



Total Aflatoxin(AFT) ELISA Kit

Catalog Number. CSB-E09923

This immunoassay kit allows for the in vitro quantitative determination of Aflatoxin concentrations in maize, corn, feed, edible oil.

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

If You Have Problems

Technical Service Contact information

Phone: 86-27-87582341

Fax: 86-27-87196150

Email: tech@cusabio.com

Web: www.cusabio.com

In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. The coupling antigen is pre-coated on the micro-well stripes. The Aflatoxin in the testing sample competes with the coupling antigens pre-coated on the micro-well stripes for the antibodies against Aflatoxin. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the testing sample has a negative correlation with the content of Aflatoxin in it. This value is compared to the standard curve and the content of the corresponding Aflatoxin is subsequently obtained.

DETECTION RANGE

0.1 ppb-8.1 ppb.

SENSITIVITY

The minimum detectable dose of the kit is typically less than 0.1 ppb.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest concentration that could be differentiated from zero. It was determined the mean O.D value of 20 replicates of the zero standard added by their three standard deviations.

Cross-reaction rate

Aflatoxin B2	100%
Aflatoxin B1	97%
Aflatoxin G1	98%
Aflatoxin G2	101%

Recovery rate

Peanuts	95% \pm 15%
Animal feed	85% \pm 15%
Edible oil	95 \pm 15%
Corn	90 \pm 15%

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

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
- 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
Assay plate (96 tests)	1
Standard	6 x 1 ml
HRP-conjugate	1 x 9 ml
Antibody	1 x 6 ml
Substrate A	1 x 9 ml
Substrate B	1 x 9 ml
Stop Solution	1 x 6 ml
Wash Buffer(10×concentrate)	1 x 40 ml

STANDARD CONCENTRATION

Standard	S0	S1	S2	S3	S4	S5
Concentration (ppb)	0	0.1	0.3	0.9	2.7	8.1

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100 ml and 500 ml graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.
- swashing ear ball
- 100ml flask rapid qualitative filter paper
- N-hexane
- mineral ether
- Methanol

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 (18-25°C) before use for 30min.
- Distilled water is recommended to be used to make the preparation for reagents or samples. Contaminated water or container for reagent preparation will influence the detection result.

REAGENT PREPARATION

1. **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 40 ml of Wash Buffer Concentrate (10 x) into deionized or distilled water to prepare 400 ml of Wash Buffer (1 x) (or just to the required volume) for use. Wash Buffer can be in good condition for 30 days in 4°C.

2. **Solution One**

Dilute Methanol with deionized water at a volume ratio of 3:2, namely 3 volume of Methanol plus 2 volume of deionized water.

3. **Solution Two**

Dilute Methanol with deionized water at a volume ratio of 7:3, namely 7 volume of Methanol plus 3 volume of deionized water.

Note:

1. CUSABIO 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.
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: Peanut

- (1) Take 10g crushed sample, add 30ml Solution One.
- (2) Shake for 5min.
- (3) Centrifuge at 3500r/min for 5min.
- (4) Dilute with deionized water(1:5). Take this solution for further analysis.
Fold of dilution of the samples: 18

B: Corn, feed

- (5) Take 3g crushed sample, add 15ml Solution Two.
- (6) Shake for 10 min.
- (7) Centrifuge at 3500r/min for 5 min.
- (8) Dilute with deionized water(1:6). Take this solution for further analysis.
Fold of dilution of the samples: 35

C: Edible oil

- (1) Take 5g sample.

- (2) Use 10ml N-hexane or mineral ether to transfer the sample to 125ml separatory funnel.
- (3) Add 15ml Solution One.
- (4) Shake for 5 min. Allow to stand to separate into two layers.
- (5) Remove the lower layer.
- (6) Dilute with deionized water(1:5). Take this solution for further analysis.
Fold of dilution of the samples: 18

ASSAY PROCEDURE

Bring all reagents and samples to room temperature 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 4°C.
3. Add 30µl of **Standard** or **Sample** per well. Standard and Samples need test in duplicate.
4. Add 70µl of **HRP-conjugate** to each well. Then 50µl **Antibody** to each well. Mix well and then incubate for 20 min at room temperature.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with **Wash Buffer** (300µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 30 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 75µl of **Substrate A** and 75µl of **Substrate B** to each well, mix well. Incubate for 15-20 minutes at room temperature. Keeping the plate away from drafts and other temperature fluctuations in the dark.

7. Add 50 μ l of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
8. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm.

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 minutes. 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 minutes). 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

Using the professional soft "Curve Expert 1.3" to make a standard curve is recommended, which can be downloaded from our web.

- (1) 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 —absorbance standard (or sample)

B₀ —absorbance zero standard

- (2) To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the Aflatoxin standards solution (ppb) as x-axis.
The Aflatoxin concentration of each sample (ppb), 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.