ENVIROLOGIX™

QuantiTox™ Kit for Trichothecenes

Catalog Number EP 100

Intended Use

The EnviroLogix QuantiTox Kit for Trichothecenes is a 96-well plate kit designed for the quantitative laboratory detection of Tricothecenes, including Roridin A, E, H and L-2, Satratoxin G and H, Isosatratoxin F, Verrucarin A and J, and Verrucarol in bulk samples. The assay's quantitation range is from 0.2 to 18.0 parts per billion (ppb) of Roridin A in the sample extract.

How the Test Works

This Kit is a competitive Enzyme-Linked ImmunoSorbent Assay (ELISA). In the test, tricothecenes in the sample compete with enzyme (horseradish peroxidase)-labeled satratoxin for a limited number of antibody binding sites on the inside surface of the test wells.

After a simple wash step, the outcome of the competition is visualized with a color development step. As with all competitive immunoassays, sample concentration is inversely proportional to color development.

Darker color = Lower concentration
Lighter color = Higher concentration

Limit of Detection

The Limit of Detection (LOD) of this kit is 0.14 ppb. The LOD was determined by interpolation at 92.3% B_0* from a standard curve. 92.3% B_0 was determined to be 3 standard deviations from the mean of a population of negative bulk samples.

*100% B_0 equals the maximum amount of Satratoxin Enzyme Conjugate that is bound by the antibody in the absence of any trichothecenes in the sample (i.e. negative control). % B_0 = (OD of Sample or Calibrator/OD of Negative Control) x 100.

Precision

Satratoxin H-fortified control solutions were repetitively analyzed both within a single assay, and in different assays on different days. The data is expressed as %CV for both the recovered concentration and for absorbance (OD).

Fortification and Recovery

Six bulk samples were fortified with Roridin to a concentration of 2.0 ppb. The average recovery was 103.6%, with a CV of 24.5%.

Cross-Reactivity

This Kit does not distinguish between various macrocyclic trichothecenes, but detects their presence to differing degrees. The table (page 2, left margin) shows the concentrations of cross-reacting mycotoxins that are approximately equivalent to the three Roridin A (RA) Calibrators run in the kit. Concentrations are in ppb.

Highlights:

- *Detection of ten different Trichothecene toxins: Satratoxin G & H, Isosatratoxin F, Roridin A, E, H & L-2, Verrucarol, and Verrucarin A & J*
- *Results in one hour—faster and more cost effective than current methods*
- *Extremely sensitive—rivals the sensitivity of current HPLC protocols*

Contents of Kit:

- *12 strips of 8 antibody-coated wells each, in plate frame*
- *1 vial of 900 ppb Roridin A Stock Solution in 50% MeOH*
- *1 bottle of Satratoxin Enzyme Conjugate*
- *1 bottle of Substrate*
- *1 bottle of Stop Solution*
- *1 packet of PBS salts (Sigma Chemical, Cat. # P-3813)*

Precision

	Recovery (%CV)	**OD** (%CV)
Intra-Assay n=7		
Control 1	21.5%	5.1%
Control 2	8.5%	4.1%
Inter-Assay n=10		
Control 1	18.7%	n/a
Control 2	16.6%	n/a

Cross-Reactivity

Mycotoxin	0.2 ppb RA Calib.	2.0 ppb RA Calib.	18 ppb RA Calib.
Satratoxin G	0.338	1.32	4.45
Satratoxin H	0.086	1.07	9.48
Isosatratoxin F	0.043	0.266	1.199
Verrucarol	16.21	561.5	9241.5
Verrucarin A	1.11	13.32	107.6
Verrucarin J	1.11	21.94	161.70
Roridin L-2	5.68	68.44	457.75
Roridin E	0.025	0.500	5.209
Roridin H	0.16	5.94	75.64

The toxins listed below were found to be non-reactive in the Trichothecenes plate kit when run at a level of 1000 ppb (roughly 50 times higher than the highest calibrator).

