

# **TotalTox™ Zearalenone**

Catalog AQ 412 BG

Part #12919

#### Matrix and Detection Summary:

Matrix Group ID	Protocol	Results reported in the range of:	Limit of Detection (LOD)*	Highest Approved Level*
ZN MG1 - Corn/	Base Range	0 - 500 ppb	50 ppb	500 ppb
Corn Common Extraction	Dilution A	0 - >2000 ppb	250 ppb	2000 ppb
ZN MG2 - Wheat	Base Range	0 - 1200 ppb	50 ppb	1200 ppb
ZN MG4- Corn Gluten Meal (CGM)	Base Range	0 - 2000 ppb	250 ppb	2000 ppb

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

#### **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 5.2 or later is required

A Summary Guide for testing is provided on Page 10. More details for each step in the process are described below and are important for achieving optimal, accurate results.

#### **Contents of Kit:**

- 50 TotalTox Strips packed in a moisture-resistant canister
- 50 reaction tubes
- 100 pipette tips (1-200 μL)
- DB5 Buffer
- Multi-Matrix Barcode Card kit lot specific

### Matrices

Note: Scanning the Multi-Matrix Barcode Card once per kit lot is required. The QuickScan software will prompt users to select a Matrix Group (MG) before proceeding to the result screen. If you only plan to test matrices within the MG1 group (Corn/Corn Common Extraction), scan the side of the MMBC card that only has the MG1 barcode. This allows the software to skip the Matrix Group selection step.

<ul> <li>Corn, Wheat</li> </ul>	<b>SET A</b> PROCEDURE: PAGE 4	• CG	M SET B PROCEDURE: PAGE 4
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### **Intended Use**

TotalTox Zearalenone is designed to quickly provide quantitative results for the presence of total Zearalenone. Matrix and Detection Summary on Page 1 lists the Limit of detection (LOD) and Assay range for each matrix.

### How the Test Works

A composite sample is collected, ground, and extracted to solubilize any Zearalenone present. The extract is further diluted into Buffer before being run on the test strip. Each strip has an absorbent pad at each end. The sample extract travels up the test strip and is absorbed into the larger pad at the top of the strip. At the end of the reaction time, the strip is cut 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, Limit of Detection (LOD), and maximum reported value. When the user selects the MG during testing, the QuickScan System software reads the test strip, retrieves information encoded in the strip's barcode and on 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:	*Available Accessories:		
• 50 EB17 dissolvable pouches	Item	Catalog No.	Part #
(1 pkt per 25g sample)	QuickScan <sup>TM</sup> System	ACC 331	12721
QuickScan System*	EB17 Extraction Buffer pouches, 50	ACC 117	12938
• Incubator (base + block)*	TotalTox Corn Gluten Meal		
Bunn grinder or equivalent	Common Extraction Set	ACC 114	12978
20-mesh screen (available through Seedburo or other vendor)	(100 tests; includes10X EB18 Extraction EB17 Extraction Buffer pouches)	on Buffer Concentrate and	d
<ul> <li>Digital scale for weighing samples</li> <li>Extraction cups with lids* or other</li> </ul>	5 oz Sample cups/lids Case of 500; for extracting samples up to 3	20-0047 80g	10167
<ul> <li>suitable vessels for sample extraction</li> <li>Graduated cylinder*</li> </ul>	10 oz Sample cups/lids Case of 100; for extracting samples >30g	20-0129	12383
Orbital/rotary shaker	Graduated cylinder (100 mL)	ACC 068	11207
• Pipette to deliver $100 \mu L^*$	MiniPet pipette 100 µL	ACC 041	11203
• Pipette to deliver larger volumes (for Dilution A if desired)*	Coffee filters (100)	ACC 083	11434
<ul> <li>Timer</li> </ul>	Centrifugation Set (50):	ACC 010	11214
Scissors	Microcentrifuge	ACC 064 E	11204
• Distilled, deionized or bottled water	50g Sample Extraction Set Additional EB17 dissolvable pouches and s	ACC 099 sample cups (100)	12409
*Available as Accessories	DB5 Buffer Additional Buffer for wheat, requires >	KR-266-7 > 100 μL per Strip	11665
	Dilution Set: Blue dilution tubes and EB17 dissolvable p	ACC 103 ouches for 50 tests	12500
	Dilution Tubes: Blue dilution tubes for non-EB17 dilution,	ACC 098 50	12236
	1 mL adjustable pipette	ACC 1303-PRO-1000	11964
	Pipette tips for 1 mL pipette (50)	20-0127	12243
	Incubator	ACC BSH301	12458

# Sample Preparation

- 1. Collect a composite sample according to your own sampling plan or USDA grain inspection guidelines. Consult USDA reference documents to help design a plan that fits your needs. Contact Technical Support for more information.
- 2. Grind samples to provide a consistency such that 95% passes through a 20-mesh sieve.
- 3. Mix ground material thoroughly before sub-sampling, to minimize variability.
- 4. Weigh 25-50g samples into containers that will allow enough head room for the liquid to move forcefully when shaken vigorously.

