FUMONISIN ELISA ASSAY

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

(Cat. No. 951FUM01C-96)

FUMONISINS

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.

INTENDED USE

The HELICA BIOSYSTEMS fumonisin ELISA is a competitive enzyme-linked immunoassay intended for the quantitative detection of fumonisins in maize. For research use only. Not for use in diagnostic procedures.

ASSAY PRINCIPLE

The HELICA BIOSYSTEMS fumonisin 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 90% methanol. 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 OD's of the kit standards and an interpretative result is determined.

REAGENTS PROVIDED

1 X 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.*
1 X Plate	**Mixing Wells**	Green	96 non-coated wells (12 eight well strips) in a microwell holder, *Ready-to-Use.*
6 X Vial	**Fumonisin Standards**	Black Cap	1.5mL/vial of fumonisin at the following concentrations: 0.0, 2.5, 7.5, 20.0, 50.0, 150.0 ng/mL in aqueous solution, *Ready-to-Use*.
1 X Bottle	**Streptavidin HRP Conjugate A**	Green Cap	12mL of peroxidase conjugated streptavidin in buffer with preservative, *Ready-to-Use*.
1 X Bottle	**Biotinylated Fumonisin Conjugate B**	White Cap	12mL of biotinylated fumonisin in buffer with preservative, *Ready-to-Use*.
1 X Bottle	**Substrate Reagent**	Blue Cap	12mL stabilized tetramethylbenzidine (TMB), *Ready-to-Use.*
1 X Bottle	**Stop Solution**	Red Cap	12mL Acidic Solution, *Ready-to-Use.*
1 X Pouch	**Washing Buffer**		PBS with 0.05% Tween20, 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: 20g measuring capability
- Graduated cylinder: 100mL
- Methanol: 36mL reagent grade per sample
- Distilled or deionized water: 4mL per sample
- Filter Paper: Whatman #1 or equivalent
- Filter Funnel

Assay Procedure

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

PRECAUTIONS

1. Bring all reagents to room temperature (19º - 27ºC) before use.
2. Store reagents at 2 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 fumonisin. Wear protective gloves and safety glasses 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

Mycotoxins (aflatoxins, trichothecenes, and others) 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 at all times 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 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 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.

EXTRACTION PROCEDURE

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

1. Prepare the Extraction Solution (90% Methanol) by adding 4mL of distilled or deionized water to 36mL of methanol (reagent grade) for each sample to be tested.
2. Grind a representative sample to the particle size of fine instant coffee (95% passes through a 20 mesh screen).
3. Weigh out a 20g ground portion of the sample and add 40mL of the Extraction Solvent (90% methanol).
   Note: The ratio of sample to extraction solvent is 1:2 (w/v).
4. Mix by shaking in a sealed container or in a blender for one minute.
5. Allow the particulate matter to settle, then filter 5 - 10mL of the extract through a Whatman #1 filter paper (or equivalent) and collect the filtrate to be tested.
6. Dilute the sample extract 1:20 in distilled water (e.g. 0.1mL + 1.9mL)
7. Diluted sample is now ready for testing.

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 Dilution Well in a microwell holder for each Standard and Sample to be tested. Place an equal number of Antibody Coated Microtiter Wells in another microwell holder.
3. Dispense 100µL of the Conjugate solution A (green) into the appropriate dilution wells followed by 100µL of Conjugate solution B (clear).
4. Using a new pipette tip for each, add 100µL of each Standard and Sample to appropriate Dilution Well containing Conjugate mixture. Mix by priming pipettor 3 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 Dilution 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 5 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 2:1 ratio with 90% methanol followed by a dilution of 20:1 in distilled water, and so the level of fumonisin shown by the standard must be multiplied by 40 in order to indicate the ng of fumonisin per gram of commodity (ppm) as follows:

standard ng/mL	Commodity µg/gm (ppm)
0.0	0.0
2.5	0.1
7.5	0.3
20.0	0.8
50.0	2.0
150.0	6.0

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 the final result.

SAMPLE	HPLC	HELICA ELISA
1	<0.1 ppm	<0.1 ppm
2	0.6 +/- 0.2 ppm	0.72 +/- 0.06 ppm
3	2.0 +/- 0.3 ppm	1.98 +/- 0.12 ppm
4	3.5 +/- 0.4 ppm	3.30 +/- 0.35 ppm