Aflatoxin B1	Fumonisin B1
Citrinin	Gliotoxin
Cyclosporin	Griseofulvin
Cytochalasin E	Ochratoxin A
Deoxynivalenol	Patulin
Emodin	T-2

Precautions and Notes

- **IMPORTANT: Roridin A is highly toxic.** All liquids should be disposed of in a plastic container containing at least 10% household bleach. All contaminated glassware should soak for 1 hour in a 30% bleach solution before being washed. Wear gloves and other protective apparel at all times. If liquid comes into contact with skin, wash immediately with water.
- Store all Plate Kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not expose Plate Kit components to temperatures greater than 37°C (99°F) or less than 2°C (36°F).
- Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before use.
- Do not use kit components after the expiration date.
- Do not use reagents or test well strips from one Plate Kit with reagents or test well strips from a different Plate Kit.
- Do not expose Substrate to sunlight during pipetting or while incubating in the test wells.
- As with all tests, it is recommended that results be confirmed by an alternate method when necessary.
- The assay has been optimized to be used with the protocol provided in the kit. Deviation from this protocol may invalidate the results of the test.
- Observe any applicable regulations when disposing of samples and kit reagents.

Items Not Provided

- disposable tip, adjustable air-displacement pipette to deliver 100 µL - 1000 µL (optional)
- positive displacement pipette to deliver 10, 20 and 100 µL (Microman™ or equivalent)
- twelve-channel pipette to deliver 50 µL and 100 µL (optional)
- marking pen (indelible)
- tape or Parafilm®
- timer
- microtiter plate reader or strip reader
- microtiter plate washer (optional)
- racked dilution tubes for loading samples into the plate with a 12-channel pipette (optional)
- distilled or deionized water
- orbital plate shaker (optional)

NOTE: It is recommended that all samples be extracted with the PBS provided in the kit. Contact EnviroLogix Tech Support if sample extracts are in a different solution or solvent.

Qualitative Use of This Kit
To use this kit in a qualitative format, prepare only the 0.2 ppb Roridin A calibrator. Run samples with both the negative calibrator (PBS) and the 0.2 ppb Roridin A calibrator in duplicate.

Remove unneeded strips

Preparation of Solutions

PBS Extraction/Dilution Buffer

Make up 1.0 liter of phosphate buffered saline (PBS), pH 7.4 ± 0.05. Add the PBS salt packet to 1.0 liter of distilled or deionized water. Stir until all of the salts have dissolved. **The Buffer should be stored refrigerated when not being used to prevent bacterial contamination.** Warm to room temperature prior to use.

Roridin A Calibrators in PBS Extraction/Dilution Buffer

Working levels of Roridin A must be made daily in PBS. A positive-displacement pipette **must** be used to dispense the Roridin A stock solution. At the end of the day, any unused calibrators should be disposed of by adding to a 10% household bleach solution.

1. Using a positive-displacement pipette, dilute the 900 ppb Roridin A Stock Solution to 18.0 ppb, 2.0 ppb and 0.2 ppb in PBS in a glass test tube, as described below.
   - **18.0 ppb Roridin A Calibrator** = 20 µL of 900 ppb Stock Solution into 980 µL PBS
   - **2.0 ppb Roridin A Calibrator =** 100 µL of 18 ppb Calibrator into 800 µL PBS
   - **0.2 ppb Roridin A Calibrator =** 10 µL of 18 ppb Calibrator into 890 µL PBS
   - **Negative Calibrator:** use an aliquot of the PBS stock

Sample Extraction and Preparation

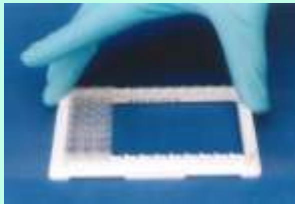
Refer to Appendix A, page 8.

