Helica® Fumonisin Hydro ELISA

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For the quantitative detection of fumonisin in barley flour, corn, corn gluten meal, white hominy corn, DDGS, flaked maize and soybean.

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Introduction – Fumonisins

The Fumonisins (B1, B2 and B3) are a group of mycotoxins produced by *Fusarium moniliforme* (*F. verticillioides*), *F. proliferatum* and other *Fusarium* species that grow on agricultural commodities in the field or during storage. Fumonisins have been found worldwide as a contaminant of maize and have been shown to cause liver cancer in experimental rats, pulmonary edema in pigs and leukoencephalomalacia (moldy corn poisoning) in horses. High levels of fumonisins in locally grown maize have been found in areas of the world that have a high prevalence of human esophageal cancer, for instance, in South Africa and China. Therefore, it's very important to estimate the levels of fumonisin in food/feed to prevent its adverse effects on human and animal health.

Several methods for quantitative detection of fumonisin have been developed including gas chromatography, high pressure liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS) and (LC-MS/MS), which are reliable and accurate for the quantification of specific compounds. However, all these methods have some challenges and limitations. For example, HPLC is time intensive, requires an extra clean-up step, is costly and needs a skilled workforce to operate/analyze.

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, and corn, designed to protect humans and animals from dangerous side effects of mycotoxins.

The Helica Fumonisin Hydro ELISA assay is a competitive enzyme-linked immunoassay intended for the quantitative detection of fumonisins in corn, corn gluten meal, barley flour, flaked maize, soybean, white hominy corn and dried distillers' grain with solubles (DDGS) in a high-throughput manner.

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 Fumonisin Hydro ELISA assay is a solid phase direct competitive enzyme immunoassay. A fumonisin-specific antibody optimized to cross-react with the three fumonisin subtypes is coated to a polystyrene microwell. Toxins are extracted from a ground sample with water. The extracted sample and horseradish peroxidase (HRP)-conjugated fumonisin are mixed and added to the antibody-coated microwell. Fumonisin from the extracted sample and HRP-conjugated fumonisin compete to bind with the antibody coated to the microwell. Microwell contents are decanted and non-specific reactants are removed by washing. An enzyme substrate (TMB) is added and color (blue) develops. The intensity of the color is directly proportional to the amount of bound conjugate and inversely proportional to the concentration of fumonisin in the sample or standard. Therefore, as the concentration of fumonisin in the sample or standard increases, the intensity of the blue color will decrease.
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-fumonisin monoclonal antibody, *Ready-to-Use*.
1X Pouch	Dilution plate	96 non-coated wells (12 eight-well strips) in a microwell holder, *Ready-to-Use*. (Mixing wells)
6X Vials	Standards	1.0 mL/vial of fumonisin at the following concentrations: 0.0, 2.5, 7.5, 20.0, 50.0 and 150.0 ng/mL in aqueous solution, *Ready-to-Use*.
2X Bottles	Conjugate	12 mL of HRP-conjugated fumonisin 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)

** An additional reagent, Hydro buffer capsules (Product No. 928XB001/ASY5068) must be purchased separately.*

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

Materials Required but Not Provided

- Grinder sufficient to render sample to particles of fine instant coffee
- Variable single and multichannel pipettors with tips: 20, 100, 300 and 1000 µL
- Distilled or deionized water
- Microtubes
- Extraction containers
- Timer
- Wash bottle
- Absorbent paper towels
- Whatman #1 filter paper and funnel
- Centrifuge
- Microcentrifuge
- Vortex mixer
- Blender
- Graduated cylinder
- Methanol, reagent grade
- Microplate reader with 450 nm filter
- Hydro buffer (Catalog# ASY5068, 90 capsules)

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 (substrate) are photosensitive and are packaged in light-protective opaque bottles. Store bottles in the dark, avoid exposure to direct light 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.

- Read this manual carefully before starting the test. The assay must be performed by specialized and trained staff.
- 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 towe

Preparation of Samples

Note: The sample must be collected according to the appropriate established sampling techniques.

