Importance of T-2 Toxin Determination

T-2 toxin is a trichothecene mycotoxin, produced naturally by *Fusarium* fungi, whose species include F. sporotrichioides, F. langsethiae, F. acuminatum, and F. poae, and is toxic to humans and other animals. Although exposure usually comes from ingesting moldy feed grain or contaminated food such as cereals, T-2 toxin can also be absorbed through intact skin and through inhalation of water droplets, aerosols, and smoke from various dispersal systems. These characteristics make it an ideal agent for biological warfare (in which it is known as "Yellow Rain"). Trichothecene compounds are generally very stable, even at high temperatures, and do not degrade during storage/milling of grains and processing/cooking of food.

Upon initial exposure to T-2 toxin, a burning sensation will occur in the mouth, throat, and stomach. Acute T-2 poisoning symptoms include vomiting, diarrhea, skin irritation, itching, rash, blisters, bleeding, fatigue, and labored breathing. Chronic exposure causes deterioration of bone marrow leading to alimentary toxic aleukia (ATA), the decrease of white blood cells and total number of leukocytes, resulting in decreased immune response and skin bleeding. T-2 toxin also affects the nervous system causing disorientation and loss of coordination. Prior to death, high fever, hemorrhaging, necrosis of the muscles and skin, bacterial infections of necrotic tissue, and/or enlarged lymph nodes may occur. There is also a high probability of death by asphyxiation due to the buildup of fluids resulting in narrowing of the larynx or bronchial pneumonia and lung bleeding. Studies have shown T-2 toxin reduces fertility of farm animals. After exposure to a high dose, ewes and heifers had delayed follicle maturation causing delayed ovulation, possibly retarding luteinization and making conceiving impossible. Bulls that were exposed to T-2 showed decreased testosterone levels and an increase in sperm cell abnormalities.

T-2 toxin has an LD50 of approximately 1 mg/kg of body weight (bw). In November 2016, the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM) set a Tolerable Daily Intake of 0.02 µg/kg bw for the sum of T-2 and its metabolite HT-2 and an acute reference dose of 0.3 µg/kg bw for T-2 toxin. The European Commission recommendation of March 2013 (2013/165/EU) set Indicative levels for the sum of T-2 and HT-2. Corn maize Indicative levels are as follows: For human consumption: Unprocessed cereals – 200 µg/kg; Cereal grains for direct consumption – 100 µg/kg; Cereal bran – 100 µg/kg; Cereal milling products – 50 µg/kg; Breakfast cereals – 75 µg/kg; Bread, pastries, biscuits, cereal snacks, and pasta – 25 µg/kg; Cereal-based foods for infants and young children – 15 µg/kg; For Animal feed: Cereal products – 500 µg/kg; Compound feed (except cats) – 250 µg/kg; Compound feed for cats – 50 µg/kg.

A minimum 10 g of corn grain sample is required for extraction. The Abraxis T-2 Toxin ELISA Assay can be performed in less than 20 minutes.

Performance Data

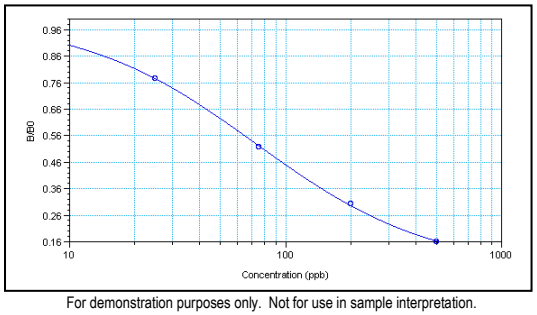
Test sensitivity: The detection limit, based on T-2 toxin, (90% B/B_0) is approximately 10 ppb (µg/kg). The middle of the test (50% B/B_0) is approximately 82 ppb. Determinations closer to the middle of the calibration curve give the most accurate results.

Test reproducibility: Intra and inter assay: < 10%

Specificity: Cross-reactivity of the Abraxis T-2 Toxin Plate Kit for various metabolites:

T-2	100%
HT-2	38%
T-2 Triol	1.6%
T-2 Tetraol	<0.04%
Verrucarol	<0.04%

Standard Curve:



For demonstration purposes only. Not for use in sample interpretation.