How to Run the Assay

- Read all of these instructions before running the kit.
- Allow all reagents to reach room temperature prior to use. Do not remove strips from foil pouch until they have warmed up).
- If more than three strips are to be run at one time, the loading time will most likely exceed 10 minutes, and the use of a multi-channel pipette is recommended in steps 3, 4, 7 and 9.
- If three or fewer strips are to be run, use a disposable-tip, air-displacement pipette and a clean pipette tip to add each Calibrator and sample extract to the wells. Conjugate, Substrate, and Stop Solution may be added in the same manner; alternatively, use a repeating pipette with a disposable tip for these three reagents.
- If fewer than all twelve strips are used, reseal the remaining strips and the desiccant in the foil pouch and refrigerate.
- Use the well identification markings on the plate frame as a guide when adding the samples and reagents. For a quantitative assay, the Negative Calibrator (NC) and three Roridan A Calibrators (C1-C3), along with 44 sample extracts (S), may be run in duplicate wells on one plate. Refer to Figure 1 for a quantitative assay example plate layout. For an example of a qualitative assay plate layout, refer to Figure 2.

1. Once all components have reached room temperature, remove the plate from the pouch. Return unneeded strips to pouch and reseal.

Add Calibrators and samples


Mix plate

Bottle Wash method

Complete protocol and add Stop Solution

2. Organize all Calibrators, sample extracts, and pipettes so that Steps 3 and 4 can be performed in 10 minutes or less.
3. Rapidly add **50 µL** of **Negative Calibrator** (NC), **50 µL** of each **Roridan A Calibrator** (C1-C3) and **50 µL** of each **sample extract** (S1-S8) to their respective wells, as shown in Figure 1. (Follow this same order of addition for all reagents.)
4. Immediately add 50 µL of Enzyme Conjugate to each well.
5. Thoroughly mix the contents of the wells by moving the plate frame in a rapid circular motion on the bench top for 30-45 seconds. Use care when mixing to avoid cross-contamination Cover the wells with tape or Parafilm and incubate at ambient temperature for 45 minutes. Use an orbital shaker at 200 rpm if available.
6. After incubation, carefully remove the covering and vigorously shake the contents of the wells into a suitable container. Flood the wells completely with tap or laboratory grade water, then shake to empty. Repeat this wash step four more times. Slap the inverted plate several times on a paper towel to remove as much water as possible.
7. Add 100 µL of Substrate to each well.
8. Thoroughly mix the contents of the wells, as in step 5. Cover the wells with new tape or Parafilm and incubate for 15 minutes at ambient temperature. Use orbital shaker if available.

Caution: Stop Solution is 1.0 N Hydrochloric acid. Handle carefully.

9. Add 100 µL of Stop Solution to each well and mix thoroughly. This will turn the well contents yellow.

NOTE: Read the plate within 30 minutes of the addition of Stop Solution.

How to Interpret the Results

For samples containing mixed tricothecenes, concentrations may only be reported as Roridin A "equivalents". Samples known to contain different trichothecenes, such as Satratoxin H, can be quantitated by re-naming the kit calibrators with the concentrations listed under "Cross-Reactivity".

Spectrophotometric Measurement

1. Set the wavelength of the microtiter plate reader to 450 nanometers (nm). (If it has dual wavelength capability, use 600, 630 or 650 nm as the reference wavelength.)
2. If the plate reader does not auto-zero on air, zero the instrument against 200 µL water in a blank well. Measure and record the optical density (OD) of each well's contents. Alternatively, measure and record the OD in every well, then subtract the OD of the water blank from each of the readings.
3. A **semi-log** curve fit for the standard curve should be used if the microtiter plate reader has data reduction capabilities. If not, calculate the results manually as described below.

How to Interpret the Qualitative Results

1. If the mean OD of the sample is lower than the mean OD of the 0.2 ppb Calibrator, it is considered positive for Trichothecenes. If the mean OD of the sample is higher than the mean OD of the 0.2 ppb Calibrator, it contains less than 0.2 ppb Roridan A equivalents.

