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Product Information

Fumonisin ELISA Kit
for maize (corn)

Catalog Number **SE120010**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Fumonisins are a group of mycotoxins produced by different *Fusarium* fungi.[1,2] Fumonisins are known to occur as natural contaminants of maize, animal feed, and maize-based foods. The most prevalent and widely investigated fuminosin is fumonisin B_1, which was historically isolated from *F. verticillioides* (formerly known as *F. moniliforme* Sheldon), along with fumonisin B_2.[3] Fumonisins have been found to cause liver and kidney cancer in experimental animals (rodents), and to lead to pulmonary edema in pigs and leukoencephalo-malacia in horses.[2]

The Fumonisin ELISA Kit is a solid-phase competitive enzyme immunoassay. A fumonisin-specific antibody, optimized to cross react with the three fumonisin subtypes B_1, B_2, and B_3, 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 a blue color 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 450 nm (OD_{450}). The optical densities of the samples are compared to the OD's of the kit standards, and an interpretative result is determined.

The Fumonisin ELISA Kit is intended for the quantitative detection of fumonisins in maize. The assay has a sensitivity of up to 150 ng/mL.

Components

1. Fumonisin Microplate (951FUM01C): 96 wells (12 × 8 well strips) in a microwell holder coated with a mouse anti-fumonisin monoclonal antibody.
2. Fumonisin Standards (953S6FU01M, Black Cap): 6 vials, 1.5 mL/vial of fumonisin at the following concentrations: 2.5, 7.5, 20.0, 50.0, 150.0 ng/mL in aqueous solution
3. Streptavidin HRP-Conjugate A (954MFU01, Green Cap): 12 mL of peroxidase-conjugated streptavidin in buffer with preservative
4. Biotinylated Fumonisin Conjugate B (954MFU02, White Cap): 12 mL of biotinylated fumonisin in buffer with preservative
5. TMB Substrate (916T001, Blue Cap): 12 mL of stabilized 3,3′,5,5′-tetramethylbenzidine (TMB)
6. Stop Solution (946P001, Red Cap): 12 mL of Acidic Solution.
7. PBST Wash Buffer Powder (915X001): 1 packet of PBS with 0.05% TWEEN® 20. Bring to 1 liter with distilled water and store refrigerated.
8. Mixing Wells (Green): 96 non-coated wells (12 × 8 well strips) in a microwell holder

Reagents and Equipment Required but Not Provided.

1. Grinder sufficient to render sample to particle size of fine instant coffee
2. Microplate reader capable of measuring absorbance at 450 nm
3. Precision pipettes to deliver 100-200 µL volumes
4. Collection Container: Minimum 125 mL capacity
5. Graduated cylinder: 100 mL
6. Methanol reagent grade (sufficient for 36 mL per sample)
7. Distilled or deionized water (sufficient for 4 mL per sample)
8. Filter Paper: Whatman® #1 or equivalent
9. Filter funnel
10. Absorbent paper towels
11. Graph paper or computer and software for ELISA data analysis
12. Balance with 20 g measuring capacity

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Consider all materials, containers, and devices that are exposed to sample or standards to be contaminated with fumonisins. Wear protective gloves and safety glasses when using this kit.

Storage/Stability
Store reagents at 2–8 °C, and do not use beyond expiration date(s). Never freeze the kit components. HRP-labelled conjugate and TMB Substrate are photosensitive and are packaged in protective opaque bottles. Store in the dark and return to storage after use.

Procedures
Before doing the assay, prepare a waste container as a receptacle for kit waste. Eject contaminated pipette tips and all other related materials into this container. Following completion of the assay, treat the container with sufficient 5-6% sodium hypochlorite (NaOCl) to saturate the container's contents, about $1/10^{th}$ the volume of the container. 5-6% NaOCl will denature the mycotoxins and neutralize the waste, which renders the waste safe for disposal. Invert the container several times to coat all waste thoroughly.

(In case of an accidental toxin spill, treat the spill surface with 5-6% NaOCl for a minimum of 10 minutes, and then with 5% aqueous acetone. Wipe dry with absorbent paper towels.)

Extraction Procedure/Sample Preparation
Note: Samples must be collected according to established sampling techniques.

1. Prepare the Extraction Solution (90% Methanol) by adding 4 mL of distilled or deionized water to 36 mL 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 20 g of ground portion of the sample. Add 40 mL 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 5-10 mL of the extract through a Whatman #1 filter paper (or equivalent). Collect the filtrate to be tested.
6. Dilute the sample extract 20-fold in distilled water (e.g. 0.1 mL plus 1.9 mL).
7. The diluted sample is now ready for testing.

Assay Procedure
Notes: A multichannel pipettor is recommended to perform the assay. If a single channel pipettor is used, it is recommended no more than a total of 16 samples and standards (2 test strips) are run.

Bring all reagents to room temperature (19–27 °C) before use. Do not return unused reagents back to their original bottles.

1. Reconstitute the PBST Wash Buffer in 1 L of distilled water. The packet contents may be washed out with a gentle stream of distilled water.
2. Place one Dilution Well in a microwell holder for each Standard and Sample to be tested. Place an equal number of Antibody-Coated Microwells in another microwell holder.
3. Dispense 100 µL of the Streptavidin HRP-Conjugate solution A (green cap) into the appropriate dilution wells, followed by 100 µL of Biotinylated Fumonisin Conjugate solution B (clear cap).
4. Using a fresh pipette tip for each, dispense 100 µL aliquots of each standard and each sample into the appropriate Dilution Wells that contain conjugate. Mix by priming pipettor 3 times.
   Note: Operator must record the location of each Standard and Sample throughout the test.
5. Using a new pipette tip for each, transfer 100 µL of contents from each Dilution Well to a corresponding Antibody-Coated Microwell. 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. Discard the contents of the microwells into an appropriate receptacle. Wash the wells with the PBST Wash Buffer solution, and discard the washings into an appropriate receptacle. Repeat for a total of 5 washings.
7. Tap the microwells (face down) on a layer of absorbent paper to remove residual wash buffer.
8. Measure the required volume of TMB Substrate (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.

9. 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.
10. Read and record the optical density (OD) of each microwell with a microplate reader using a 450 nm filter.

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 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 20-fold dilution in distilled water. Thus 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 considered when expressing the final result.

Reproducibility:

Intra-Assay: CV <15%
Inter-Assay: CV <10%

References

1. Woloshuk, C.P., and Shim, W.-B., Aflatoxins, fumonisins, and trichothecenes: a convergence of knowledge. *FEMS Microbiol. Rev.*, **37(1)**, 94-109 (2013).
2. Voss, K.A., and Riley, R.T., Fumonisin Toxicity and Mechanism of Action: Overview and Current Perspectives. *Food Safety*, **1(1)**, 49-69 (2013).
3. Gelderblom, W.C. et al., Fumonisins - novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Appl. Environ. Microbiol., **54(7)**, 1806-1811 (1988).

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