# QUANTITATIVE ASSAY FOR - FUMONISIN IN URINE

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

**(Cat. No. 951FUM01U-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. Urinary Fumonisin ($UFB_1$) has been used as a biomarker of Fumonisin intake.

## INTENDED USE

The HELICA Fumonisin in urine ELISA is a competitive enzyme-linked immunoassay intended for the quantitative detection of Fumonisins in urine. For research use only. Not for use in diagnostic procedures.

## ASSAY PRINCIPLE

The HELICA Fumonisin in urine 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. Fumonisins are purified from urine and concentrated by a clean-up column. The concentrated sample and conjugate are mixed and added to the antibody-coated microwell. Fumonisin from the urine 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.

## 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 B1 at the following concentrations: 0.0, 0.2, 0.6, 1.5, 4.0, 8.0 ng/mL in aqueous solution, *Ready-to-Use*. |
| 2 X Bottle | **Fumonisin HRP Conjugate** | Green Cap | 2 X 12mL of peroxidase conjugated 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% Tween 20, bring to 1 liter with distilled water and store refrigerated. |
| 1 X Bottle | **Sample Buffer** | Brown Cap | 12mL Neutralization Buffer, *Ready-to-Use.* |

- Clean-up columns: 50 columns are provided in a separate box.

## MATERIALS REQUIRED BUT NOT PROVIDED

### Purification Procedure

- Centrifuge capable of 3500 rpm or above
- Centrifuge tube (15mL)
- Serological pipette
- Distilled or deionized water: 5mL per sample
- Vacuum manifold
- Vacuum pump
- Nitrogen gas
- Water bath
- Methanol
- Acetic acid

### Assay Procedure

- A single-channel pipette and a multi-channel pipette with tips: 100µL - 300µL
- Timer
- 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 1/10$^{th}$ 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.

## PURIFICATION PROCEDURE

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

1. Thaw frozen urine sample at room temperature.
2. Centrifuge sample for 15minutes at 3500rpm.
3. Transfer 1mL of sample in a new tube and add 3mL of 100% MeOH. Mix well.
4. Let it sit for 10minutes and mix well.
5. Centrifuge for 15minutes at 3500rpm.
6. Set clean-up column on top of manifold.
7. Condition with 3 mL of 75% MeOH. (Note: Flow rate should not exceed 1mL/min for all steps.)
8. Load 4 mL of urine sample from step 5 (only supernatant).
9. Wash with 3mL of 75% MeOH.
10. Wash with 3mL of 100% MeOH.
11. Drain residual MeOH completely using vacuum.
12. Elute with 5mL of 1% Acetic acid in 100% MeOH and collect.
13. Dry the eluate completely by a gentle stream of nitrogen in water bath (50°C).
14. Reconstitute with 0.2mL of Sample Buffer.
15. Sample is now ready for testing.

## ASSAY PROCEDURE

Note: It is recommended to utilize a multi-channel pipettor 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 deionized water into a 1-Liter container.
2. Crystallization in standard solutions may be observed. Vortex standard vials well before use. Place mixing wells in a microwell holder for standard and sample to be tested.
3. Place an equal number of antibody coated wells in another microwell holder.
4. Dispense 200µL of the Conjugate into the appropriate mixing wells.
5. Using a new pipette tip, add 100 µL of each standard and prepared sample to the appropriate mixing well containing the Conjugate. Mix by priming pipettor at least 3 times.
6. Using a new pipette tip for each, transfer 100µL of contents from each mixing well to a corresponding Antibody Coated Microwell. Incubate for 60 minutes. The mixing wells contain enough solution to run each standard and/or sample in duplicate if so desired. Cover to avoid direct light.
7. Decant the contents from the microwells into a discard basin. Using a multi-channel pipette, filling each microwell with 300µL of Washing 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 buffer.
9. Place Substrate Reagent in a separate container. Add 100µL of Substrate Reagent to each microwell. Incubate for 10 minutes. Cover to avoid direct light.
10. Place Stop Solution in a separate container. Add 100µL of Stop Solution to each microwell.

11. Read the optical density (OD) of each microwell with a microplate reader using a 450nm filter. Record the OD value of each microwell.

## INTERPRETATION OF RESULTS

Construct a standard curve (4-parameter curve fit) using either the OD values or the OD values expressed as a percentage of the OD of zero (0.0) standard against the Fumonisin content of the standard (%B/$B_0$). 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 concentrated 5 times, and so the level of Fumonisin shown by the standard must be divided by 5 in order to indicate the pg of Fumonisin per milliliter of urine (ppt) as follows:

| **Standard (ng/mL, ppb)** | Concentration factor | ng/mL (ppb) in sample | **pg/mL (ppt) in sample** |
|---|---|---|---|
| **0** | 5 | 0 | **0** |
| **0.2** | 5 | 0.04 | **40** |
| **0.6** | 5 | 0.12 | **120** |
| **1.5** | 5 | 0.3 | **300** |
| **4** | 5 | 0.8 | **800** |
| **8** | 5 | 1.6 | **1600** |

The sample concentration results in a standard curve from 0ppt to 1600ppt. If a sample contains Fumonisin at greater than the highest standard, it should be diluted appropriately in the Sample Buffer and retested. The extra dilution step should be taken into consideration when expressing the final result.

## ASSAY CHARACTERISTICS

Data from 7 consecutive standard curves gave the following results:

| Standard (ppb) | OD | %B/B0 | %CV |
|---|---|---|---|
| 0 | 1.804 | 100 | 1.17 |
| 0.2 | 1.569 | 87 | 1.43 |
| 0.6 | 1.209 | 67 | 1.49 |
| 1.5 | 0.764 | 42 | 1.53 |
| 4 | 0.375 | 21 | 3.50 |
| 8 | 0.207 | 12 | 2.44 |

Fumonisin B1 was spiked in urine followed by purification and assay procedures.

| Fumonisin B1 (ppt, pg/mL) | Recovery (%)* |
|---|---|
| 50 | 83 |
| 100 | 84 |
| 200 | 79 |
| 1000 | 81 |

*Average recoveries were calculated from both male and female urines spiked with different levels of Fumonisin B1. (n = 7 for each spike level)