QuickTox Kit for Zearalenone

Catalog AQ 332 BG *Part #13078*

Matrices and Detection Ranges:

Matrix Group ID	Matrices	Results reported in the range of:	Limit of Detection (LOD)*	Maximum Reported Value of Base Range*
ZN MG2	Wheat	0 - 520 ppb	50 ppb	520 ppb
ZN MG3	DDGS	0 - 1000 ppb	300 ppb	1000 ppb
ZN MG7	Corn Fermented Protein	0 - 1000 ppb	300 ppb	1000 ppb
ZN MG8	Barley	0-500 ppb	50 ppb	500 ppb

**Do not assume accuracy for results reported below the protocol's LOD or above the protocol's highest approved level.*

Scan to View Verified Matrices:

https://www.envirologix.com/verified

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Contents of Kit:

- 50 QuickTox Strips packed in a moisture-resistant canister
- 50 reaction tubes
- 100 pipette tips (1-200 µL)
- DB4 Buffer
- Multi-Matrix Barcode Card - kit lot specific

Important Notes:

- Before testing, the enclosed Multi-Matrix Barcode Card (MMBC) must be scanned just once for each kit lot to upload information to the QuickScan
- QuickScan Software Version 5.9, or later is required
- DB4 Buffer is matched with specific Zearalenone kit lot numbers. Be sure to use DB4 with the kit it is provided with. There is a "use with" label on the DB4 that will indicate the matching Zearalenone Lot Number.
- **Verified Matrix** is a customer-specific sample type that has been rapidly and reliably tested using EnviroLogix strips through our agile Matrix Addition process—delivering fit-for-purpose protocol guidance where full validation is not required. Because the samples used in verification may not represent global sample variation, Verified Matrices are not covered under the EnviroLogix limited warranty. We recommend evaluating performance using your own samples prior to incorporating a Verified Matrix into your operations.
- **Validated Matrix** are listed in the table on page 1 and are covered under the EnviroLogix limited warranty.

Table A on page 4 is provided as a Summary Guide for testing. More details for each step in the process are described below, and are important for achieving optimal, accurate results.

Intended Use

This kit is designed to quickly extract and screen milled samples for the presence of Zearalenone residues. It will then provide quantitative results when used in conjunction with the QuickScan System.

How the Test Works



A composite sample is first collected, then extracted to solubilize any Zearalenone present. Each sample should be ground to a fineness of 20 mesh and extracted following the protocol specified for the matrix being run. This extract is further diluted for testing with the QuickTox Kit.

Each QuickTox Strip has an absorbent pad at each end. The protective tape with the arrow indicates which end of the strip to insert into the reaction tube. The sample extract travels up the membrane strip and is absorbed into the larger pad at the top of the strip. At the end of the test time, the strip is cut off at the top of the arrow tape, the bottom pads are discarded, and the strip is inserted into the QuickScan reader to obtain quantitative results.

Matrix specific extractions and analysis protocols are chosen for accuracy and precision. Each matrix is assigned to a Matrix Group (MG). Each MG has a common standard curve and maximum reported value. When the user selects the MG during testing, the QuickScan System software reads the test strip, retrieves the lot specific information that was uploaded using the Multi-Matrix Barcode Card (MMBC), and uses the appropriate curve to obtain a result for the matrix being tested.

Precautions – Read First!

SAFETY

1. **Disposal of Zearalenone-contaminated materials.** Follow your facility's safety procedures for disposal of samples and extracts potentially containing or known to contain Zearalenone.

Items Not Provided:

- QuickScan System*
- Incubator (base + block)*
- Bunn grinder or equivalent
- 20-mesh screen (available through Seedburo or other vendor)
- Digital scale for weighing samples
- Solvent: 50% reagent-grade ethanol†
- Extraction cups with lids* or other suitable vessels for sample extraction
- Graduated cylinder*
- Orbital/rotary shaker
- Pipette to deliver 100 µL*
- Timer
- Scissors
- Distilled, deionized or bottled water

**Available as Accessories*

***Available Accessories:**

Item	*Catalog No.*	*Part #*
QuickScan™ System	ACC 351	13065
5 oz Sample cups/lids *Case of 500; for samples up to 30g*	20-0047	10167
Graduated cylinder (100 mL)	ACC 068	11207
MiniPet pipette 100 µL	ACC 041	11203
Coffee filters (100)	ACC 083	11434
Centrifugation Set (50):	ACC 010	11214
Microcentrifuge	ACC 064 E	11204
Incubator	ACC BSH301	12458

†Purchase reagent-grade 50% ethanol, or prepare using 100% ethanol as directed in Notes section.