Barley Flour

1. Weigh out a 20 g ground portion of the sample into a sealable extraction container and add 200 mL of distilled water.
   Note: The ratio of sample to distilled water is 1:10 (w/v).
2. Seal the container and shake for 5 minutes.
3. Allow the particulate matter to sediment for 5 minutes, then carefully filter 5 mL of the top layer of the extract through a Whatman #1 filter paper (or equivalent) and collect the filtrate (sample extract) to be tested.
4. Dilute the sample extract 1:4 with distilled water (e.g., 0.1 mL + 0.3 mL)
5. The diluted sample is now ready for testing.
6. The final dilution to be used for calculations is **1:40**.

Corn

1. Grind a representative sample to the particle size of fine instant coffee (95% passes through a 20-mesh screen).
2. Weigh out a 20 g ground portion of the sample into a sealable extraction container and add 100 mL of distilled water.
   Note: The ratio of sample to distilled water is 1:5 (w/v).
3. Seal the container and vortex for 3 minutes.
4. Allow the particulate matter to settle for 5 minutes, then centrifuge 5 mL of the top layer of the extract for 5 min at 3500 rpm and carefully transfer the supernatant (sample extract) to a clean tube.
5. Dilute the sample extract 1:8 in distilled water (e.g., 0.1 mL + 0.7 mL)
6. The diluted sample is now ready for testing.
7. The final dilution to be used for calculations is **1:40**.

Corn Gluten Meal

1. Weigh out a 10 g ground portion of the sample into a sealable extraction container, add 8 capsules of hydro buffer and then add 50 mL of distilled water.
   Note: The ratio of sample to distilled water is 1:5 (w/v).
2. Seal the container and vortex for 5 minutes.
3. Allow the particulate matter to settle for 5 minutes, then microcentrifuge approximately 1 mL of the top layer of the extract for 1 minute and carefully transfer the supernatant (sample extract) to a clean tube.
4. Dilute the sample extract 1:8 in distilled water (e.g., 0.1 mL + 0.7 mL)
5. The diluted sample is now ready for testing.
6. The final dilution to be used for calculations is **1:40**.

DDGS

Note: This is an organic solvent-based extraction procedure (DDGS is extracted using 70% methanol).

1. Grind a representative sample to the particle size of fine instant coffee (95% passes through a 20-mesh screen).
2. Weigh out a 20 g ground portion of the sample into a sealable extraction container. Add 80 mL of 70% methanol (30 mL of water + 70 mL reagent grade methanol).
   Note: the ratio of sample to 70% methanol is 1:4 (w/v).
3. Seal the container and vortex for 3 minutes.
4. Allow the particulate matter to settle for 5 minutes, and then use Whatman #1 filter paper to filter approximately 5 mL of the top layer and collect filtrate in a clean tube.
5. Dilute the filtrate (sample extract) 1:10 in distilled water (e.g., 0.1 mL + 0.9 mL)
6. The diluted sample is now ready for testing.
7. The final dilution to be used for calculations is **1:40**.

DDGS

Note: This is a water-based extraction procedure (DDGS is extracted using distilled water and hydro buffer).

1. Weigh out a 10 g ground portion of the sample into a sealable extraction container. Add 4 capsules of hydro buffer and then add 40 mL of deionized water.
   Note: the ratio of sample to distilled water is 1:4 (w/v).
2. Seal container and vortex for 2 minutes.
3. Allow particulate matter to settle for 2 minutes, then carefully pipette 1 mL of the extract to an Eppendorf tube. Microcentrifuge for 1 minute; transfer the supernatant (sample extract) to a clean tube.
4. Dilute the sample extract 1:10 in distilled water (e.g., 0.1 mL + 0.9 mL)
5. The diluted sample is now ready for testing.
6. The final dilution to be used for calculations is **1:40**.

