Total Aflatoxins ELISA Kit

Catalog Number. EKC40002

This immunoassay kit allows for the in vitro quantitative determination of total Aflatoxins concentrations in rice, corn, millet, soy sauce, vinegar, edible oil(rapeseed oil, sesame oil, salad oil, peanut oil), liquor(wine, beer, rice wine), peanuts, moon cakes, walnut cakes, peanut buffer, flour, feed(including feedstuff), feed(formula/concentrated feed), milk(including fresh milk, milk powder, cream).

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

The manual may be updated as a result of continuous improvements.
Please refer to hard copy manual included in the kit for your experiment.

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 inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with Aflatoxins antigen. Standards or samples are added to the appropriate microtiter plate wells with an Aflatoxins specific antibody and Horseradish Peroxidase (HRP) conjugated anti-antibody. The competitive inhibition reaction is launched

between pre-coated Aflatoxins and Aflatoxins in standards or samples with the Aflatoxins special antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of Aflatoxins in the standards or samples. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

0.025 ppb-2.025 ppb.

SENSITIVITY

The minimum detectable dose of the kit is typically less than 0.025 ppb.

The sensitivity of this assay, or Lower Limit of Detection (LOD) 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

Total Aflatoxins	100%

RECOVERY RATE

Feed	75±10%
Food	85±10%

LIMIT OF DETECTION

Rice, corn, millet	2.5 ppb
Soy sauce, vinegar	0.5 ppb
Edible oil(rapeseed oil, sesame oil, salad oil, peanut oil)	2.5 ppb

Liquor(wine, beer, rice wine)	0.5 ppb
Peanuts	2.5 ppb
Moon cakes, walnut cakes, peanut buffer	1 ppb
Flour	1 ppb
Feed(including feedstuff)	2.5 ppb
Feed(formula/concentrated feed)	1 ppb
Milk(including milk powder, cream)	0.5 ppb

PRECISION

Intra-assay Precision (Precision within an assay): CV%<15%
Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<15%
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 on the kit label.
- 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(96T)
Assay plate	1
Standard	6 x 1 mL
HRP-conjugate	1 x 12 mL
Antibody	1 x 7 mL
Substrate A	1 x 7 mL
Substrate B	1 x 7 mL
Stop Solution	1 x 7 mL
Wash Buffer(20×)	1 x 40 mL
Redissolving Solution(2×)	1 x 50 mL
Adhesive Strip	4
Instruction Manual	1

STANDARD CONCENTRATION

Standard	S0	S1	S2	S3	S4	S5
Concentration (ppb)	0	0.025	0.075	0.225	0.675	2.025

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 one month at 2 - 8° C.

***Provided this is within the expiration date of the kit.**

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- An incubator which can provide stable incubation conditions up to 25°C±0.5°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- Analytical balance,2 decimal place
- Rotary evaporator or nitrogen gas
- Centrifuge, Vortex mixer
- 50 mL and 500 mL graduated cylinders.
- Single-channel micropipette(20 μL-200 μL、100 μL-1000 μL)
- 250 μL multichannel micropipette
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.
- Filter paper
- Methanol
- Trichloromethane
- N-hexane

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 or samples. 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 15 mL of **Wash Buffer (20x)** into 285 mL deionized or distilled water to prepare 300 mL of **Wash Buffer (1x).** Keep it at 4℃ for one month.
- **Redissolving Solution**: Dilute 1 mL of **Redissolving Solution(2x)** into 1 mL deionized or distilled water to prepare 2 mL of **Redissolving Solution**.
- **50% Methanol:** Dilute **Methanol** with deionized or distilled water at a volume ratio of 1:1, namely 50 mL of **Methanol** plus 50 mL deionized or distilled water.

Note:

1. Biomatik 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

For food (from A to G)

A. Low fat food(rice, corn, millet)

1. Weigh 1.00±0.05g of the homogenized sample.

2. Add 10 mL of **50% Methanol** and shake strongly for 5 min
3. Collect the solution through filter paper and stand for 10 min
4. Transfer 50 μL of supernatant into a new centrifugal tube, add 450 μL of **Redissolving Solution(1x)**, shake well for 30s.
5. Take 50 μL of sample for further analysis.
   Dilution factor of the samples: 100

B. Soy sauce, vinegar

1. Weigh 2.00±0.05g of the sample, add 3 mL deionized or distilled water and shake well.
2. Add 10 mL of **Trichloromethane** and shake properly for 5 min.
3. Centrifuge at more than 4000 rpm for 10 min at room temperature
4. Transfer 5 mL of under layer and the sample can be dried by blowing nitrogen gas 50°C.
5. Add 1 mL of **50% Methanol** and shake well.
6. Transfer 50 μL of solution into a new centrifugal tube, add 950 μL of **Redissolving Solution(1x)**, shake well for 30s.
7. Take 50 μL of sample for further analysis.
   Dilution factor of the samples: 20

