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Cat No.: EKF1106

Fumonisin B1 (FB1) ELISA Kit

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

Email (Order) order@enkilife.com

Email (Techsupport) techsupport@enkilife.com

Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.
Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Product description

This ELISA kit is rapid, quantitative enzyme-linked immunosorbent assays (ELISA) for the determination of Fumonisin B1 content in Food samples.

Key Features

- Sensitivity：1 ppb (ng/mL)
- Detection range：1 ppb~160 ppb
- Specificity：

Fumonisin B1	100%

- Detection limits：

Cereal, feed	50 ppb

- Recovery rate：

Feed	95±15%
Cereal	100±15%

Storage and Shelf Life

The kit should be stored at 2~8°C and must not be frozen. The shelf life of this product is 1 year. Do not use the kit beyond the expiration date.

Test Principle

This assay employs the competitive inhibition enzyme immunoassay technique. The kit consists of an enzyme-labeled plate pre-coated with coupled antigens, horseradish enzyme markers, antibodies, standard substances, and other supporting reagents. During detection, standard substances or sample solutions are added. Fumonisin B1 in the samples competes with the pre-coated coupled antigens on the enzyme-labeled plate for Fumonisin B1 specific antibody. After adding the enzyme markers, TMB substrate is used for color development. The absorbance value of the sample is negatively correlated with the content of Fumonisin B1 it contains, and the residual amount of Fumonisin B1 in the sample can be obtained by comparing with the standard curve.

Materials Supplied

Item	Quantity
Assay Plate	96T
Standard	6 x 1 mL
HRP Conjugate	1 x 5.5 mL
Antibody	1 x 5.5 mL
TMB Substrate Reagent (A\B)	2 x 6 mL
Stop Solution	1 x 6 mL
Wash Buffer (20×)	1 x 40 mL
Reconstitution Buffer (10×)	1 x 50 mL
Plate Sealer	1 piece
Instruction Manual	1 piece
Sealed Bag	1 copy

Standard Concentration

Standard	1	2	3	4	5	6
Concentration (ppb=ng/mL=ng/g)	0	1	5	20	80	160

Materials Required, Not Supplied

- Instruments: Microplate reader, printer, homogenizer, nitrogen blow-drying device, vortex, centrifuge, constant temperature incubator, balance (sensitivity 0.01g)
- Single-channel micropipette (20 μL-200 μL,100 μL-1000 μL)
- 300 μL multichannel micropipette
- Distilled or deionized water

ⓘNotes:

- Before using the kit, read the instructions carefully.
- Do not use expired kits, and do not mix reagents in kits with different batch numbers.
- Kindly use graduated containers to prepare the reagent.
- Bring all reagents to room temperature (20-25°C) before use for 30 min.
- Only the disposable tips can be used for the experiments and the tips must be changed when used for different reagents.
- Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.
- The stop solution is acidic. Wear eyes, hands, face, and clothing protection when using

the product.

- EnkiLife is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

Reagent Preparation

- **Wash Buffer (1x):** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 10 mL of Wash Buffer (20x) into 190 mL deionized or distilled water to prepare 200 mL of Wash Buffer (1x). Keep it at 4 °C for one month.
- **Reconstitution Buffer:** Dilute the **Reconstitution Buffer (10×)** with deionized water. (Reconstitution Buffer (10×): Deionized water =1:9). The Reconstitution buffer can be store at 4℃ for a month.

Sample Preparation and collection

The prepared sample may be stored for up to one day at 4°C.

Cereal, feed: Weigh 2±0.05 g of homogenate feed into 50 mL centrifuge tube, add 10 mL of deionized water. Vortex for 5 min, centrifuge at 4000 rpm for 10 min at room temperature. Take 100 μL of supernatant to new centrifuge tube, add 900 μL of **Reconstitution Buffer**, mix fully. Take 50 μL for analysis. Dilution factor of sample: 50.

Assay Protocol

ⓘNotes:

- Take the required reagent out of the refrigerated environment at 4°C and place it at room temperature for more than 30 min. If the reagent is crystalline, allow it to dissolve sufficiently at room temperature. Shake each liquid reagent well before use.
- It is recommended that all standards and samples be run at least in duplicate.
- Remove the required number of assay plates, put the unused assay plates in sealed bags, and store at 4°C.
- Avoid using metal packaging and stirring reagents.
- Mix the liquid well and complete removal of liquid at each step is essential to good performance.

- Discard the substrate with any color that indicates the degeneration of this solution. When the absorbance value of 0ppb standard less than 0.5 indicates its degeneration.
- The sample addition time for each step shall not exceed 3 min.

1、**Number:** The corresponding microwells of the sample and the standard are numbered in sequence, and each sample and standard are parallel to each well with 2 wells, and the location of the standard well and the sample well is recorded.

2、**Start the reaction:** Add 50 µL of **Standard** or **Sample** per well to the respective microwells, then add 50 µL of **HRP Conjugate** per well, followed by 50 µL of **Antibody** to each well. Cover the assay plate with a plate sealer, gently shake for 5s to mix well, and react in the dark at 25 °C for 30 min.

3、**Washing:** Carefully remove the plate sealer, discard the liquid in the plate wells. Wash by filling each well with 350 µL of **Wash Buffer (1X)** using a squirt bottle, multi-channel pipette, manifold dispenser, or auto washer, and let it stand for 30 s. Aspirate or decant the liquid in the plate wells, pat it dry against clean absorbent paper, and complete one wash. Repeating the process 5 times.

Tips: After the plate wells is dried, the next step should be carried out immediately.

4、**Add Substrate Reagent:** Add 50 µL of **Substrate solution A** to each well, then add 50 µL of **Substrate Reagent B**, gently shake for 5s to mix well. Incubate for 15 min at 25°C. Protect from light.

Tips: Adjust the incubation time according to the color change, but do not exceed 30 min. Once the standard wells show a clear gradient, the incubation can be stopped.

5、**Stop the reaction:** Add 50 µL of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.

6、**Measure the OD value:** Determine optical density (OD) result at 450 nm within 10 min. (Recommend reading the OD value at the dual-wavelength: 450/630 nm).

Calculation of results

1、**Calculation of percent absorbance:**

The percent absorbance of the standard or sample is equal to the average of the percent absorbance value of the standard or sample (double well) divided by the absorbance value of the first standard (0 ppb), multiplied by 100%.

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

A—The average absorbance value of the standard or sample solution
A_0—Average absorbance value of 0 ppb standard

2、**Create of standard curve:**
Take the percentage absorbance of the standard substance as the Y-axis and the logarithm of the corresponding standard substance concentration (ppb) as the X-axis to plot the semi-logarithmic curve of the standard substance. The percentage absorbance of the sample is substituted into the standard curve, and the concentration corresponding to the sample is read out from the standard curve, and multiplied by its corresponding dilution factor to get the actual concentration of the analyte in the sample. Substitute the percentage absorbance of the sample into the standard curve, read the concentration corresponding to the sample from the standard curve, and multiply it by the corresponding dilution factor to obtain the actual concentration of the analyte in the sample.

ℹ We are always committed to providing high-quality products and thank you for your understanding and support. If you have any questions, please feel free to contact our technical support team.