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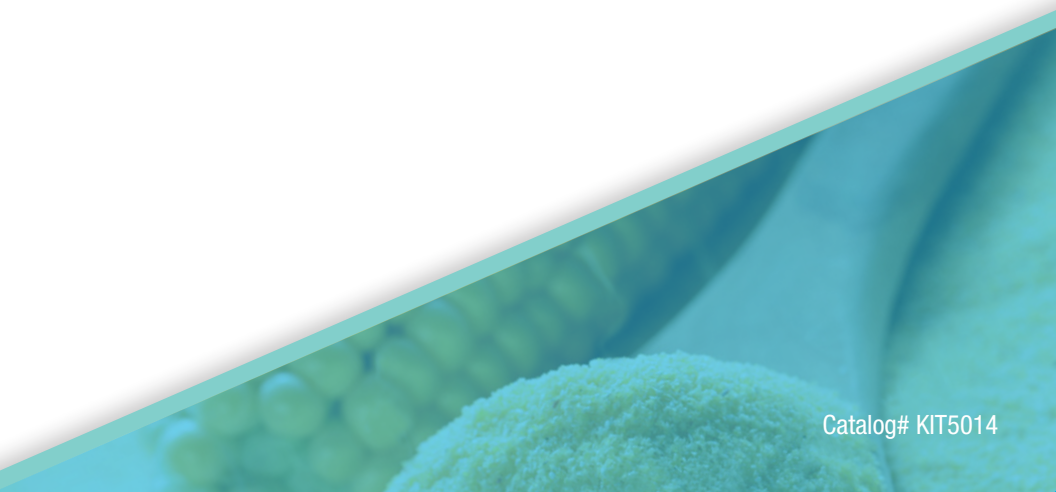
Helica™ Fumonisin Hydro ELISA

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



Catalog# KIT5014

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This package insert must be read in its entirety before using this product.

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Intended Use

The fumonisins (B_1, B_2, and B_3) are a group of mycotoxins produced by *Fusarium moniliforme*. Fumonisins have been found world-wide as a contaminant of maize and have been shown to cause liver cancer in experimental rats, pulmonary edema in pigs and leukoencephalomalacia in horses. High levels of fumonisins in locally grown maize have been found in areas of the world which 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 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, costly and needs skilled workforce to operate/analyze.

The Helica™ Fumonisin Hydro ELISA 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.

Field of Use

Data obtained from Helica™ assays should not be used for human diagnostic or human treatment purpose. Assays are not approved by the United States Food and Drug Administration or any other U.S. or non-U.S. regulatory agency for use in human diagnostics or treatment. Helica™ 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 supervision of technically qualified persons.

Principle of the Method

The Helica™ Fumonisin Hydro ELISA 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 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 chromagen 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 an interpretative result is determined.

Storage and Shelf Life

- Store reagents at 2°C to 8°C, and do not use beyond expiration date(s).
- Never freeze kit components.

Precautions

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

- Bring all reagents to room temperature (19-25˚C) before use.
- Do not return unused reagents back into their original bottles.
- The assay procedure details volumes required.
- Adhere to all time and temperature conditions stated in the procedure.
- Handle the test kit in accordance with good laboratory practices (GLP).
- Do not interchange reagents between kits of different lot numbers.
- Do not use reagents beyond expiration date of the kit. The alteration of a reagent can cause inaccurate results.
- Do not exchange the vial caps.
- Use sterile pipette tips.
- Do not use solutions if they become cloudy or precipitate.
- Substrate solution is light sensitive. Avoid exposure to direct light.
- Do not allow wells to dry completely.
- Handle any solution with gloves.
- Never pipette reagents or samples by mouth.
- During the sample extraction, avoid cross-contamination.
- Devices such as a blender must be cleaned after each sample preparation.
- Substrate Solution contains TMB, which is highly toxic if inhaled, ingested, or comes with toxic or irritating substances, rinse the affected skin area with plenty of water. Please refer to SDS.
- Stop solution contains sulphuric acid, which is corrosive. Please refer to the SDS.
- Avoid incubating on cold work benches.

Kit Safety and Waste Disposal Instructions

Mycotoxins are well known carcinogens in humans and are thus considered highly toxic. 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. It is strongly advised that gloves, a lab coat, and safety glasses be worn always while handling mycotoxin 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 enough 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 mycotoxins 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.