# Sample Clarification

Depending on the sample matrix, there may be multiple acceptable methods for removing particulate from the extract. Refer to Matrix Group instructions (Page 4) or Summary Table (Page 8).

Centrifugation	Filtration
<ol> <li>Fill a microcentrifuge tube with extract.</li> <li>Centrifuge for the specified time at 2000 x g (rcf, <u>not rpm</u>).</li> <li>Use the top layer of extract for all matrices; there may be a white floating layer above that extract that should not be used for testing.</li> </ol>	<ol> <li>Add an approved coffee filter (e.g. BUNN Part #BUNBCF100B) to a clean vessel.</li> <li>Pour extract into filter. Wait no more than 2 min.</li> <li>Pull back the filter to access the filtered extract.</li> </ol>

# Testing in Base Range

Refer to Matrix Group instructions (Pages 5 and 6) or Summary Table (Page 10) for base range testing.



### **Range with Dilution**

If after running and reading the test, the initial result is greater than the upper end of the Base Range, some samples can be diluted and retested to extend quantitation (see Summary on Page 1). Combine extract with the appropriate Dilution Reagent to create a diluted extract. Measure carefully and mix well.

Dilution Reagent					
Corn/MG1					
<b>EB17</b> Dilution Solution: Dissolve 1 EB17 pouch in 150 mL of water and mix well; Dilution Solution mixture will appear cloudy. Label, date, and document the preparation. Dilution					
Solution can be stored at ambient temperature for 30 days. Thoroughly mix before use.					

Corn Extended Range: Testing samples at levels greater than 500 ppb corn

- 1. Mix 800 µL Dilution Reagent + 100 µL clarified extract in a blue Dilution Tube or other suitable vessel.
- 2. Rerun assay as before (see Page 4). Example: for corn, pipette  $100 \mu L DB5 + 100 \mu L$  of the diluted extract into a new reaction tube; place tube in 22°C incubator for 2 min^, add a new test strip, and wait 4 minutes for test results.
- 1. In the QuickScan Results Screen, use the dilution tab pull down menu to select Dilution A (1:A). The System will adjust and display the Zearalenone level from diluted samples. Adjusted results are valid in the range of **500-2000 ppb for Corn.**

### Use of the QuickScan System

Detailed instructions for use of the QuickScan System are supplied with each unit and can also be found at <u>www.envirologix.com/quickscan</u>. The lot-specific Multi-Matrix Barcode Card (MMBC) must be scanned into the system prior to testing. In summary, a strip is inserted into the reader and the strips are read by touching or clicking on the "Read Test" area of the screen. The "Select Matrix Groups" screen will appear if more than one barcode was scanned into the system from the MMBC. Select the group that displays the matrix run. Results are then recorded in an electronic worksheet, allowing each user to report and track data easily.

## Kit Storage

This Kit should be stored refrigerated. Note the shelf life on the kit box. Prolonged exposure to high temperatures may adversely affect the test results; protect all components from extreme hot or cold temperatures. Do not leave in direct sunlight or in a vehicle. 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, DON (deoxynivalenol), T2, Zearalenone.

### Notes

- 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.
- Immediate shaking of the sample after water addition is critical to ensure the EB17 packet does not cause clumps which may interfere with test results.
- This assay is calibrated against reference samples supplied by Trilogy Analytical Laboratory, Washington, MO, and other vendors and associated HPLC data.
- 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, timing, and accurate pipetting 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.

- **IMPORTANT:** If used, the 10X EB18 Extraction Buffer should be considered an irritant (SDS available at <u>https://www.envirologix.com/?attachment\_id=3004</u>). Avoid contact with the skin, eyes, or clothing. Wear personal protective equipment including safety glasses, gloves, and a lab coat when handling.
  - **To prepare 1X EB18 Buffer Solution:** Mix 1 part 10X EB18 Extraction Buffer with 9 parts of water. 1X solution expires one week from date of mixing when stored at room temperature, or 4 weeks when stored at 2-8°C. Bring to room temperature before using.



