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User's Manual

Fumonisin ELISA Kit

REF **DEIA3492**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Fumonisin ELISA Kit is a competitive binding enzyme immunoassay for the quantitative measurement of Fumonisin.

General Description

Fumonisins B1, B2 and B3 are the main members of mycotoxins produced by Fusarium moniliforme. Fumonisins appear to be widespread in corn, and have been detected in corn-based products including feed and human foods. The presence of fumonisins in foods could have implications for human health. The Fumonisin EIA is intended for the quantitative detection of Fumonisin levels in rice, corn and cornmeal.

Principles of Testing

The enzyme immunoassay for Fumonisin is based on the competition between the Fumonisin in the sample and the Fumonisin horseradish peroxidase conjugate, for binding to antibody directed against Fumonisin, coated onto microwells. The sample containing the Fumonisin, and the Fumonisinhorseradish peroxidase conjugate, when added to the microtiter wells, compete for binding to a limiting number of antibody sites. After incubation, each well is rinsed in order to remove non-bound components. The bound enzymatic activity is then measured by the addition of a chromogenic substrate. If no or small amount of Fumonisin is present in the sample more enzyme labeled Fumonisin will bind the antibody on the solid surface. On the other hand, if large or significant amount of Fumonisin is present in urine sample, less enzyme labeled Fumonisin will bind to the antibody, producing less color signal. Therefore, the intensity of the color developed is inversely proportional to the concentration of Fumonisin in the sample. The concentration is calculated on the basis of a standard curve.

Reagents And Materials Provided

1. 96-well microtiter plate. Twelve strips of 8 detachable wells, coated with anti-Fumonisin antibody.
2. One vial of 1.2 mL. Negative Calibrator containing 0 µg/mL of fumonisin.
3. Three vials of Positive Calibrator (0.9 mL) containing 1.0, 5.0 and 9.0 µg/mL of fumonisin.
4. One bottle. containing 10.5 mL of Fumonisin-Horseradish Peroxidase conjugate (FNS-HRP).
5. One bottle. containing 10.5 mL of Tetramethylbenzidine (TMB) substrate.
6. One bottle. containing 15 mL of Wash Buffer (10×PBS-Tween). Dilute 10 fold with distilled or deionized water to 150 mL prior to use.
7. One bottle. containing 10.5 mL of Stop Solution, 3N HCl.

Materials Required But Not Supplied

1. Pipetters capable of delivering 50 µL and 100 µL.
2. Microtiter plate reader (wavelength 405 nm).

3. Plate washer or squeezable wash bottle.
4. Timer.
5. Absorbent paper towels.

Storage

All reagents of the kit are stable, if stored at 2-8°C, until the expiration date stated on the kit. For more detailed information, please download the following document on our website.

Reagent Preparation

Preparation of Fumonisin Standard Solutions

1. A 1 mg standard can be dissolved in 1 mL of methanol to give a 1 mg/mL solution.
2. Intermediate dilutions are then made in 70% methanol to give concentrations of 100 µg/mL (ppm) and 10 µg/mL (10 ppm). To obtain these concentrations:

1) Add 100 µL of the 1 mg/mL solution to 900 µL 70% Methanol giving a 100 µg/mL solution.
2) Add 100 µL of the 100 µg/mL solution to 900 µL 70% Methanol giving a 10 µg/mL solution.
3) Add 500 µL of the 10 µg/mL solution to 500 µL 70% Methanol giving a 5 µg/mL solution.
4) Add 100 µL of the 10 µg/mL solution to 900 µL 70% Methanol giving a 1 µg/mL solution.

Assay Procedure

1. Carefully add 50 µL of standard or samples to the bottom of each well. Slightly tap the side of the strip holder to evenly distribute the sample.
2. Avoid touching the well with pipette tip and add 100 µL of FNS-HRP to each well. Slightly tap the side of the strip holder to properly mix the sample and enzyme conjugate.
3. Incubate at room temperature for 30 minutes.
4. After incubation, dispose the solution in the wells by inverting and shaking. Wash microtiter wells 3 times with wash buffer to remove the non-bound conjugate. Washing may be done manually as follows: use squeeze bottle to fill wells gently with wash buffer, dumping the wells between each wash by inverting and shaking. After the third wash, tamp holder with washed strips onto a piece of absorbent paper.
5. Add 100 µL of TMB substrate to each well and incubate at room temperature for 15 min. To avoid contamination, place the needed amount of substrate into a test tube and dispense only from the tube itself.
6. Add 100 µL of Stop Solution to each well and tap the strip holder for proper mixing.
7. Read absorbance at 450 nm using an ELISA reader.

Typical Standard Curve

1. Calculation

1) Average the absorbance (ODs) for each standard concentration of fumonisin including 0 µg/mL (OD0).
2) % of Inhibition = 100 -(ODs / OD0) ×100

2. Plot values of % of Inhibition, step 1 (b), against their corresponding concentrations on Log10 paper.
3. Calculate fumonisin concentration in the sample by interpolation and multiply by the sample's dilution factor to obtain the actual quantity of fumonisin.