Kit Contents

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 Plate	Mixing wells	Green	96 non-coated wells (12 eight-well strips) in a microwell holder, *Ready-to-Use*.
6X Vials	Fumonisin standards	Black cap	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*.
1X Bottle	Fumonisin HRP-conjugate	Green cap	12 mL of peroxidase conjugated fumonisin in buffer with preservative, *Ready-to-Use*.
1X Bottle	Substrate reagent	Blue cap	12 mL stabilized tetramethylbenzidine (TMB), *Ready-to-Use*.
1X Bottle	Stop solution	Red cap	12 mL acidic solution, *Ready-to-Use*.
1X Pouch	Washing buffer		PBS with 0.05% Tween20®, bring to 1 liter with distilled water and store refrigerated.

Materials Required But Not Provided

- Grinder sufficient to render sample to particle of fine instant coffee
- Single or multi-channel pipettor with 20, 100, 300 and 1000µL tips
- Microtubes
- Timer
- Wash bottle
- Absorbent paper towels
- Whatman #1 filter paper
- Centrifuge
- Microcentrifuge
- Vortex mixer
- Blender
- Distilled water or deionized water
- Graduated cylinder
- Microplate reader equipped with a 450nm filter
- Methanol reagent grade
- Hydro buffer (Catalog# ASY5068, 90 capsules)

Preparation of Samples

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

Barley Flour

1. Weigh out a 20g ground portion of the sample and add 200mL of distilled water.

   Note: The ratio of sample to distilled water is 1:10 (w/v).
2. Shake in a sealed container for 5 minutes.
3. Allow the particulate matter to sediment for 5 minutes, then carefully filter 5mL 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. 0.1mL + 0.3mL)
5. Diluted sample is now ready for testing.
6. Final dilution to be used for calculations is (1:40).

Corn

1. Weigh out a 20g ground portion of the sample and add 100mL of distilled water.

   Note: The ratio of sample to distilled water is 1:5 (w/v).
2. Shake by vortex in a sealed container for 3 minutes.
3. Allow the particulate matter to sediment for 5 minutes, then centrifuge 5mL of the top layer of the extract for 5min at 3500 rpm and carefully transfer the supernatant (sample extract) to a clean tube.
4. Dilute the sample extract 1:8 in distilled water (e.g. 0.1mL + 0.7mL)
5. Diluted sample is now ready for testing.
6. Final dilution to be used for calculations is (1:40).

Preparation of Samples, con't.

Corn Gluten Meal

1. Weigh out a 10g ground portion of the sample and add about 8 capsules of hydro buffer and then add 50mL of distilled water.

   Note: The ratio of sample to distilled water is 1:5 (w/v).
2. Shake by vortex in a sealed container for 5 minutes.
3. Allow the particulate matter to sediment for 5 minutes, then microcentrifuge about 1mL of the top layer of the extract for a minute and carefully transfer the supernatant (sample extract) to a clean tube.
4. Dilute the sample extract 1:8 in distilled water (e.g. 0.1mL + 0.7mL)
5. Diluted sample is now ready for testing.
6. Final dilution to be used for calculations is (1:40).

Note: Hydro buffer can be purchased separately from the supplier. (Catalog# ASY5068, 90 capsules)

DDGS

Note: This extraction procedure is organic solvent based where DDGS is extracted using 70% methanol. To prepare 70% methanol, add 30mL of water to 70mL reagent grade 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 20g ground portion of the sample. Add 80mL of 70% methanol.

   Note: the ratio of sample to 70% methanol is 1:4 (w/v).
3. Shake by vortex in a sealed container for 3 minutes.
4. Allow the particulate matter to sediment for 5 minutes, and then use Whatman #1 filter paper to filter about 5mL of top layer and collect filtrate in a clean tube.
5. Dilute the filtrate (sample extract) 1:10 in distilled water (e.g. 0.1mL + 0.9mL)
6. Diluted sample is now ready for testing.
7. Final dilution to be used for calculations is (1:40).

Preparation of Samples, con't.

DDGS

Note: This extraction procedure is hydro buffer based where DDGS is extracted using distilled water and hydro buffer. Hydro buffer can be purchased separately from the supplier. (Catalog# ASY5068, 90 capsules)

1. Weigh out a 10g ground portion of the sample. Add 4 capsules of hydro buffer and then add 40mL of deionized water.

   Note: the ratio of sample to distilled water is 1:4 (w/v).
2. Shake by vortex in a sealed container for 2 minutes.
3. Allow the particulate matter to sediment for 2 minutes, then carefully pipette out 1mL of the extract in an Eppendorf tube and microcentrifuge for a minute and then transfer the supernatant (sample extract) to a clean tube.
4. Dilute the sample extract 1:10 in distilled water (e.g. 0.1mL + 0.9mL)
5. Diluted sample is now ready for testing.
6. 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 20g ground portion of the sample and add 80mL of distilled water.