Assay Preparation

Table A on page 4 is provided as a Summary Guide for testing. More details for each step in the process are described below, and are important for achieving optimal, accurate results.

Preparation of the Sample

Turn on the incubator and set to 22°C for a minimum of 10 minutes before testing. Ensure the temperature display has stabilized and indicates "OK" before starting the assay. Make sure all reagents including samples, strips, buffer, and sample extractant are at room temperature and ready for use before starting the assay. The sample extract should be tested shortly after dilution with buffer.

Determine number and size of sub-samples and weigh out

1. Collect a composite sample according to your own sampling plan or FGIS/AMS (formerly GIPSA) guidelines. Consult FGIS/AMS reference documents to help design a plan that fits your needs.
2. Grind samples using a Bunn grinder or mill which provides a sample such that ≥95% passes through a 20-mesh sieve. Mix ground material thoroughly before sub-sampling.
3. Weigh sub-samples into containers allowing enough head room for liquid to move forcefully when shaken vigorously.

Extract samples

1. **For Wheat:** Add 50% reagent-grade ethanol* at a 3X ratio: Subsample weight (in grams) x 3 = mL of 50% ethanol; for example, a 20g sample requires 60 mL. *May be purchased at 50%, or instructions on making your own can be found in the Notes section.
2. **For DDGS:** Add 100 ml of 50% ethanol to 25g sample. Shake vigorously for 10 seconds by hand.
3. **For Corn Fermented Protein**: Add 40 mL of 50% ethanol to 10g sample. Shake vigorously for 10 seconds by hand.
4. Choose mechanical shaker or hand shaking. Liquid should be moving forcefully through the matrix to extract the Zearalenone.
   a. Shaker table: mix at highest speed (>300 rpm) for 1 minute
   b. By hand: shake vigorously for 2 minutes
5. Samples that are not thoroughly mixed and fully wetted may adversely affect test results due to inconsistent extraction.

Clarify extracts

1. **Centrifugation:**

   For Wheat: Fill a microcentrifuge tube with extract and centrifuge for 30 sec at 2000 x *g* (***not rpm***). The clear layer is the extract that will be used in the testing.

   For DDGS and Corn Fermented Protein: Fill a microcentrifuge tube with extract and centrifuge for 1 minute at 2000 x *g* (***not rpm***). The clear layer is the extract that will be used in the testing.

2. **Filtration**:

   Wheat Only: Add an approved coffee filter (e.g. BUNN Part #BUNBCF100B) to a clean vessel. Pour extract into filter, and wait no more than 2 minutes. Pull back the filter to access the filtered extract.

Add reagents to reaction tube

NOTE: Take care not to contaminate the DB4 Buffer. Keep Buffer covered when not in use, and use a new pipette tip for each test. **Please note**: DB4 Buffer is matched with specific Zearalenone kit lot numbers; be sure to use the DB4 that is provided with the kit (do not mix and match buffers with different kit lots). There is a "use with" label on the DB4 that will indicate the matching Zearalenone lot number.

1. Gently invert the DB4 buffer 10 times prior to use (slowly to avoid creating bubbles).
2. Add 100 µL DB4 to the Reaction Tube (discard tip).
3. Add 100 µL clarified extract to the Reaction Tube.
4. Mix Buffer and sample extract thoroughly by stirring or drawing the liquids up and down in the pipette tip. Samples that are not thoroughly mixed and/or accurately pipetted will adversely affect test results.
5. Do not reuse diluted samples. Use a new reaction tube for each sample.

How to Run the QuickTox Test

- Be sure the incubator has already stabilized at 22°C. If testing is planned throughout the day it recommended to turn the incubator on in the morning and leave it on until testing is complete.
- Be sure you have allowed refrigerated canisters to come to room temperature before opening.

1. Add the Reaction Tube containing the diluted sample to the incubator. If the temperature of the testing environment is unknown or outside of the range of 20-24°C (68-75°F), **allow the sample to acclimate in the incubator for 2 minutes before proceeding.**
2. Remove the QuickTox Strips to be used. Avoid bending the strips. Reseal the canister immediately.
3. Add test strip to tube, arrows down, and wait 5 minutes (run time).
4. Immediately cut off and discard the bottom section of the strip covered by the arrow tape. Insert strip into the QuickScan reader for quantitation.

