T-2 Toxin ELISA Quantitative

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Cat. No. 951T201GF-96

T-2 TOXIN

T-2 toxin belongs to the type A trichothecene mycotoxins and is one of the most toxic secondary metabolites produced by several fungi of the genus *Fusarium*. Due to the typical stability of the trichothecene family, T-2 toxin is able to withstand processing procedures and can commonly be found in animal feed and food. Its contamination has been implicated to be responsible for health problems primarily because of its ability to inhibit protein synthesis in both human and animal. T-2 toxin can cause symptoms such as weight loss or reduced weight gain, diarrhea, dermal necrosis, and dyspnea. Therefore, it is necessary to use sensitive methods for its detection.

INTENDED USE

The assay provides a quantitative method for determining the presence of T-2 toxin in cereals and animal feed.

ASSAY PRINCIPLE

The HELICA T-2 toxin ELISA Assay is a quantitative, indirect immunoassay whereby a mouse anti-T-2 toxin antibody that predominantly cross reacts with T-2 toxin (see cross-reactivity information), is coated to a polystyrene microwell. Toxins are extracted from a ground sample with 70% methanol. The extracted sample and horse-radish peroxidase (HRP)-conjugated T-2 toxin are mixed and added to the appropriate well. T-2 toxin from the extracted sample and HRP-conjugated T-2 toxin compete to bind to the antibody coated to the microwell. After this incubation period, the contents are decanted, washed and an HRP substrate is added which develops a blue color in the presence of the enzyme. The intensity of the color is directly proportional the amount of bound conjugate and inversely proportional to the amount of T-2 toxin in the sample or standard. An acidic stop solution is added which changes the chromogen color from blue to yellow. The microwells are measured optically by a microplate reader with an absorbance filter of 450nm (OD_{450}). The optical densities of the samples are compared to the ODs of the kit standards and a result is determined by interpolation from the standard curve.

MATERIALS PROVIDED

1 X Pouch	**Antibody Coated Microwell Plate**	Silver Pouch	96 wells (12×8 well strips) in a microwell holder coated with a mouse anti-T-2 toxin monoclonal antibody, *Ready-to-Use*.
1 X Plate	**Mixing Wells**	Green	96 non-coated wells (12×8 well strips) in a microwell holder, *Ready-to-Use*.
6 X Vial	**T-2 toxin Standards**	Black Cap	1.5mL/vial of T-2 toxin at the following concentrations: 0.0, 0.25, 1.0, 2.0, 6.0, 12.0ng/mL in 35% methanol, *Ready-to-Use.*
2 X Bottle	**T-2 toxin-HRP Conjugate**	Green Cap	2 x 12mL of T-2 toxin conjugated to peroxidase in buffer with preservative, *Ready-to-Use.*
1 X Bottle	**Substrate Reagent**	Blue Cap	12mL stabilized tetramethylbenzidine (TMB) with H_2O_2, *Ready-to-Use.*
1 X Bottle	**Stop Solution**	Red Cap	12mL Acidic Solution, *Ready-to-Use.*
1 X Pouch	**Washing Buffer**	White Pouch	PBS with 0.05% Tween 20, bring to 1 liter with distilled water and store refrigerated.

MATERIALS REQUIRED BUT NOT PROVIDED

Extraction Procedure

- Grinder sufficient to render sample to particle size of fine instant coffee
- Collection Container: Minimum 125mL capacity
- Balance: 10g measuring capability
- Graduated cylinder: 100mL
- Methanol
- Distilled or deionized water
- Centrifuge

Assay Procedure

- Pipettor with tips: 100µL and 200µL
- Timer
- Wash bottle
- Dilution tubes
- Absorbent paper towels
- Microplate reader with 450nm filter

