Helica® T-2 Toxin ELISA

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For the quantitative detection of T-2 toxin in cereals and animal feed.

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Introduction – 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*, the most important species being *F. sporotrichioides*, *F. langsethiae*, *F. acuminatum* and *F. poae*. These fungi are found in grains such as barley, wheat and oats. Due to the typical stability of the trichothecene family, T-2 toxin can 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 animals. 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

Hygiena's Helica® Mycotoxin ELISA kits are user-friendly, cost-effective kits for the detection of mycotoxins in a wide range of commodities including animal feeds, grains, corn and animal urine, designed to protect humans and animals from dangerous side effects of mycotoxins.

The Helica T-2 toxin ELISA Assay is a quantitative, indirect immunoassay for determining the presence of T-2 toxin in cereals and animal feed.

Data obtained from assays should not be used for human diagnostic or human treatment purposes. Assays are not approved by the United States Food and Drug Administration or any other US or non-US regulatory agency for use in human diagnostics or treatment. Assays should not be used as the sole basis for assessing the safety of products for release to consumers. The information generated is only to be used in conjunction with the user's regular quality assurance program.

Not approved for clinical diagnosis. Use for research and development, quality assurance and quality control under the supervision of technically qualified persons.

Principle of the Method

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 on page 8), 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 to 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 450 nm (OD450). 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.

Kit Contents

Package/ Number	Component	Description
1X Pouch	Antibody- coated microwell plate	96 wells (12 eight-well strips) in a microwell holder coated with a mouse anti-T-2 toxin monoclonal antibody, *Ready-to-Use*.
1X Plate	Dilution plate	96 non-coated wells (12 eight-well strips) in a microwell holder, *Ready-To-Use*. (Mixing wells)
6X Vials	Standards	1.5mL/vial of T-2 toxin at the following concentrations: 0.0, 0.25, 1.0, 2.0, 6.0 and 12.0 ng/mL in 35% methanol, *Ready-to-Use*.
2X Bottles	Conjugate	2 x 12 mL of T-2 toxin conjugated to peroxidase in buffer with preservative, *Ready-to-Use*.
1X Bottle	Substrate	12 mL stabilized tetramethylbenzidine (TMB), *Ready-to-Use*.
1X Bottle	Stop solution	12 mL acidic solution, *Ready-to-Use*.
1X Pouch	PBS-T powder	PBS with 0.05% Tween® 20*, bring to 1 Liter with distilled water and store refrigerated. (Wash buffer)

**TWEEN® 20 is a registered trademark of CRODA International Plc.*

Materials Required but Not Provided

- Grinder sufficient to render sample to a particle size of fine instant coffee
- Collection container: minimum 125 mL capacity
- Balance: 10 g measuring capacity
- Graduated cylinder: 100 mL
- Methanol (reagent grade)
- Distilled or deionized water
- Centrifuge
- Single or multichannel pipettors with tips: 100 µL and 200 µL
- Timer
- Wash bottle
- Dilution tubes
- Absorbent paper towels
- Microplate reader with 450 nm filter

Storage and Shelf Life

- Store reagents at 2 to 8 °C. Do not freeze.
- Reagents should be used by the expiration date stamped on the individual labels.
- HRP-labeled conjugate and TMB-substrates are photosensitive and are packaged in a light-protective opaque bottle. Store in the dark and return to storage after use.

Precautions and Waste Disposal

General Precautions:

- Bring all reagents to room temperature (19 to 25 °C) before use.
- Do not interchange reagents between kits of different lot numbers.
- Do not use solutions if cloudy or precipitate is present.
- Do not return unused reagents to their original bottles. The assay procedure details volumes required.
- Adhere to all time and temperature conditions stated in the procedure.
- During the sample extraction, avoid cross-contamination.
- Devices, such as a blender, must be cleaned after each sample preparation.
- Samples tested should have a pH of 7.0 (± 1.0). Excessive alkaline or acidic conditions may affect the test results.

Safety Precautions:
Mycotoxins (aflatoxins, trichothecenes and others) are well-known human carcinogens and are considered highly toxic. Because mycotoxins can cause human illness, appropriate safety precautions must be taken and personal protective equipment worn when handling samples, reagents, glassware and other supplies and equipment that potentially could be contaminated with mycotoxins.

- Before using this assay, please review the Safety Data Sheet(s) available at www.hygiena.com.
- Refer to your site practices for safe handling of materials.
- It is strongly advised that protective gloves, a lab coat and safety glasses be worn at all times while handling mycotoxin kits and their respective components. Consider all materials, containers and devices that are exposed to samples or standards to be contaminated with mycotoxins.
- Never pipette reagents or samples by mouth.
- Standards are flammable. Caution should be taken in the use and storage of these reagents.
- The stop solution contains sulfuric acid, which is corrosive. Please refer to the SDS. Do not allow to contact skin or eyes. If exposed, flush with water.

