



User's Manual

Aflatoxin B1 ELISA Kit



DEIA054



96T





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

Aflatoxin B1 ELISA Test Kit is a competitive enzyme immunoassay for the quantitative analysis of Aflatoxin B1 in cereals, feed, milk, milk powder, enzyme, cookies, soy sauce, vinegar, oils, peanuts. The unique features of the kit are:

1. High recovery (75-125%), rapid(10-40minutes), cost-effective extraction methods.
2. High sensitivity (0.02 ng/g or ppb).
3. High reproducibility.
4. A quick ELISA assay (only 1 hour regardless of number of samples).

Principles of Testing

The method is based on a competitive colorimetric ELISA assay. The Aflatoxin B1-BSA has been coated in the plate wells. During the analysis, sample and Aflatoxin B1 antibody (Antibody#1) and HRP-Conjugate (HRP-Conjugated Antibody #2) are added to the wells for incubation. If the Aflatoxin B1 residue is present in the sample, it will compete with the Aflatoxin B1 on the plate wells for the Aflatoxin B1 antibody, The secondary antibody, tagged with a peroxidase enzyme, targets the primary antibody that is complexed to the drug coated on the plate wells. The resulting color intensity, after addition of the HRP substrate (TMB), has an inverse relationship with the Aflatoxin B1 residue concentration in the sample.

Reagents And Materials Provided

Aflatoxin B1 ELISA Test Kit has the Aflatoxin B1 capacity for 96 determinations or testing of 42 samples in duplicate (assuming 12 wells for standards). Return any unused microwells to the foil bag and reseal them with the desiccant provided in the original package.

1. Aflatoxin B1-BSA-Coated Plate: 1x 96-well Plate (8 wells x 12 strips)
2. Aflatoxin B1 Standards: 1.0 mL for each
Negative control (white cap tube)
0.02ng/mL (yellow cap tube)
0.06 ng/mL (orange cap tube)
0.2 ng/mL (pink cap tube)
0.6 ng/mL (purple cap tube)
1.5 ng/mL (blue cap tube)
3. Antibody #1: 6 mL
4. HRP-Conjugated Antibody #2: 6 mL
5. 20X Wash Solution: 30 mL
6. 10 X Sample Dilution Buffer: 25mL
7. Stop Buffer: 12 mL

8. TMB Substrate: 12 mL

Materials Required But Not Supplied

1. Microtiter plate reader (450 nm)
2. Incubator
3. Tissue Mixer (e.g. Omni TissueMaster Homogenizer)
4. Vortex mixer (e.g. Gneie Vortex mixer from VWR)
5. 10, 20, 100 and 1000µL pipettes
6. Multi-channel pipette: 50-300 µL (Optional)
7. Methanol

Storage

Store the kit at 2-8°C. The shelf life is 12 months when the kit is properly stored.

Specimen Collection And Preparation

Be sure samples are properly stored. In general, samples should be refrigerated at 2-4°C for no more than 1-2 days. Freeze samples to a minimum of -20°C if they need to be stored for a longer period. Frozen samples can be thawed at room temps (20 – 25°C / 68 – 77°F) or in a refrigerator before use.

1. Preparation of 1X Wash Solution:

Mix 1 volume of 20X Wash Buffer concentrate with 19 volumes of distilled water.

2. Preparation of 70% Methanol:

Mix 7 volumes of 100% Methanol with 3 volumes of distilled water.

3. Preparation of Solution A:

Mix 3 volumes of 100% Methanol with 7 volumes of 1X Wash Solution.

4. Preparation of 1X Sample Dilution Buffer:

Mix 1 volume of 10X Sample Dilution Buffer with 9 volumes of distilled water.

5. Preparation of 30% Methanol:

Mix 3 volumes of 100% Methanol with 7 volumes of distilled water.

When preparing solutions, vortex vigorously for at least one minute in an air-tight container.

Milk

- 1) To 1 mL of milk, add 1 mL of Solution A.
- 2) Vortex vigorously for 1 minute.
- 3) Centrifuge for 5 minutes at 4,000 x g at room temperature (20 – 25°C / 68 – 77°F).
- 4) Use 50 µL of the sample per well for the assay, avoiding any fat that may have formed on top of the sample.