PRECAUTIONS

1. Bring all reagents to room temperature (19°C - 27°C) before use.
2. Store reagents at 2°C to 8°C, and do not use beyond expiration date(s). Never freeze kit components.
3. Do not return unused reagents back into their original bottles. The assay procedure details volumes required.
4. Adhere to all time and temperature conditions stated in the procedure.
5. Never pipette reagents or samples by mouth.
6. The Stop Solution contains acid. Do not allow to contact skin or eyes. If exposed, flush with water.
7. Consider all materials, containers and devices that are exposed to sample or standards to be contaminated with T-2 toxin. Wear protective gloves when using this kit.
8. Dispose of all materials, containers and devices in the appropriate receptacle after use.
9. HRP-labeled conjugate and TMB-substrate are photosensitive and are packaged in a protective opaque bottle. Store in the dark and return to storage after use.

KIT SAFETY AND WASTE DISPOSAL INSTRUCTIONS

T-2 toxin is considered highly toxic. Do not dispose of these materials down the drain.

Please note that there is a potential for T-2 toxin contamination in or on any of the kit components provided. It is strongly advised that gloves, a lab coat, and safety glasses be worn at all times while handling T-2 toxin kits and their respective components.

1. Before conducting the assay, prepare a waste container to act as a receptacle for your kit waste. Eject contaminated pipette tips and all other related materials into this container.
2. Once the assay is completed, the container should be treated with a sufficient amount of 5-6% sodium hypochlorite (NaOCl) to saturate the contents of the container (approximately 1/10th the volume of the container). 5-6% NaOCl will denature the T-2 toxin and neutralize the waste, making it safe for disposal. Invert the container several times to thoroughly coat all waste.
3. In the case of an accidental toxin spill, treat the spill surface with 5-6% NaOCl for a minimum of 10 minutes followed by 5% aqueous acetone. Wipe dry with absorbent paper towels.

EXTRACTION PROCEDURE

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Animal feed, Corn, and Wheat

1. Grind a representative sample to the particle size of fine instant coffee (95% passes through a 20 mesh screen)
2. Prepare extraction solvent (70% methanol) by adding 60mL of distilled or deionized water to 140mL of methanol for each sample to be tested.
3. Transfer 200mL of extraction solvent to a container and add 8g of the ground sample.
   Note: The ratio of sample to extraction solvent is a 1:25 dilution (w/v).
4. Mix by shaking in a sealed container for a minimum of 5 minutes.
5. Centrifuge the sample at 3,500rpm for 2 minutes to pellet the particulate matter.
6. Collect the supernatant containing T-2 toxin for analysis.
7. Dilute an aliquot of the extract 2-fold in distilled or deionized water.
8. Final dilution for use in calculation = 1:50.

ASSAY PROCEDURE

1. Bring the reagents to room temperature before use. Reconstitute the PBS-Tween packet by washing out the contents with a gentle stream of distilled water into a 1-Liter container. Q.S. to 1 Liter with distilled water and store refrigerated when not in use.
2. Place one mixing well in a microwell holder for each Standard and Sample to be tested. Place twice the number of antibody coated microwells in another microwell holder to run standards and samples in duplicate.
3. Return the unused wells to the pouch and re-seal to avoid the entry of moisture. Retain the well holder for future use.
4. Dispense 200µL of conjugate into each mixing well.
5. Using a fresh pipette tip for each, dispense 100µL of standards and samples into the appropriate wells and mix by aspirating three times.
6. Using a multichannel pipette, transfer 100µL of the mixture to the corresponding antibody coated microtiter well. Incubate at room temperature for 20 minutes. The mixing wells contain enough solution to run each standard and/or sample in duplicate. If running each standard and/or sample in singlets or more replicates is desired, the volumes of conjugate and sample/standard should be scaled accordingly.
7. Decant the contents from microwells into a discard basin. Wash the microwells by filling each with PBS-Tween wash buffer, then decanting the wash into a discard basin. Repeat wash for a total of 3 washes.
8. Tap the microwells (face down) on a layer of absorbent towels to remove residual wash buffer.