Read strips or plate in a Plate Reader within 30 minutes of the addition of Stop Solution

How to Calculate the Quantitative Trichothecenes Results

1. After reading the wells, average the OD of each set of Calibrators and samples, and calculate the $\%B_0$ as follows:

$\%B_0$ = (average OD of Calibrator or sample divided by the average OD of Negative Calibrator) x 100

The $\%B_0$ calculation is used to equalize different runs of an assay. While the raw OD values of the Calibrators and samples may differ from run to run, the $\%B_0$ relationship of Calibrators and samples to the Negative Calibrator should remain fairly constant. The $\%B_0$ of each Calibrator should fall within these ranges:

Calibrator	$\%B_0$
18 ppb	14 – 25
2.0 ppb	42 – 57
0.2 ppb	76 – 90

The coefficient of variation (CV) for each pair of Calibrator and sample OD values should not exceed 15% (%CV=[standard deviation/mean] x 100).

2. Graph the $\%B_0$ of each Calibrator against its concentration on a semi-log scale (see Figure 4). Use the values for Roridin A or substitute the Calibrator equivalents for Satratoxin or other mycotoxins as appropriate.

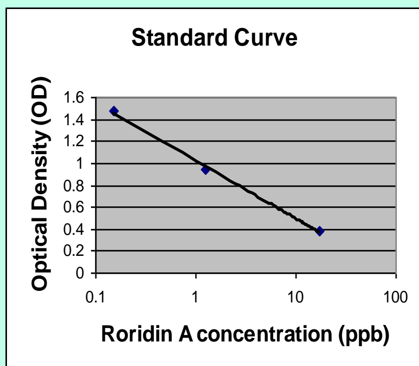
3. Determine the trichothecene concentration of each sample by finding its $\%B_0$ value and the corresponding concentration level on the graph. Multiply the results by any dilution factor incurred during sample extraction.

4. Interpolation of sample concentration is only possible if the $\%B_0$ of the sample falls within the $\%B_0$ range of the Calibrators.

   If the $\%B_0$ of a sample is higher than that of the lowest Calibrator, the sample should be reported as less than the concentration of the lowest Calibrator.

   If the $\%B_0$ of a sample is lower than that of the highest Calibrator, the sample should be reported as greater than the concentration of the highest Calibrator. If a concentration must be determined for these high-level samples, dilute the sample 1:20 in PBS and repeat the immunoassay. If the result now falls within the $\%B_0$ range of the Calibrators, multiply the concentration measured in the diluted sample by a factor of 20.

Figure 4.
Illustrative standard curve



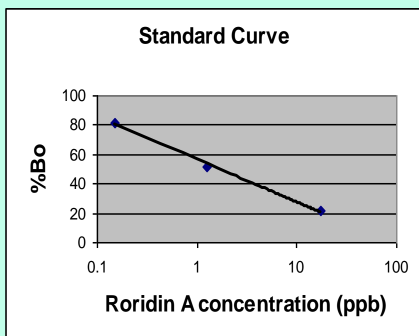


Figure 1. Example of a typical Quantitative assay setup

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	NC										
B	C1	C1										
C	C2	C2										
D	C3	C3										
E	S1	S1										
F	S2	S2										
G	S3	S3										
H	S4	S4										

Figure 2. Example of a typical Qualitative assay setup

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	NC										
B	C1	C1										
C	S1	S1										
D	S2	S2										
E	S3	S3										
F	S4	S4										
G	S5	S5										
H	S6	S6										

Figure 3. Illustrative calculations

Well contents	OD	Average OD	%CV	$\%B_0$	Roridin A Concentration (ppb)
Negative Calibrator	1.473 1.588	1.531	5.313	100	0
0.2 ppb Calibrator	1.283 1.287	1.285	0.220	83.93	0.2
2.0 ppb Calibrator	0.768 0.814	0.791	4.112	54.67	2.0
18.0 ppb Calibrator	0.293 0.320	0.307	6.229	20.05	18.0
Sample	0.521 0.556	0.539	4.596	35.21	6.28

Actual values may vary; this data is for demonstration purposes only.