Note: Dilution factor = 2

Milk Powder/ Enzyme

- 1) To 1 g of milk powder in a vial, add 2mL of 70% Methanol. Vortex vigorously for 5 minutes to obtain a homogeneous suspension.
- 2) Centrifuge the samples for 5 minutes at 4,000 x g at room temperature (20 – 25°C / 68 – 77°F).
- 3) Dilute 200 µL of the obtained supernatant with 300 µL of distilled water.

Vortex for 1 minute.

- 4) Use 50 µL of the diluted sample per well in the test.

Note: Dilution factor = 5

Cereal, Feed, Seed, Cookies

- 1) Grind and mix a representative sample (according to accepted sampling techniques).
- 2) Weigh out 1 g of the ground sample and place into a suitable container.
- 3) Add 10 mL of 70% Methanol and vortex vigorously for 3 minutes manually or 10 minutes using a multi-vortexer.
- 4) Centrifuge the samples for 5 minutes at 4,000 x g at room temperature (20 – 25°C / 68 – 77°F). Dilute 200 µL of the obtained supernatant with 800 µL of 1X Sample Dilution Buffer. Vortex for 1 minute.
- 5) Use 50 µL of the diluted sample per well in the test.

Note: Dilution Factor = 50

**The sample size may be increased if required, but the volume of methanol/water must be changed accordingly, for example, if a 10 g sample is to be used, 100 mL of 70% methanol should be used. If the Aflatoxin B1 concentration is expected to be high, the sample can be further diluted with 14% Methanol in 1X Sample Dilution Buffer.*

Vegatable/bean/peanut/oils

- 1) Weigh out 1g of the oil sample, add 5 ml of 30% Methanol
- 2) Vortex vigorously for 2 minutes at maximum speed manually, then add 3 ml of n-hexane and Vortex vigorously for 2 minutes at maximum speed manually.
- 3) Centrifuge the samples for 5 minutes at 4,000 x g at room temperature (20 – 25°C / 68 – 77°F).
- 4) Dilute 500 µL of the lower aqueous layer with 500 µL of 1X Sample Dilution Buffer. Vortex for 1 minute.
- 5) Use 50 µL of the diluted sample per well in the test.

Note: Dilution factor: 10

Soy sauce/vinegar

- 1) Weigh out 1mL of the ground sample and place into a suitable container.
- 2) Add 4mL of 70% Methanol and vortex vigorously for 3 minutes manually or 10 minutes using a multi-vortexer.
- 3) Centrifuge the samples for 5 minutes at 4,000 x g at room temperature (20 – 25°C / 68 – 77°F). Dilute 200 µL of the obtained supernatant with 800 µL of distilled water. Vortex for 1 minute, test the pH, the acid is adjusted to 6.8-7.2 with 1M sodium hydroxide; the alkaline is adjusted to 6.8-7.2 with 1M hydrochloric acid.
- 4) Use 50 µL of the diluted sample per well in the test.

Note: Dilution Factor = 25

Chili powder

- 1) Weigh out 1mL of the ground sample and place into a suitable container.
- 2) Add 5mL of 70% Methanol and vortex vigorously for 3 minutes manually or 10 minutes using a multi-vortexer.
- 3) Centrifuge the samples for 5 minutes at 4,000 x g at room temperature (20 – 25°C / 68 – 77°F). Dilute 200 µL of the obtained supernatant with 800 µL of 1X Sample Dilution Buffer. Vortex for 1 minute.
- 4) Use 50 µL of the diluted sample per well in the test.

Note: Dilution Factor = 25

Reagent Preparation

IMPORTANT: All reagents should be brought up to room temperature before use (1 – 2 hours at 20 – 25°C / 68 – 77°F); Make sure you read "Precautions" before use. Solutions should be prepared just prior to ELISA test. All reagents should be mixed by gently inverting or swirling prior to use. Prepare volumes that are needed for the number of wells being run. Do not return the reagents to the original stock tubes/bottles. Using disposable reservoirs when handling reagents can minimize the risk of contamination and is recommended.

1. Preparation of 1X Wash Solution

Mix 1 volume of 20X Wash Buffer concentrate with 19 volumes of distilled water.