   Note: The ratio of sample to distilled water is 1:4 (w/v).
3. Shake by vortex in a sealed container for 3 minutes.
4. Allow the particulate matter to sediment for 2 minutes, then carefully pipette out 1mL of the extract in an Eppendorf tube and microfuge for one minute and then transfer the supernatant (sample extract) to a clean tube.
5. Dilute the sample extract 1:10 in distilled water (e.g. 0.1mL + 0.9mL)
6. Diluted sample is now ready for testing.
7. Final dilution to be used for calculations is (1:40).

Preparation of Samples, con't.

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 20g ground portion of the sample and add 200mL of distilled water.

   Note: The ratio of sample to distilled water is 1:10 (w/v).
3. Blend in a sealed container for 3 minutes.
4. Allow the particulate matter to sediment for 5 minutes, then centrifuge about 5ml from the top for 5-10 min at 3500 rpm and collect the top layer (sample extract) in a fresh clean tube
5. Dilute the sample extract 1:4 in distilled water (e.g. 0.1mL + 0.3mL)
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 20g ground portion of the sample and add 200mL of distilled water.

   Note: The ratio of sample to distilled water is 1:10 (w/v).
3. Shake by vortex in a sealed container for 5 minutes.
4. Allow the particulate matter to sediment for 5 minutes, then carefully filter 5 - 10mL of the top layer of the extract through a Whatman #1 filter paper (or equivalent) and collect the filtrate (sample extract) to be tested.
5. Dilute the sample extract 1:4 in distilled water (e.g. 0.1mL + 0.3mL)
6. Diluted sample is now ready for testing.
7. 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. Reconstitute the PBS-Tween packet by washing out the contents with a gentle stream of distilled water into a 1-Liter container.
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 per standard and sample, please scale down the reagent volumes accordingly.
3. Dispense 220µL of the conjugate solution into the appropriate mixing wells.
4. Using a new pipette tip for each, add 20µL of each Standard and Sample to appropriate mixing well containing conjugate mixture. Mix by priming pipettor 20 times.

   Note: Operator must record the location of each Standard and Sample throughout test.
5. Using a new pipette tip for each, transfer 100µL of contents from each mixing well to a corresponding Antibody Coated Microtiter Well. Incubate at room temperature for 10 minutes. The mixing wells contain enough solution to run each standard and/or sample in duplicate if so desired.
6. 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.
7. Tap the microwells (face down) on a layer of absorbent towels to remove residual buffer.
8. Measure the required volume of Substrate Reagent (1mL/strip or 120µL/well) and place in a separate container. Add 100µL to each microwell. Incubate at room temperature for 10 minutes. Cover to avoid direct light.
9. 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 at the same pace as the Substrate was added.
10. 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.

Interpretation of Results

Construct a dose-response curve using either the unmodified OD values or the OD values expressed as a percentage of the OD of the zero (0.0) 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 greater 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 result.

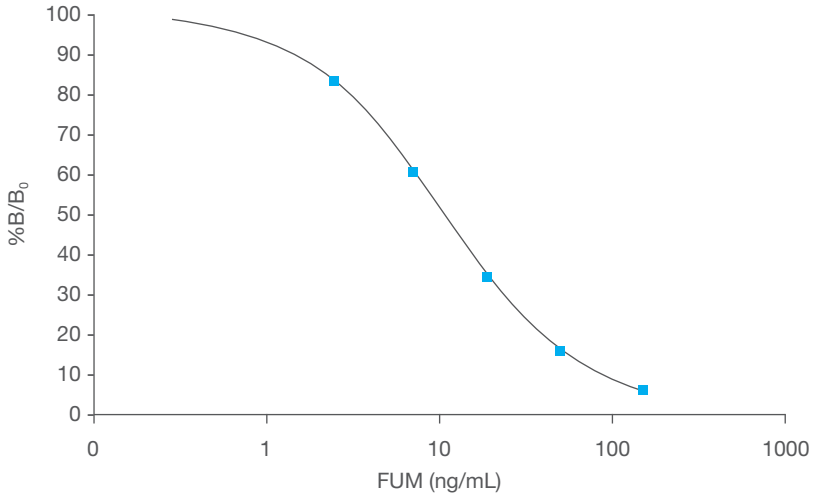
Standard (ng/mL)	Commodity µg/g (ppm) (1:40)
0	0
2.5	0.1
7.5	0.3
20	0.8
50	2
150	6

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:
B_1 - 100%, B_2 - 60%, B_3 - 74%.

Recovery

Mean recoveries along with %CV are listed in the table below. Bulk commodities have been tested using FUM 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	6	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 call 1-714-578-7830 or email techsupport@hygiena.com. Technical support can also be requested at https://www.hygiena.com/hygiena/technical-support-request.html.

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