Flaked Maize

1. Grind a representative sample to the particle size of fine instant coffee (95% passes through a 20-mesh screen).
2. Weigh out a 20 g ground portion of the sample into a sealable extraction container and add 80 mL of distilled water.
   Note: The ratio of sample to distilled water is 1:4 (w/v).
3. Seal container and vortex for 3 minutes.
4. Allow particulate matter to settle for 2 minutes, then carefully pipette 1 mL of the extract to an Eppendorf tube. Microcentrifuge for 1 minute; transfer the supernatant (sample extract) to a clean tube.
5. Dilute the sample extract 1:10 in distilled water (e.g., 0.1 mL + 0.9 mL).
6. The diluted sample is now ready for testing.
7. The final dilution to be used for calculations is **1:40**.

Soybean

1. Grind a representative sample to the particle size of fine instant coffee (95% passes through a 20-mesh screen).
2. Weigh out a 20 g ground portion of the sample into a sealable extraction container and add 200 mL of distilled water.
   Note: The ratio of sample to distilled water is 1:10 (w/v).
3. Seal container and blend for 3 minutes.
4. Allow the particulate matter to settle for 5 minutes, then centrifuge approximately 5 ml from the top for 5-10 min at 3500 rpm; transfer the top layer (sample extract) to a fresh clean tube.
5. Dilute the sample extract 1:4 in distilled water (e.g., 0.1 mL + 0.3 mL)
6. Diluted sample is now ready for testing.
7. Final dilution to be used for calculations is **1:40**.

White Hominy Corn

1. Grind a representative sample to the particle size of fine instant coffee (95% passes through a 20-mesh screen).
2. Weigh out a 20 g ground portion of the sample into a sealable extraction container and add 200 mL of distilled water.
   Note: The ratio of sample to distilled water is 1:10 (w/v).
3. Seal container and vortex for 5 minutes.
4. Allow the particulate matter to settle for 5 minutes, then carefully filter 5-10 mL of the top layer of the extract through a Whatman #1 filter paper (or equivalent); collect the filtrate (sample extract) to be tested.
5. Dilute the sample extract 1:4 in distilled water (e.g., 0.1 mL + 0.3 mL).
6. The diluted sample is now ready for testing.
7. The final dilution to be used for calculations is **1:40**.

Assay Procedure

Note: It is recommended that a multi-channel pipettor be utilized to perform the assay. If a single channel pipettor is used, it is recommended that no more than a total of 16 samples and standards (2 test strips) are run.

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 to run duplicates. 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 220 µL of the conjugate solution into the appropriate mixing wells.
5. Using a new pipette tip for each, add 20 µL of each standard and sample to the appropriate mixing well containing conjugate mixture. Mix by priming the pipettor 20 times.
   Note: The operator must record the location of each standard and sample throughout the test.
6. Using a new pipette tip for each, transfer 100 µL of contents from each mixing well to a corresponding antibody-coated microtiter well (use 2 wells for each to run duplicates). Incubate at room temperature for 10 minutes.
7. Decant the contents from microwells into a discard basin. Wash the microwells by filling each with PBS Tween wash buffer then decanting the buffer 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 buffer.
9. Measure the required volume of substrate reagent (1 mL/strip or 120 µL/well) and place in a separate container. Add 100 µL to each microwell. Incubate at room temperature for 10 minutes. Cover to avoid direct light.
10. Measure the required volume of Stop Solution (1 mL/strip or 120 µL/well) and place in a separate container. Add 100 µL in the same sequence and at the same pace as the substrate 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.

Interpretation of Results

Construct a dose-response curve using the OD values expressed as a percentage of the OD of the zero (0.0 ng/mL) standard against the fumonisin content of the standard. Unknowns are measured by interpolation from the standard curve.

The information contained on the label of a standard vial refers to the contents of that vial. However, the sample has been diluted at a certain ratio with extraction buffer followed by a dilution in distilled water and so the level of fumonisin shown by the standard must be multiplied by 40 in order to indicate the µg of fumonisin per gram of commodity (ppm). The sample dilution results in a standard curve from 0.0 ppm to 6.0 ppm. If a sample contains fumonisin at a greater concentration than the highest standard, it should be diluted appropriately in distilled water and retested. The extra dilution step should be taken into consideration when expressing the result.