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T-2 Toxin ELISA, Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of T-2 Toxin in Corn and Corn Meal Samples

Product No. 53013B

1. General Description

The Abraxis T-2 Toxin ELISA Plate Kit is an immunoassay for the quantitative and sensitive screening of T-2 toxin in corn, corn meal, corn germ meal, corn gluten meal, and corn/soy blend. This test is suitable for the quantitative and/or qualitative screening of T-2 toxin in extracted corn/corn meal samples (please refer to Sample Preparation, Section C). Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of T-2 toxin. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted hydrochloric acid. Avoid contact of these solutions with skin and mucous membranes. If these reagents come in contact with skin, wash thoroughly with water.

3. Storage and Stability

The T-2 Toxin ELISA Kit should be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box. Avoid prolonged (> 24 hours) storage at room temperature. Consult state, local, and federal regulations for proper disposal of all reagents.

4. Test Principle

The test is a direct competitive ELISA based on the recognition of T-2 toxin by specific antibodies. T-2 toxin is extracted from homogenized corn/corn meal samples. T-2 toxin, when present in the sample extract, and a T-2 toxin-enzyme conjugate compete for the binding sites of anti-T-2 toxin antibodies in solution. The T-2 toxin antibodies are then bound by a second antibody (anti-rabbit) immobilized on the microtiter plate. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of T-2 toxin present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the T-2 Toxin ELISA, Possible Test Interference

Although many organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects cannot be completely excluded.

Mistakes in handling the test can also cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, exposure to direct or indirect sunlight during the substrate reaction, or extreme temperatures (lower than 10°C or higher than 30°C) during the test performance.

Each reagent is optimized for use in the Abraxis T-2 Toxin Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Abraxis T-2 Toxin Plate Kits with different lot numbers.

Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.

The Abraxis T-2 Toxin ELISA kit provides screening results. As with any analytical technique (GC/MS, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.

A. Reagents and Materials Provided

1. Microtiter plate coated with a secondary antibody (anti-rabbit), in a re-sealable aluminum pouch
2. T-2 Toxin-HRP Enzyme Conjugate, 8 mL
3. Rabbit Anti-T-2 Toxin Antibody, 8 mL
4. T-2 Toxin Standards (5): 0, 25, 75, 200, 500 ppb or µg/kg (0, 0.025, 0.075, 0.200, 0.500 ppm or mg/kg), 2 mL each (*Note: To compensate for the 1:5 dilution of the corn/corn meal sample extraction, the actual concentration of the standards are 1/5th of the stated value; correction of the results to obtain the original sample concentration is therefore not required.)*
5. Color (Substrate) Solution (TMB), 14 mL
6. Stop Solution, 14 mL (handle with care)

B. Additional Materials (not delivered with the test kit)

1. Laboratory grade deionized or distilled water
2. Methanol, ACS grade
3. Graduated cylinder, 100 mL or larger
4. 60 mL glass vial with Teflon-lined cap for sample extraction (see Sample Preparation, Section C)
5 20 mL (or larger) glass vial with Teflon-lined cap for extract collection (see Sample Preparation, Section C)
6. Glass fiber filter paper, Whatman GF/A or equivalent (with appropriate size glass funnel)
7. 4 mL glass vials with Teflon-lined screw caps for 1:10 dilutions of standards and sample extracts (see Test Preparation, Section D)
8. Tap water
9. Micro-pipettes with disposable plastic tips (10-200 and 200-1000 µL)
10. Multi-channel pipette (10-300 µL), stepper pipette (10-300 µL), or electronic repeating pipette with disposable plastic tips (capable of delivering 50-300 µL)
11. Paper towels or equivalent absorbent material
12. Timer
13. Microtiter plate reader (wavelength 450 nm)

C. Sample Preparation: Extraction of Corn/Corn Meal Samples

(corn, corn meal, corn germ meal, corn gluten meal, and corn/soy blend)

Prepare Extraction Solution (70% Methanol)

1. Determine the volume of extraction solution to be prepared (50 mL per sample extraction is required) and obtain an appropriate sized glass container.
2. Measure 30 mL of deionized or distilled water for each 100 mL being prepared and add to the container.
3. Measure 70 mL of Methanol for each 100 mL being prepared and add to the container. Cover and swirl to mix completely. Store tightly sealed to minimize evaporation.

