used. To ensure their safety, a table will list all safety hazards. Table one below contains the necessary safety information for performing the aflatoxin ELISA test.

Table 1. Important Safety Information	
Flammable 	Highly flammable liquids or vapors. Use caution when working with 70% methanol, it is highly flammable when near a heat source.
Toxic 	The chemicals and reagents used are toxic. Avoid swallowing, inhaling, and contact with exposed skin. If on skin or hair, immediately take off all contaminated clothing. Rinse skin with water and or use the safety shower.
Health 	A carcinogen or substance with respiratory, reproductive or organ toxicity that causes damage over time. It can be a chronic or long-term health hazard.

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