# Set A: Corn, Wheat

- Review Sample Preparation on Page 2 for grinding consistency and notes.
- Turn on the incubator and set to 22°C for a minimum of 10 minutes before testing. Ensure that the temperature display has stabilized and indicates "OK" before starting the assay. All reagents should be at room temperature.
- Use distilled, deionized, or flat (non-carbonated) bottled water.
- If testing 50-gram samples, additional EB17 Buffer pouches and larger extraction vessels are required (50g Sample Set, order Catalog No. ACC-099).

#### **Sample Extraction**

C WI	25g	Add 1 EB17 pouc Add 75 mL water	•	shaking for 10 se	nediately, by vigorously econds by hand. If e sample against the palm
Corn, Wheat	50g	Add 2 EB17 pouches to sample Add 150 mL water		of your other har loosen up any dr	nd or a hard surface to
Shake: choose mechanical shaker or hand shaking		Shaker Table:	mix at highest	By Hand: shake	

Clarify Extract: choose centrifuge or filter (wheat, centrifuge only)

#### **Combine Buffer and Extract, then Run Test Strips**

- 1. Add DB5 to the Reaction Tube (discard tip)
- 2. Add clarified extract to the Reaction Tube
- 3. Mix thoroughly with extract pipette tip, discard tip
- 4. Place the Reaction Tube in the 22°C incubator; equilibrate for 2 minutes. Note: tube acclimation step is only required if the temperature of the testing environment is unknown or outside of 20 - 24°C (68 - 75°F)
- 5. Add test strip to tube, arrows down, wait 4 minutes (run time)
- 6. Immediately cut strips at the top of the arrow tape (discard bottom pads)
- 7. Insert strip into QuickScan Reader

#### TIPS!

speed ( $\geq$  300rpm) for 1 minute

Centrifuge: 30 seconds

at 2000 x g (rcf, *not rpm*)

#### **Get Complete Extraction**

Fully wet samples before the next shaking step

vigorously for 2 minutes

Filter: Pour through approved

coffee filter (ACC 083); wait

no more than 2 minutes

- Avoid delay between water addition and shaking
- Assure liquid is moving forcefully though the sample while shaking

#### **For Best Performance**

- Pipette up and down while mixing
- Read strips promptly after run time

#### **Avoid Contamination**

- Use a new Reaction Tube per test
- Keep DB5 capped, when possible
- Use new pipette tips for each step

Matrix	LOD (ppb)	-	straction vessel in se 25 or 50g):	Shake	Clarify	Reaction Tube	Run
Corn	50	A1. 25g sample A2. 1 x EB17 A3. 75 mL water	B1. 50g sample B2. 2 x EB17 B3. 150 mL water	1 min – shaker	Filter <u>or</u> Centri- fuge	100 μL DB5 + 100 μL extract	4 min
Wheat	50	A4. Immediately shake 10 sec.	B3. 130 IIIL water B4. Immediately shake 10 sec.	or 2 min – by hand	Centri- fuge	200 μL DB5 + 100 μL extract	4 11111

#### TABLE A: Set A Matrix Summary (base range)



## Set B: Corn Gluten Meal

- Review Sample Preparation on Page 2 for grinding consistency and notes.
- Turn on the incubator and set to 22°C for a minimum of 10 minutes before testing. Ensure that the temperature display has stabilized and indicates "OK" before starting the assay. All reagents should be at room temperature.
- For ease of extraction, purchase ACC-114, CGM Common Extraction Set; includes both extraction buffers required. Prepare EB18 Extraction Buffer from concentrate before proceeding (see Notes, Page 4, for preparation instructions and SDS). Use distilled, deionized, or flat (non-carbonated) bottled water.

#### **Sample Extraction**

CornAdd 1 EB17 pouch to sample10 seconds by handGluten25gAdd 50 mL 1X EB18 Extractionagainst the palm of	<ul> <li>diately, by vigorously shaking for</li> <li>d. If needed, shake the sample</li> <li>F your other hand or a hard surface</li> <li>ry sample areas. <u>Immediately</u></li> <li>king step.</li> </ul>
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Shake: choose mechanical shaker or hand shaking

Shaker Table: mix at highest Bv Hand: shake speed ( $\geq$  300rpm) for 1 minute vigorously for 2 minutes

*Centrifuge:* 60 seconds at

Clarify Extract: Centrifuge:

#### **Combine Buffer and Extract, then Run Test Strips**

- 1. Add DB5 to the Reaction Tube (discard tip)
- 2. Add clarified extract to the Reaction Tube
- 3. Mix thoroughly with extract pipette tip, discard tip
- 4. Place the Reaction Tube in the 22°C incubator; equilibrate for 2 minutes. Note: tube acclimation step is only required if the temperature of the testing environment is unknown or outside of 20 - 24°C (68 - 75°F)
- 5. Add test strip to tube, arrows down, wait 5 minutes (run time)
- 6. Immediately cut strips at the top of the arrow tape (discard bottom pads)
- 7. Insert strip into QuickScan Reader

#### TIPS!

#### **Get Complete Extraction**

2000 x g (rcf, *not rpm*)

- Fully wet samples before the next shaking step
- Avoid delay between EB18 buffer addition and shaking
- Assure liquid is moving forcefully though the sample while shaking

#### **For Best Performance**

- Pipette up and down while mixing
- Read strips promptly after run time

#### **Avoid Contamination**

- Use a new Reaction Tube per test
- Keep DB5 capped, when possible
- Use new pipette tips for each step

Matrix	LOD (ppb)	Add to sample extraction vessel in order:	Shake	Clarify	Reaction Tube	Run
CGM	250	<ol> <li>25g sample</li> <li>1 x EB17</li> <li>50 mL EB18</li> <li>(immediately shake by hand 10 sec)</li> </ol>	1 min – shaker <u>or</u> 2 min – by hand	Centri- fuge	100 μL DB5 + 100 μL extract	5 min

#### TABLE B: Set B Matrix Summary (base range)



#### For Technical Support Contact Us At:

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website: www.envirologix.com



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EnviroLogix has developed this kit using proprietary reagents.

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Appropriate respiratory protection should be determined according to local conditions using risk analysis protocols. An approved disposable air purifying particulate respirators may be used as a backup to engineering controls. Always use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Contain spills, do not allow into environmen

#### TotalTox Zearalenone Page 8 of 10

	LOGI	×	Date	sion nr.2 d 04/29/2019 e n. 2 / 6		
SECTION 3. Composition	linformati	on on ingre				
3.2 Mixture		on on nigro	allento.			
See Bellowerk ( 022440)	CAS No	EC No	Classification According to 29CFR 1910.1200	Amount (%)		
Sodium Tetraborate Decahydrate	1303-96-4	215-540-4	H360 Rep 1B	< 3 %		
p-tertiary Octylphenoxy polyethyl alcohol (Triton X-100)	9002-93-1		H302 Acute Tox. Oral 4 H315 Skin Irrit. 2 H318 Eye Dam. 1	1 %		
	9014-85-1		H411 Aquatic Chronic 2 H315 Skin irritation 2 H318 Eye damage 1	2 %		
	2634-33-5	220-120-9	H335 STOT SE 3 H302 Acute Tox. 4; H315 Skin Irrit. 2	0.048 %		
one (Proxel- GXL)			H317 Skin Sens. 1 (C≥ 0.05%)			
			H318 Eye Dam. 1 ; H400 Aquatic Acute 1			
SECTION 4. First aid mea						
After inhalation :	ires	In cas	e of inhalation. Remove to fresh air. If not breathing	g give artificial		
After skin contact :		In cas	ation. Get medical attention immediately. e of skin contact. Remove contaminated clothing and	shoes immediate		
848 - 10 - 10		evider	affected area with mild soap or detergent for at least ice of chemical remains.			
After eye contact :		minut	e of eye contact, immediately flush eyes with plenty es. Lifting eyelids occasionally, until no evidence of	or water for at lea chemical remain		
After swallowing :		Get m	edical attention immediately.			
		medic Call a	<u>e of ingestion</u> , DO NOT Induce vomiting unless dire al personnel. Never give anything by mouth to an u physician immediately.	nconscious perso		
2 Most important symptoms an ute and delayed:	nd effects, be					
are and delayed: 3 Indication of any immediate i	medical atte					
d special treatment needed:		None				
SECTION 5. Firefighting r	measures	š				
1 Extinguishing media:		CO2, exting or alcohol r	guishing powder or water spray. Fight larger fires wit esistant foam.	h water spray		
2 Special hazards arising from obstance or mixture:	the					
ubstance or mixture:		None				
3 Advice for firefighters:		Wear protective g	ctive gear appropriate for fire conditions including re gear.	spiratory		
3 Advice for firefighters: SDS DB5 Dilution Buffer		Wear protective g	tive gear appropriate for fire conditions including re	spiratory		
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Breathing Equipment:

8.2.3 Environmental exposure controls:



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SECTION 12. Ecological inform	ation.			
12.1 Toxicity: Triton X-100		bhales promelas (fathead minnow) Daphnia - 26 mg/l - 48 hr	- 8.9mg/l - 96.0 hr	
12.2 Persistence and degradability :	No Data Available			
12.3 Bio accumulative potential:	No Data Availabl	e		
12.4 Mobility in soil :	No Data Availabl	e		
12.5 Results of PBT and vPvB assessment:	Not available as a	chemical safety assessment, not re	quired/not conducted.	
12.6 Other adverse effects:	No Data Availabl	e		
SECTION 13. Disposal conside	rations.			
Waste treatment methods:	material. Disp	nsed professional waste disposal se osal of surplus or waste solutions n al, state, and national laws and regu	nust be in accordance with	
SECTION 14. Transport informa	tion.			
14.1 UN-Number DOT, ADR, ADN, IM 14.2 UN proper shipping name DOT. A		Not Hazardous for Transport		
IATA :	DK, ADN, IMDO,	Not Hazardous for Transport		
14.3 Transport hazard class(es) DOT, / IATA): 14.4 Packing group (DOT, ADR, IMDO		Not Hazardous for Transport Not Hazardous for Transport		
14.5 Environmental hazards		No environmental hazard.		
14.6 Special precautions for user :		None		
14.7 Transport in bulk according to An MARPOL73/78 and the IBC code:	mex II of	No information available.		
SECTION 15. Regulatory inform	nation.			
15.1 Safety, health, and environmental US Federal Regulations OSHA SARA 313 US State Regulations European/International Regulations	regulations	Not a hazardous material Not listed		
European labeling in accordance with EC	Directives	Not hazardous according to E	aropean directives	
15.2 Chemical Safety Assessment		Not carried out		
SDS DB5 Dilution Buffer				

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SECTION 16. Other infor	rmation.	
completeness. Persons receivin	ng this information must exercise their indepe use. This document shall not constitute a gue	Logix makes no representation of its accuracy or endent judgment in determining the product's safety trantee for any specific product features and shall n
EHS Department EnviroLogix Inc.		
Codes:		
H302 Harmful if swallowed H318 Causes Serious Eye Damag	H315 Causes skin irritation H31 ge H335 May cause respiratory irritation H41	7 May cause an allergic skin reaction 1 Toxic to Aquatic Life with Long Lasting Effects

### **Summary Guide for Approved Matrices**

Approved Matrix	Add to Sample Extraction Vessel (in this order)	Then shake immediately	Clarify	Run the Base Range Protocol First, followed by Dilution Protocol, if necessary	Transfer to Reaction Tube	Add Reaction Tube to Incubator Set at 22°C	Add Strip for	Read in QuickScan: Dilution Tab on the Result Page Should Display
	<ol> <li>25g sample</li> <li>1 EB17 pouch</li> <li>75 mL water*</li> <li>Immediately shake 10 seconds</li> <li>0R</li> <li>50g sample</li> <li>2 EB17 pouches</li> <li>150 mL water*</li> <li>Immediately shake 10 seconds</li> </ol>	1 min highest speed on shaker table or 2 min by hand	Filter or Centrifuge 30 sec. at 2000 x g	Base Range 50-500 ppb	100 μL DB5 buffer + 100 μL clarified extract in Reaction Tube	Acclimate tube for 2 min <sup>^</sup>	4 min.	1:1 (this is software default)
Corn (MG1)				Dilution A 250-2000 ppb	<u>Pre-Mix</u> 800 μL Dil'n Sol'n <sup>+</sup> + 100 μL clarified extract <u>Transfer</u> 100 μL of this Pre- Mix and 100 μL DB5	Acclimate tube for 2 min <sup>^</sup>	4 min.	1:A (this must be selected)
Wheat (MG2)			Centrifuge 30 sec. at 2000 x g	Base Range 50-1200 ppb	200 µL DB5 buffer + 100 µL clarified extract in Reaction Tube	Acclimate tube for 2 min <sup>^</sup>	4 min.	1:1 (this is software default)
Corn Gluten Meal (MG4)	<ol> <li>25g sample</li> <li>1 EB17 pouch</li> <li>50 mL of 1X EB18</li> <li>Immediately shake vigorously for 10 seconds by hand</li> </ol>		Centrifuge 60 sec. at 2000 x g	Base Range 250-2200 ppb	100 μL DB5 buffer + 100 μL clarified extract in Reaction Tube	Acclimate tube for 2 min^	5 min.	1:1 (this is software default)

Notes:

\*Use distilled, deionized, or flat (non-carbonated) bottled water

^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)

**†**Refer to page 3 for Dilution Solution instructions