Sample Extraction

1. Grind the corn/corn meal sample to pass through a 20 mesh sieve. Samples not being immediately analyzed should be stored in the refrigerator (4–8°C).
2. Thoroughly mix the ground sample. Weigh 10 g and add to a 60 mL glass vial with a Teflon-lined cap.
3. Add 50 mL of the Extraction Solution to the glass vial and seal tightly.
4. Vigorously shake for 3 minutes.
5. Allow the sample to settle for 2-3 minutes.
6. Filter at least 15 mL of the extract through a glass fiber filter (Whatman GF/A or equivalent) and collect in a clean glass vial with Teflon-lined cap.
7. Extract must be diluted 1:10 prior to analyzing (see Test Preparation, Section D).

D. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. A multi-channel pipette or a stepping pipette is recommended for adding the enzyme conjugate, antibody, substrate, and stop solutions in order to equalize the incubation periods across the entire microtiter plate. Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Allow the microtiter plate, reagents, and sample extracts to reach room temperature before use.
2 Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed.
3. The enzyme conjugate, antibody, substrate and stop solutions are ready to use and do not require any further dilutions.
4. ***Dilute all standards and sample extracts 1:10 with deionized or distilled water*** in appropriately labeled 4 mL glass vials with Teflon-lined caps. For example, add 100 µL of standard or sample extract to 900 µL of deionized or distilled water. Cap tightly and vortex. The diluted standards and sample extracts are now ready to be analyzed (see Assay Procedure, Section F).
5. The stop solution must be handled with care as it contains diluted Hydrochloric Acid.
6. After analysis, store the remaining kit components in the refrigerator (4-8°C).

E. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std4: Standards

Samp1, Samp2, etc.: Sample Extracts

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4										
B	Std 0	Std 4										
C	Std 1	Samp1										
D	Std 1	Samp1										
E	Std 2	Samp2										
F	Std 2	Samp2										
G	Std 3	etc.										
H	Std 3	etc.										

F. Assay Procedure

1. Add **50 µL of the enzyme conjugate solution** to the individual wells successively using a multi-channel pipette, stepping pipette, or electronic repeating pipette.
2. Add **50 µL of the 1:10 diluted standards or sample extracts** into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
3. Add **50 µL of the antibody solution** to the individual wells successively using a multi-channel pipette, stepping pipette, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 60 seconds. Be careful not to spill the contents. Incubate the strips for **10 minutes** at room temperature.
4. Remove the covering and decant the contents of the wells into a sink. Wash the strips **five times** using tap water. Please use at least a volume of **250 µL of tap water** for each well and each washing step. Remaining tap water in the wells should be removed by patting the inverted plate on a stack of paper towels.
5. Add **100 µL of substrate (color) solution** to the individual wells successively using a multi-channel pipette, stepping pipette, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for **5 minutes** at room temperature. Protect the strips from sunlight.
6. Add **100 µL of stop solution** to the wells in the same sequence as for the substrate (color) solution using a multi-channel pipette, stepping pipette, or electronic repeating pipette.
7. Read the absorbance at 450 nm using a microtiter plate ELISA photometer within 15 minutes after the addition of the stopping solution.

G. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-Parameter (preferred) or Logit/Log). For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on a vertical linear (y) axis versus the corresponding T-2 toxin concentration on horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for the samples will then yield levels in ppb of T-2 toxin by interpolation using the standard curve. Results can also be determined using a spreadsheet macro available from Abraxis upon request.

The concentrations of the samples are determined using the standard curve run with each test. Results for sample extracts do not require a correction factor; the results obtained are the original sample concentration. (Standard concentrations are 1/5th of the stated value to correspond with the extraction dilution.) Samples showing a lower concentration of T-2 toxin than standard 1 (25 ppb or 0.025 ppm) should be reported as containing < 25 ppb (0.025 ppm) of T-2 toxin. Samples showing a higher concentration than standard 4 (500 ppb or 0.5 ppm) should be reported as containing > 500 ppb (0.5 ppm) of T-2 toxin or must be diluted using deionized or distilled water (and the results multiplied by the corresponding dilution factor) to obtain accurate quantitative results.

Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the standards. Samples with lower absorbances than a standard will have concentrations of T-2 toxin greater than that standard. Samples which have higher absorbances than a standard will have concentrations of T-2 toxin less than that standard.

As with any analytical technique (GC/MS, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.