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Appendix A: Guidelines for Sample Extraction

Note: Contact EnviroLogix before testing samples in extraction solutions other than the PBS supplied in the kit.

Surface Mold:

- Collect a small sample with a cotton-tipped swab or other implement.
- Insert the sample into 6.0 mL of PBS Extraction/Dilution Buffer in a glass test tube, then swirl it to release the sample and extract any mycotoxins into the PBS.
- Vortex or vigorously shake the sample for approximately 20-30 seconds.
- Microcentrifuge an aliquot of the sample for 5 minutes at 10,000 rpm.
- Run the supernatant in the assay (follow product insert).

Ceiling Tile:

- Cut off a small portion of the ceiling tile sample (just large enough to fit through a 30 mL extraction vial opening).
- Place the sample into a 30 mL extraction vial containing two steel ball bearings (such as provided in EnviroLogix' ACC 001 Soil Extraction Kit).
- Add 20 mL of PBS to the extraction vial.
- Use mechanical means (orbital shaker) to shake the sample vigorously for 10 minutes.
- Allow the particulates to settle. Microcentrifuge the supernatant for 5 minutes at 10,000 rpm.
- Run the supernatant in the assay.

Carpet:

- Cut off a small portion of the carpet sample (just large enough to fit through a 30 mL extraction vial opening).
- Place the sample into a 30 mL extraction vial containing two steel ball bearings (such as provided in ACC 001).
- Add 20 mL of PBS to the extraction vial.
- Use mechanical means (orbital shaker) to shake the sample vigorously for approximately 10 minutes.
- Allow the particulates to settle. Microcentrifuge the supernatant for 5 minutes at 10,000 rpm.
- Dilute the supernatant 1:2 in PBS (1 mL of supernatant + 1 mL PBS) and run in the assay.

Drywall:

- Using a razor blade, pocket knife, or other implement, carefully scrape the contaminated surface of a one sq. inch drywall sample over a plastic weigh boat or weighing paper.
- Carefully pour the scraped sample into a graduated polypropylene conical tube or equivalent.
- Add PBS to bring the total volume to approximately 10 mL. Thoroughly vortex or vigorously shake the sample for approx. 20-30 seconds.
- Allow any particulates to settle. Microcentrifuge the supernatant for approximately 5 minutes and 10,000 rpm
- Run the supernatant in the assay.

Dust:

- Vacuum a representative dust sample with a Mitest™ dust collector and filter from Indoor Biotechnologies Inc. (http://inbio.com) or other filtering device.
- If using the Mitest, snap the bottom cap onto the dust collector (make sure not to tip the dust collector down when the vacuum is off or the filter will fall out). Add 8.0 mL of PBS to the dust sample trapped in the collector. Cap the top. Shake vigorously for two minutes.
- If not using the Mitest, place approximately 1 gram of dust into a graduated conical tube, add 8 mL of PBS, cap and shake vigorously for 2 minutes.
- Allow the particulates to settle for 3-5 minutes.
- Dilute the supernatant 1:5 (300 µl sample extract into 1.2 mL PBS) with PBS in a microcentrifuge tube.
- Vortex or vigorously shake the diluted sample. Microcentrifuge the sample for approximately 5 minutes and 10,000 rpm.
- Run the supernatant in the assay.

Note: Any results exceeding the 18.0 ppb Roridin A Calibrator can be diluted further and retested.

Calculations: Assay results will indicate the nanograms (ng) of Trichothecenes per mL of sample extract (ppb). To calculate total ng of Trichothecenes on/in the solid bulk sample, multiply assay results by the volume of Extraction Buffer.

Example: A carpet extract tested in the assay yields 10 ppb (ng/mL) Roridin A. A 1:2 post-extraction dilution of the sample was done before it was run in the assay, which means the results must be multiplied by 2, giving a concentration of 20 ppb (ng/mL). The carpet extract total volume after extraction is 20 mL. 20 ng/mL x 20 mL = 400 ng of Roridin A equivalent Trichothecenes toxin extracted from the 0.5 sq. inch carpet sample.