Disposal:
Decontaminate materials and dispose of waste per your site practices and as required by local regulations. Do not dispose of these materials down the drain. Please note that there is a potential for mycotoxin contamination in or on any of the kit components provided.

- Dispose of all materials, containers and devices in the appropriate receptacle after use.
  Before conducting the assay, prepare a waste container to act as a receptacle for your kit waste.
  Eject contaminated pipette tips and discard all other related materials into this container.
- 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). The NaOCl will denature the mycotoxins and neutralize the waste, making it safe for disposal. Invert the container several times to thoroughly coat all waste.
- 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.

Preparation of Samples

Extraction Procedure

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 60 mL of distilled or deionized water to 140 mL of methanol for each sample to be tested.
3. Transfer 200 mL of extraction solvent to a container and add 8 g of the ground sample.
4. Note: The ratio of sample to extraction solvent is a 1:25 dilution (w/v).
5. Mix by shaking in a sealed container for a minimum of 5 minutes.
6. Centrifuge the sample at 3,500 rpm for 2 minutes to pellet the particulate matter.
7. Collect the supernatant containing T-2 toxin for analysis.
8. Dilute an aliquot of the extract 2-fold in distilled or deionized water for testing.
9. The final dilution for use in calculation is **1:50**.

Assay Procedure

Note: It is recommended that a multichannel pipettor be utilized to perform the assay.

1. Bring all the reagents to room temperature before use. Prepare wash buffer by reconstituting the PBS-T powder packet by washing out the contents with a gentle stream of distilled or deionized water into a 1-Liter container. Fill to 1 Liter with distilled or deionized 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 microtiter wells in another microwell holder. If running a single well, adjust volumes accordingly. Return unused wells to the foil pouch with desiccant and reseal.
3. Mix each reagent by swirling the reagent bottle prior to use.
4. Dispense 200 µL of conjugate into each mixing well.
5. Using a fresh pipette tip for each, dispense 100 µL of standards and samples into the appropriate wells and mix by aspirating three times.
6. Using a new pipette tip for each, transfer 100 µL of contents from each mixing well to the corresponding antibody-coated microtiter well. Incubate at room temperature for 20 minutes.
   Note: The mixing wells contain enough solution to run each standard and/or sample in duplicate (recommended). If running each standard or sample in singlets or more replicates are 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-T 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 into a separate container. Add 100 µL 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 into a separate container. Add 100 µL in the same sequence and at 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 450 nm 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 the OD values expressed as a percentage (%B/B_0) of the OD of the zero 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 1:25 ratio by extraction solvent as instructed in the extraction procedure and 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.

Standard (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 a 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.

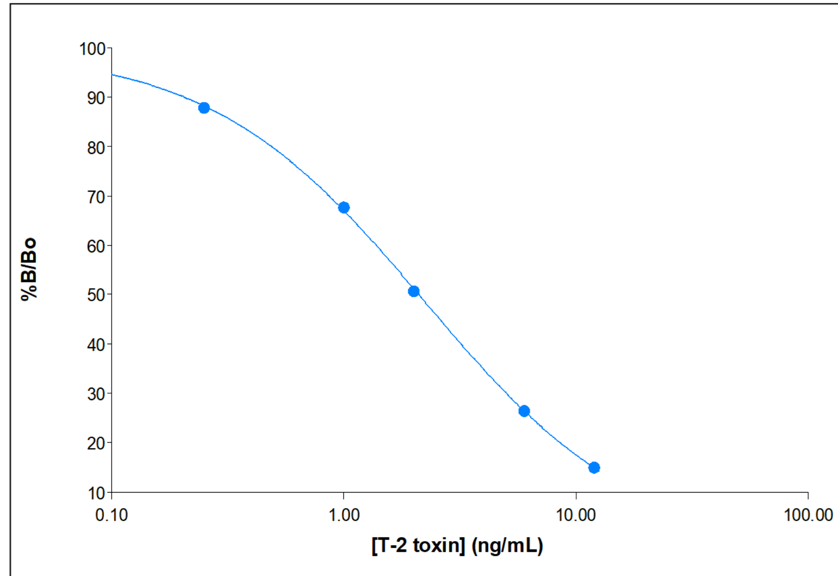
Assay Characteristics

Performance

Data from the average of 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 200 ppb, 50 ppb and 10 ppb 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-reactivity

The assay will cross-react with T-2 toxin analogs as follows:

- T-2 toxin: 100%
- HT-2 toxin: 3%

References

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- Krska R., Malachova A., Berthiller F., and van Egmond H. P. (2014). Determination of T-2 and HT-2 toxins in food and feed: an update. *World Mycotoxin Journal* 7 (2), 131-142.
- 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.

Technical Assistance

For questions or comments, please contact your local distributor. You can also email techsupport@hygiena.com, visit our Contact Us page for regional phone numbers or request technical support at https://www.hygiena.com/hygiena/technical-support-request.html.