ENVIROLOGIX™

QuickTox™ Kit for QuickScan Zearalenone Flex

Catalog AQ 312 BG *Part # 12696*

Matrices and Detection Ranges:

Matrix Group ID	Matrices	Limit of Detection (LOD)	Maximum Reported Value of Base Range
ZN MG1	Corn	50 ppb	520 ppb
ZN MG2	Wheat, wheat bran, sorghum		

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
- Scan the full MMBC to enable selection of target matrix during analysis; or fold the MMBC and scan only the MG1 or MG2 barcode if you want QuickScan to skip the matrix selection and default to only the matrices associated with the selected group
- QuickScan Software Version 4.11.0 Update 3 or later is required
- DB4 Buffer is matched with specific Zearalenone Flex 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 Flex Lot Number.

If only testing MG1 or MG2 exclusively, fold the Multi Matrix Barcode and scan only the ZN MG1 or ZN MG2 barcode. This allows the software to skip the step which prompts users to select a Matrix Group.

Matrix Group ID	Matrices
DF MG1	Corn

Matrix Group ID	Matrices
DF MG2	Wheat, wheat bran, sorghum

Table A on page 6 is provided as a Summary Guide for testing each matrix. More details for each step in the process are described below, and are important for achieving optimal, accurate results.

Contents of Kit:

- 50 QuickTox Strips packed in a moisture-resistant canister
- 50 clear Reaction Tubes
- 100 pipette tips
- DB4 Buffer, kit lot specific
- Multi-Matrix Barcode Card, kit lot specific

Items Not Provided:

- QuickScan System*
- Incubator (block + base)*
- Bunn grinder or equivalent
- 20-mesh screen (available through Seedburo or other vendors)
- Digital scale for weighing samples
- Plastic sample cups with lids* or other extraction vessels
- Graduated cylinder*
- Orbital/rotary shaker
- Pipette to deliver 100 µL*
- Ethanol 50%* (Reagent Alcohol)
- Timer
- Scissors
- Microcentrifuge*

*Available as Accessories

Available Accessories:

Item	*Catalog No.*	*Part #*
QuickScan™ System	ACC 131	10050 + 10198
Sample cups w/ lids (500/case) *For extracting samples up to 30g; extracting larger samples requires different vessels. Sample cups may also be used to collect filtrate.*	ACC 012-CS	10167
Graduated cylinder (100 mL)	ACC 068	11207
MiniPet pipette 100 µL (one/location free)	ACC 041	11202
50% Ethanol	ACC E26902-1X	11156
Centrifugation Set: Disposables for 50 tests	ACC 010	11214
Microcentrifuge	ACC 064 E	11204
Incubator	ACC BSH 301	12458

Intended Use

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

- Limit of detection (LOD) = 50 ppb
- Assay range = up to 520 ppb

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.

A unified extraction and analysis protocol for most matrices avoids sample preparation error without compromising test 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.

Assay Preparation

Table A on page 6 is provided as a Summary Guide for testing each matrix. 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 USDA/GIPSA guidelines. Consult USDA/GIPSA reference documents to help design a plan that fits your needs. Note, Corn Silage procedure was qualified using samples with a moisture content in the range of 30-50%, which is a typical range for this matrix.
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 samples into containers that will allow enough head room for the liquid to move forcefully when shaken vigorously.

Extract samples

1. All commodities require 50% ethanol extraction.
    a. MG1: Corn – 2X extraction ratio; for example, 20g sample requires 40 mL 50% ethanol
    b. MG2: Wheat, wheat bran, sorghum – 3X extraction ratio; for example 20g sample, 60 mL 50% ethanol
2. Make sure the grain is completely wet, and then mix thoroughly as stated in the table. Liquid should be moving forcefully through the matrix to extract the Zearalenone.
3. The order of addition has been optimized. Please refer to and follow Summary Guide instructions for each matrix regarding the order of addition.
4. Samples that are not thoroughly mixed and fully wetted may adversely affect test results due to inconsistent extraction.

Clarify extracts (adhere to the Summary Guide table for optimal performance)

1. Centrifugation: Fill a microcentrifuge tube with extract and centrifuge for the specific time at 2000 x *g* (***not rpm***). The clear layer is the extract that will be used in the testing.
2. Settling: Allow the sample to sit undisturbed until it separates into two layers.. The top layer containing the Zearalenone residues will be used in testing In some instances, a foamy layer will float above the desired top layer. The best technique to retrieve this extract is to tip the extraction cup at a 45 degree angle, exposing the supernatant beneath the foamy layer, avoiding particulates.

Add reagents to reaction tube

1. 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 Flex 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 Flex lot number.
2. Follow Table A instructions for Buffer and extract order of addition.
3. Use two pipette tips (one for Buffer, one for extract) for each sample.
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 Strip Test

A minimum of 10 minutes before testing is to start, turn on the incubator and set to 22°C (follow manufacturer's instructions for setting temperature). Ensure the temperature display has stabilized and indicates "OK" before starting the

assay. If testing is planned throughout the day it recommended to turn the incubator on in the morning and leave it on throughout the day.

1. Allow refrigerated canisters to come to room temperature before opening.
2. 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.**
3. Remove the QuickTox Strips to be used. Avoid bending the strips. Reseal the canister immediately.
4. Place the strip into the reaction tube containing the Buffer and sample extract. The arrow tape on the end of the strip should point into the reaction tube.
5. Allow the strip to develop for the time noted in Table A.
6. Immediately cut off and discard the bottom section of the strip covered by the arrow tape. Insert strip into the QuickScan reader for quantitation.

Use of the QuickScan System

Detailed instructions for use of the QuickScan System are supplied with each unit, and can also be found at www.envirologix.com/support/quickscan . 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.

Results are reported from 50 to 520 ppb. Results below 50 ppb are reported as "<50 ppb." Results greater than 520 ppb are reported as ">520 ppb."

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.
- This assay is calibrated against wheat and corn 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.

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License

EnviroLogix has developed this kit using proprietary reagents.

Table A: Validated Matrices

Table A: Approved Matrices	Matrix Group	Limit of Detection – LOD (ppb)	Add Grain to Vessel First, then 50% ethanol	Fully wet sample, then mix	Clarify/dilute	Add to the reaction tube and mix	Add reaction tube to Incubator set at 22°C	Add strip for
Corn	ZN MG1	50	20g to 50g *then* **2X** volume of 50% ethanol (e.g. 20g sample, then 40 mL ethanol)	1 minute at the highest speed on shaker table, or 2 minutes vigorously by hand	Centrifuge (30 sec) *or* Settle	100 µL of buffer + 100 µL of clarified extract	Acclimate tube for 2 min^	5 min
Wheat, Wheat Bran, Sorghum	ZN MG2		20g to 50g *then* **3X** volume of 50% ethanol (e.g. 20g sample, then 60 mL ethanol)		Centrifuge (30 sec)			

Notes:

^ 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)