Assay Procedure

Label the individual strips that will be used and aliquot reagents as the following example:

Component	Volume per Reaction	24 Reactions
Antibody #1	50 µL	1.2 mL
HRP-Conjugated Antibody #2	50 µL	1.2 mL
1X Wash Solution	1.0 mL	24mL
Stop Buffer	100 µL	2.4 mL
TMB Substrate	100 µL	2.4 mL

1. Add 50 µL of each Aflatoxin B1 Standards in duplicate into different wells (Add standards to plate only in the order from low concentration to high concentration).
2. Add 50 µL of each sample in duplicate into different sample wells.
3. Add 50 µL HRP-Conjugated Antibody #2 to each well .
4. Add 50 µL Antibody #1 to each well and mix well by gently rocking the plate manually for 1 minute.
5. Incubate the plate for 15 minutes at room temperature (20 – 25°C / 68 – 77°F).
6. Wash the plate 4 times with 250 µL of 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels (Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps).
7. Add 100 µL of TMB substrate. Time the reaction immediately after adding the substrate. Mix the solution by gently rocking the plate manually for 1 minute while incubating. (Do not put any substrate back to the original container to avoid any potential contamination. Any substrate solution exhibiting coloration is indicative of deterioration and should be discarded. Covering the microtiter plate while incubating is recommended).

- After incubation for 10 minutes in room temperature, add 100 µL of Stop Buffer to stop the enzyme reaction.
- Read the plate as soon as possible following the addition of Stop Buffer on a plate reader with 450 nm wavelength (Before reading, use a lint-free wipe on the bottom of the plate to ensure no moisture or fingerprints interfere with the readings).

Calculation

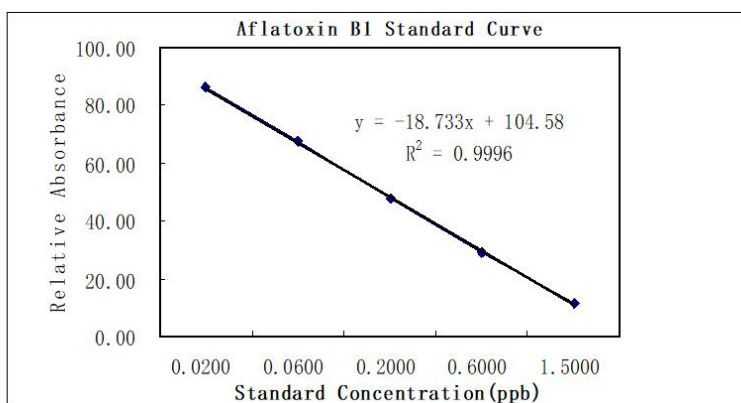
A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/mL on a logarithmic curve.

$$\text{Relative absorbance (\%)} = \frac{\text{absorbance standard (or sample)} \times 100}{\text{absorbance zero standard}}$$

When use computing software, recommends Log/Log standard curves for each sample to determine the corresponding concentration of the tested drug in ng/mL.

Typical Standard Curve

The following figure is a typical Aflatoxin B1 standard curve



Sensitivity

Analytes	Cross-Reactivity (%)
Aflatoxin B1	100.0
Aflatoxin B2	49
Aflatoxin G1	37
Aflatoxin G2	8

Specificity

Precautions

- The standards contain Aflatoxin B1 . Handle with particular care.
- Do not use the kit past the expiration date.

3. Do not intermix reagents from different kits or lots except for components with the same part No's within their expiration dates.
4. Try to maintain a laboratory temperature of 20–25°C (68°–77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation.
5. Make sure you are using only distilled or deionized water since water quality is very important.
6. When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.
7. Incubations of assay plates should be timed as precisely as possible. Be consistent when adding standards to the assay plate. Add your standards first and then your samples.
8. Add standards to plate only in the order from low concentration to high concentration as this will minimize the risk of compromising the standard curve.
9. Always refrigerate plates in sealed bags with a desiccant to maintain stability. Prevent condensation from forming on plates by allowing them equilibrate to room temperature (20 – 25°C / 68 – 77°F) while in the packaging.