Table A – Summary Guide

<table>
<tr><th>Matrix / Grou</th><th>Add Grain to Vessel First, then 50% ethanol</th><th>Fully wet sample, then mix</th><th>Clarify/dilute</th><th>Add to the reaction tube and mix</th><th>Add reaction tube to Incubator set at 22°C</th><th>Add strip for</th></tr>
<tr><td>Wheat - ZN MG2</td><td>20g to 50g
then
3X volume of 50% ethanol
(e.g. 20g sample, then 60 mL ethanol)</td><td rowspan="4">1 minute at the highest speed on shaker table, or 2 minutes vigorously by hand</td><td>Centrifuge (30 sec at 2000 x g) or Filter</td><td rowspan="4">100 µL of DB4 buffer
+
100 µL of clarified extract</td><td rowspan="4">Acclimate tube for 2 min^</td><td rowspan="4">5 min</td></tr>
<tr><td>DDGS - ZN MG3</td><td>25g to 50g
then
4X volume of 50% ethanol
(e.g. 25g sample, then 100 mL ethanol)</td><td rowspan="3">Centrifuge
(1 min at 2000 x g)</td></tr>
<tr><td>Corn Fermented Protein – ZN MG7</td><td>10 g sample
then
40 mL of 50% ethanol
Immediately shake vigorously for 10 seconds by hand</td></tr>
<tr><td>Barley- ZN MG8</td><td>20g to 50g
then
3x volume of 50% ethanol
(e.g. 20g sample then 60g of ethanol)</td></tr>
</table>

^ The tube acclimation step is only required if the temperature of the testing environment is unknown or outside of 20-24°C (68-75°F)

Use of the QuickScan System

Detailed instructions for use of the QuickScan System are supplied with each unit, and can also be found at envirologix.com/support. The Multi-Matrix Barcode Card must be scanned prior to testing.

In summary, a strip is inserted in the carrier and the carrier is inserted into the reader; the strips are read by touching or clicking on the "Read Test" area of the screen. If the "Select Matrix Groups" screen appears, select the group that displays the matrix run for each device. Results are then recorded in an electronic worksheet, allowing each user to report and track data easily.

Kit Storage

This QuickTox Kit should be stored refrigerated. Note the shelf life on the kit box. Prolonged exposure to high temperatures may adversely affect the test results. Do not open the desiccated canister until ready to use the strips.

Cross-reactivity

The following mycotoxins have been tested with this kit and no false positive results occurred at the 100 ppm level: Aflatoxin B_1, Fumonisin B_1, Ochratoxin A, Vomitoxin (DON), T-2 and HT-2.

Precautions and Notes

- Strips must be read wet promptly at the specified time for the matrix run to ensure accurate results.
- This product is currently not applicable for use in testing any other crops beyond those specified in this Product Insert.
- Pipettes lose calibration accuracy over time. Calibrate or replace pipettes at least annually.
- This assay is calibrated against reference samples supplied by Trilogy Analytical Laboratory, Washington, MO, and other vendors and associated HPLC data. Where possible, performance in other sample matrices has been validated using naturally contaminated samples. Where naturally contaminated samples are not available, performance has been validated using fortified samples.
- As with all screening tests, it is recommended that results be confirmed by an alternate method when necessary.
- The assay has been optimized for use with the protocols provided in the kit. Deviation from these protocols may invalidate the results of the test. Room-temperature components, proper and thorough mixing, accurate pipetting, and using the kit lot specific DB4 Buffer provided in the kit are essential to accurate results.
- The results generated through the proper use of this diagnostic tool reflect the condition of the working sample directly tested. Extrapolation as to the condition of the originating lot, from which the working sample was derived, should be based on sound sampling procedures and statistical calculations which address random sampling effects, non-random sampling effects and assay system uncertainty. A negative result obtained when properly testing the working sample does not necessarily mean the originating lot is entirely negative for the analyte in question.
- Protect all components from hot or cold extremes of temperature when not in use. Do not leave in direct sunlight or in vehicle.
- Observe any applicable regulations when disposing of samples and extracts.
- 50% Ethanol Preparation Instructions: For 100 mL, measure 50 mL 100% ethanol [reagent grade or better, 200 proof or 99.5% purity]; pour into suitable container with cap. Add 50 mL deionized or distilled water. Cap tightly and shake to mix. Use care when uncapping.



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