

AFB1 ELISA Kit

Cat #: orb411503 (manual)

For Research Use Only. Not For Use in Diagnostic Procedures!

This immunoassay kit allows for the in vitro quantitative determination of Aflatoxin B1 concentrations in feedstuff, feed, grain, edible oil.

This package insert must be read in its entirety before using this product.

PRINCIPLE OF THE ASSAY

This assay employs the competitive inhibition enzyme immunoassay technique.

The microtiter plate provided in this kit has been pre-coated with Aflatoxin B1 antigen. Standards or samples are added to the appropriate microtiter plate wells with Aflatoxin B1 specific antibody and Horseradish Peroxidase (HRP) conjugated anti-antibody. The competitive inhibition reaction is launched between pre-coated Aflatoxin B1 and Aflatoxin B1 in standards or samples with the Aflatoxin B1 special antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of Aflatoxin B1 in the standards or samples. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

0.15 µg/kg-4.05 µg/kg.

SENSITIVITY

The minimum detectable dose of the kit is typically less than 0.15 µg/kg.

The sensitivity of this assay, or Lower Limit of Detection (LOD) was defined as the lowest concentration that could be differentiated from zero.

CROSS-REACTION RATE

Aflatoxin B1	100%
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RECOVERY RATE

Edible oil	80%~120%
Feedstuff, feed, grain	70%~100%

LIMIT OF DETECTION

Edible oil	1 µg/kg
Feedstuff, feed, grain	2 µg/kg

PRECISION

Intra-assay Precision (Precision within an assay): CV%<10%

Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<10%

Three samples of known concentration were tested in twenty assays to assess.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Reagent	Quantity	Quantity
Assay plate	96T	48T
Standard	5 x 1 mL	5 x 0.5 mL
HRP-conjugate	1 x 7 mL	1 x 3.5 mL
Antibody	1 x 7 mL	1 x 3.5 mL
TMB Substrate	1 x 12 mL	1 x 6 mL
Stop Solution	1 x 10 mL	1 x 5 mL
Sample Diluent	1 x 50 mL	1 x 25 mL
Wash Buffer(10×)	1 x 50 mL	1 x 25 mL
Adhesive Strip	4	4
Instruction Manual	1	1

STANDARD CONCENTRATION

Standard	S1	S2	S3	S4	S5
Concentration ($\mu\text{g}/\text{kg}$)	0	0.15	0.45	1.35	4.05

STORAGE

Unopened kit	Store at 2 - 8°C. Do not use the kit beyond the expiration date
Opened kit	May be stored for up to 1 month at 2 - 8°C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- An incubator which can provide stable incubation conditions up to 25°C
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Centrifuge, Vortex mixer
- Analytical balance, 2 decimal places
- Single-channel micropipette (20 μL -200 μL , 100 μL -1000 μL , 1000 μL -5000 μL)
- 300 μL multichannel micropipette
- Deionized or distilled water.
- Pipette and pipette tips.
- Ethanol
- NaOH

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Note:

- 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.

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 (10x)** into 90 mL deionized or distilled water to prepare 100 mL of **Wash Buffer (1x)**. Keep it at 4°C for one month.
- **70% Ethanol:** Transfer 700 mL of **Ethanol** into 300 mL deionized or distilled water, shake well.
- **40% Ethanol:** Transfer 400 mL of **Ethanol** into 600 mL deionized or distilled water, shake well.

Note:

1. We are 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.
2. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
3. 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.

SAMPLE COLLECTION AND STORAGE

A. Edible oil

1. Take 5.00 ± 0.05 g of edible oil and add 25 mL of **70% Ethanol**, vortex well for 2 min.
2. Centrifuge at 4000 rpm for 5 min.
3. Transfer 100 μ L of supernatant into a new centrifugal tube, add 100 μ L of **Sample Diluent**, shake well.
4. Take 50 μ L of sample for further analysis.

Dilution factor of the samples: 10

B. Feedstuff, feed, grain

1. Weigh 5.00 ± 0.05 g of the homogenized sample.
2. Add 25 mL of **40% Ethanol**, vortex properly for 2 min.
3. Centrifuge at 4000 rpm for 5 min.
4. Transfer 200 μ L of supernatant into a new centrifugal tube, add 600 μ L of **Sample Diluent**, shake well.
5. Take 50 μ L of sample for further analysis.

Dilution factor of the samples: 20

(Adjust the pH to 6-7 by adding NaOH when the pH<6)

ASSAY PROCEDURE

Bring all reagents and samples to room temperature (20-25°C) before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 2-8°C.

3. Add 50 μ L of **Standard** or **Sample** per well. Then add 50 μ L of **HRP-conjugate** to each well and 50 μ L of **Antibody** to each well. Cover the microtiter plate with a new adhesive strip and mix well, then incubate for 15 min at 25°C.
4. Aspirate each well and wash, repeating the process four times. Wash by filling each well with 250 μ L of **Wash Buffer (1x)** and let it stand for 30 seconds, complete removal of liquid at each step is essential to good performance.
5. Add 100 μ L of **TMB Substrate** to each well, mix well. Incubate for 5 minutes at 25°C. Protect from light.
6. Add 50 μ L of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well within 5 min, using a microplate reader set to 450nm (Recommend reading the OD value at the dual wavelength: 450/630 nm within 5 min).

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 min. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, **DO NOT** let the strips **DRY** at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding Substrates (e.g. observation once every 10 min). Substrates should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. Substrates are easily contaminated. Substrates should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the Substrates. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrates.

CALCULATION OF RESULTS

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

$$\text{Absorbency value (\%)} = \frac{B}{B_0} \times 100\%$$

B —the average absorbance value of the sample or standard

B₀ —the average absorbance value of the 0 µg/kg standard

To draw a standard curve: Take the absorbency value of standards as y-axis, the concentration of the Aflatoxin B1 standards solution (µg/kg) as x-axis.

The Aflatoxin B1 concentration of each sample (µg/kg), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

Note:

- Discard the substrate with any color that indicates the degeneration of this solution; when the absorbance value of S1 less than 0.5 indicates its degeneration.
- The optimum reaction temperature is 25°C, and too high or too low will result in the changes in the absorbance value and detecting sensitivity.