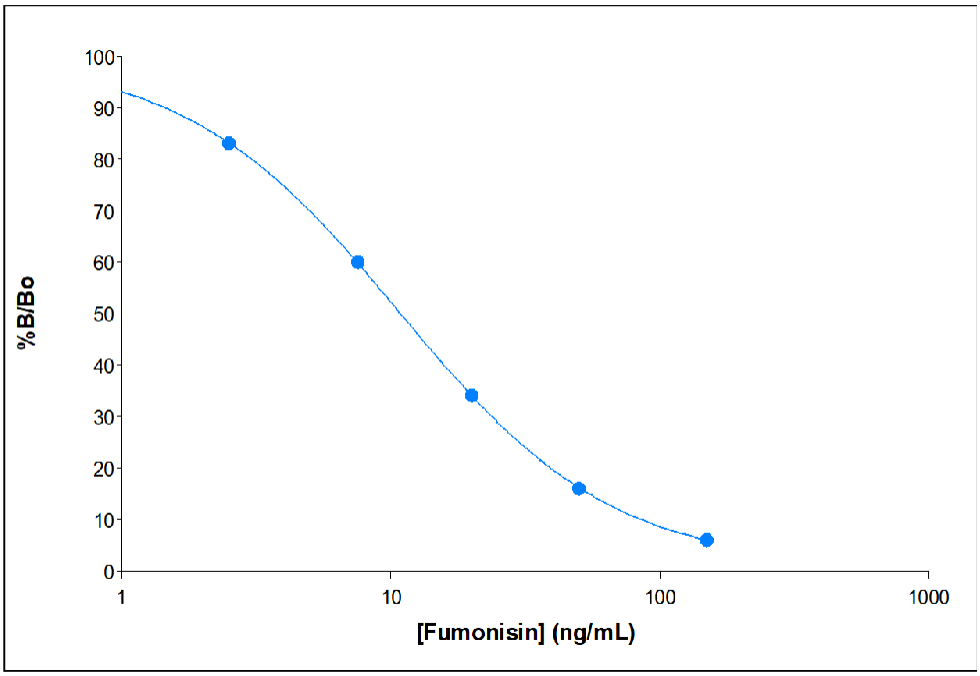
Standard (ng/mL)	Commodity (µg/g) (ppm) (1:40)
0	0
2.5	0.1
7.5	0.3
20	0.8
50	2
150	6

Assay Characteristics

Intra-assay from 16 consecutive standard curves gave the following results:

Standard (ng/mL)	Concentration in Commodity (ppm) (1:40)	%B/B$_0$	CV (%)
0	0	100	-
2.5	0.1	83	2.6
7.5	0.3	60	3.2
20	0.8	34	2.7
50	2	16	2.7
150	6	6	3.3

The graph below represents the data in the table above.



Cross-reactivity

The assay will cross-react with Fumonisin analogues as follows:

- B1: 100%
- B2: 60%
- B3: 74%

Recovery

Mean recoveries along with % CV are listed in the table below. Bulk commodities have been tested using the Fumonisin Hydro ELISA by at least three independent extractions (n). Recoveries for the bulk commodities in independent extractions (n) were as follows:

Type of Commodity	Spike Level (ppm)	Mean Recovery (%)	CV (%)	n	Extraction Solvent
Barley	0.8	116	5.7	3	Distilled water
	2.5	98	6.4		
	5.0	94	2.9		
Corn	0.27	82	4.2	3	Distilled water
	2.63	105	5.2		
Corn gluten meal	0.8	105	12.2	3	Hydro buffer
	2.5	105	7.2		
	5.0	121	0.6		
DDGS	0.8	101	10.4	3	70% methanol
	2.5	108	8.3		
	5.0	98	4.7		
DDGS	0.8	119	8.7	3	Hydro buffer
	2.5	106	2.6		
	5.0	95	3.5		
Flaked maize	0.8	116	5.1	3	Distilled water
	2.5	107	2.9		
	5.0	89	2.3		
Soybean	0.8	116	4.3	3	Distilled water
	2.5	111	3.7		
	5.0	98	2.9		
White hominy corn	1.0	109	3.1	3	Distilled water
	2.5	106	4.3		
	5.0	86	1.5		

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.