Fumonisin B1 (FB1) ELISA Kit

Catalog No.: abx364880

Size: 96 tests

Storage: Store all components at 4°C for up to 6 months.

Application: For detection and quantification of FB1 content in corn, feed and oil.

Detection range: 0.5 ppb – 40.5 ppb

Detection limit: Corn – 75 ppb, Feed – 75 ppb, Oil – 10 ppb.

Principle of the Assay: This kit is based on competitive enzyme-linked immuno-sorbent assay technology. An antigen is pre-coated onto a 96-well plate. Standards, test samples, and biotin-conjugated reagent are added to the wells and incubated. The HRP-conjugated reagent is then added, and the whole plate is incubated. Unbound conjugates are removed using wash buffer at each stage. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient FB1 will produce a blue coloured product, which then changes to yellow after adding the acidic stop solution. The intensity of the yellow colour is inversely proportional to the FB1 amount bound on the plate. The Optical Density (OD) is measured spectrophotometrically at 450 nm in a microplate reader, from which the concentration of FB1 can be calculated.

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Kit components

1. 96-well microplate
2. Detection Reagent A: 5.5 ml
3. Detection Reagent B: 5.5 ml
4. TMB Substrate A: 6 ml
5. TMB Substrate B: 6 ml
6. Stop Solution: 6 ml
7. Wash Buffer (20X): 40 ml
8. Reconstitution Buffer (10X): 50 ml
9. Standard: 6 × 1 ml
10. Plate sealer: 3
11. Hermetic bag

Materials Required But Not Provided

1. Microplate reader (450 nm)
2. Distilled water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Homogenization medium (10 mM Tris-HCl, pH 7.4, containing 10 mM NaCl, 10 mM sucrose, 0.1 mM EDTA)
5. Pipette and pipette tips
6. Vials/tubes
7. Sonicating water bath
8. Centrifuge
9. Vortex mixer
10. Methanol
11. N-hexane

Protocol

A. Preparation of samples and reagents

1. Reagents

Bring all reagents to room temperature before use. Mix all reagents immediately before use.

- **Reconstitution Buffer:** Dilute the 10X Reconstitution buffer 1:9 with deionized water. The resulting solution can be stored at 4°C for up to 1 month.
- **Wash Buffer:** Dilute the 20X Reconstitution buffer 1:19 with deionized water.

2. Samples

Isolate the test samples soon after collecting and analyze immediately.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Corn and feed samples:** Homogenize the sample by hand, using a mechanical homogenizer, or by ultrasonication. Weigh the homogenate. For each 1 g of homogenate, add 5 ml deionized water, and vortex for 5 minutes. Centrifuge the homogenate at 4,000 rpm at room temperature for 10 minutes. Collect 0.1 ml of the supernatant, add 0.9 ml of Reconstitution Buffer, and vortex for 2 minutes. Take 50 µl and analyze immediately. *Sample dilution factor: 50*
- **Oil samples:** Add 8 ml of N-hexane and 5 ml of 70% Methanol to 5 ml of sample. Vortex for 5 minutes. Centrifuge the homogenate at 4,000 rpm at room temperature for 10 minutes. Discard the top layer supernatant, and take 100 µl of the bottom layer to a new centrifuge tube. Add 1.9 ml of Reconstitution Buffer and mix fully. Take 50 µl and analyze immediately. *Sample dilution factor: 20*

We recommend carrying out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.
- If the TMB substrate reagents change to a blue colour, they have expired. When the OD value of the 0 ppb standard is < 0.5, the TMB substrate or detection reagents may have deteriorated.

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B. Assay Procedure

1. Set the standard wells (0 ppb, 0.5 ppb, 1.5 ppb, 4.5 ppb, 13.5 ppb, 40.5 ppb), and sample wells and record their positions. We recommend testing each sample and standard in duplicate.
2. Add 50 µl of standard to the standard wells.
3. Add 50 µl of sample to each sample well.
4. Add 50 µl of Detection Reagent A to all wells.
5. Add 50 µl of Detection Reagent B to all wells.
6. Cover the plate with a plate sealer, tap the plate gently to mix.
7. Incubate at 25°C for 30 minutes in the dark.
8. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. *Fill each well completely with Wash buffer (300 µl) using a multi-channel Pipette or autowasher (1-2 mins soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels. Note: the wells should not become completely dry at any point.*
9. Add 50 µl of TMB Substrate A to all wells.
10. Add 50 µl of TMB Substrate B to all wells. Tap the plate gently to mix.
11. Incubate at 25°C for 15 minutes in the dark.
12. Add 50 µl of Stop Solution to all wells and mix fully.
13. Immediately read and record the absorbance at 450 nm. The absorbance must be read within 10 minutes of adding the Stop Solution.

C. Calculation of Results

The standard curve can be plotted as the Absorbance (%) of each standard solution (y) vs. the log of the respective concentration of the standard solution (x). A linear fit is recommended for the standard curve ($y = ax + b$). The FB1 concentration of the samples can be interpolated from the standard curve. Where samples have been diluted, the concentration of the sample calculated from the standard curve must be multiplied by the dilution factor.

$$\text{Absorbance (\%)} = \frac{A}{A_0} \times 100$$

where:

A Absorbance of sample

A_0 Absorbance of the 0 ppb standard

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