9. Measure the required volume of Substrate Solution (1mL/strip or 120µL/well) and place in a separate container. Add 100µL to each microwell. Incubate for 10 minutes. Cover to avoid direct light.
10. Measure the required volume of Stop Solution (1mL/strip or 120µL/well) and place in a separate container. Add 100µL in the same sequence and the same pace as the Substrate Solution was added.
11. Read the optical density (OD) of each microwell with a microtiter plate reader using a 450nm filter. Record the optical density (OD) of each microwell.
12. Setting the zero standard as 100% binding (B_0), calculate % binding (%B) for each standard and sample as a percentage of the zero binding (%B/B_0).

INTERPRETATION OF RESULTS

Construct a dose-response curve using either the unmodified OD values or the OD values expressed as a percentage (%B/B_0) of the OD value of the zero (0.0) standard against the T-2 toxin content of the remaining standards and samples. Unknowns are measured by interpolation from the standard curve.

The information contained on the label of each standard vial refers to the contents of that vial. However, the sample has been diluted at a 25:1 ratio by extraction solvent as instructed in the EXTRACTION PROCEDURE and also 2-fold in distilled or deionized water and so the level of T-2 Toxin shown by the standard must be multiplied by 50 in order to indicate the ng per gram (ppb) of the commodity as follows:

Standards (ng/mL)	Animal Feed or Cereal Diluted 1:50 (ppb in sample)
0	0
0.25	12.5
1.0	50
2.0	100
6.0	300
12.0	600

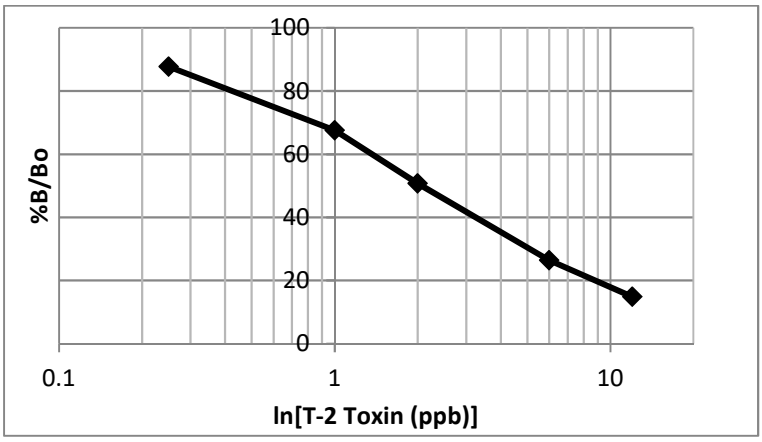
If a sample contains T-2 toxin at greater concentration than the highest standard, it should be diluted appropriately in 35% methanol and retested. The extra dilution step should be taken into consideration when expressing the final result.

ASSAY CHARACTERISTICS

Data from twelve consecutive standard curves gave the following results:

Standard (ng/mL)	%B/B_0	%CV
0	100	-
0.25	87.8	3.2
1.0	67.6	3.3
2.0	50.7	4.8
6.0	26.4	4.9
12.0	14.9	4.6

The below figure is a representative standard curve for T2-toxin based on the above data table.



Recoveries of 200ppb, 50ppb, and 10ppb T-2 toxin spiked into feed samples based on five independent experiments were as follows (n= 5):

ppb	% Recovery
200	98.8
50	92.0
10	98.4

Recoveries from certified reference materials (corn and wheat) were as follows based on four independent experiments (n=4):

Matrix	ppb	% Recovery
Corn	425	100.9
	187	97.7
Wheat	340.7	96.5
	57.1	84.4

CROSS-REACTIONS

The assay will cross- react with T-2 toxin analogues as follows:
T-2 toxin 100%, HT-2 toxin: 3%

REFERENCES

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3. McKean C., Tang L., Billam M., Tang M., Theodorakis C. W., Kendall R. J., and Wang J.-S. (2006). Comparative acute and combinative toxicity of aflatoxin B1 and T-2 toxin in animals and immortalized human cell lines. J. Appl. Toxicol. 26, 139-147.