C. Edible oil(rapeseed oil, sesame oil, salad oil, peanut oil)

1. Weigh 1.00±0.05g of the sample.
2. Add 2 mL of **N-hexane** and 5 mL of **50% Methanol**, shake strongly for 5 min.
3. Centrifuge at more than 4000 rpm for 10 min at room temperature
4. Transfer 50 μL of under layer into a new centrifugal tube, add 200 μL of **Redissolving Solution(1x)**, shake well for 30s.
5. Take 50 μL of sample for further analysis.
   Dilution factor of the samples: 25

D. Liquor(wine, beer, rice wine)

1. Weigh 2.00±0.05g of the sample and add 10 mL of **Trichloromethane** and shake properly for 5 min.
2. Centrifuge at more than 4000 rpm for 10 min at room temperature
3. Transfer 5 mL of under layer and the sample can be dried by blowing nitrogen gas 50°C.
4. Add 1 mL of **50% Methanol** and shake well.
5. Transfer 50 μL of solution into a new centrifugal tube, add 950 μL of **Redissolving Solution(1x)**, shake well for 30s.
6. Take 50 μL of sample for further analysis.

Dilution factor of the samples: 20

E. **Peanuts**

1. Weigh 1.00±0.05g of the homogenized sample and 5 mL of **N-hexane** and 10 mL of **50% Methanol**, shake strongly for 5 min
2. Centrifuge at more than 4000 rpm for 10 min at room temperature
3. Transfer 50 μL of under layer into a new centrifugal tube, add 450 μL of **Redissolving Solution(1x)**, shake well for 30s.
4. Take 50 μL of sample for further analysis.

Dilution factor of the samples: 100

F. **Moon cakes, walnut cakes, peanut buffer**

1. Weigh 2.00±0.05g of the homogenized sample and add 5 mL of **N-hexane** and 10 mL of **50% Methanol**, shake strongly for 5 min
2. Centrifuge at more than 4000 rpm for 10 min at room temperature
3. Transfer 5 mL of under layer and add 10 mL of **Trichloromethane**, shake properly for 5 min.
4. Centrifuge at more than 4000 rpm for 10 min at room temperature
5. Transfer 5 mL of under layer and the sample can be dried by blowing

nitrogen gas 50°C.

6. Add 1 mL of **50% Methanol** and shake well.
7. Transfer 50 μL of solution into a new centrifugal tube, add 950 μL of **Redissolving Solution(1x)**, shake well for 30s.
8. Take 50 μL of sample for further analysis.

Dilution factor of the samples: 40

G. Flour

1. Weigh 2.00±0.05g of the sample and 10 mL of **50% Methanol,** shake strongly for 5 min
2. Collect the solution through filter paper.
3. Transfer 5 mL of solution, add 10 mL of **Trichloromethane**, shake properly for 5 min.
4. Centrifuge at more than 4000 rpm for 10 min at room temperature
5. Transfer 5 mL of under layer and the sample can be dried by blowing nitrogen gas 50°C.
6. Add 1 mL of **50% Methanol** and shake well.
7. Transfer 50 μL of solution into a new centrifugal tube, add 950 μL of **Redissolving Solution(1x)**, shake well for 30s.
8. Take 50 μL of sample for further analysis.

Dilution factor of the samples: 40

For feed (from H to I)

H. Feed(including feedstuff)

1. Weigh 1.00±0.05g of the homogenized sample and 10 mL of **50% Methanol,** shake strongly for 5 min

2. Collect the solution through filter paper and stand for 10 min.
3. Transfer 50 μL of supernatant into a new centrifugal tube, add 450 μL of **Redissolving Solution(1x)**, shake well for 30s.
4. Take 50 μL of sample for further analysis.
   Dilution factor of the samples: 100

I. Feed(formula/concentrated feed)

1. Weigh 2.00±0.05g of the sample and 10 mL of **50% Methanol**, shake strongly for 5 min
2. Collect the solution through filter paper.
3. Transfer 5 mL of solution, add 10 mL of **Trichloromethane**, shake properly for 5 min.
4. Centrifuge at more than 4000 rpm for 10 min at room temperature
5. Transfer 5 mL of under layer and the sample can be dried by blowing nitrogen gas 50°C.
6. Add 1 mL of **50% Methanol** and shake well.
7. Transfer 50 μL of solution into a new centrifugal tube, add 950 μL of **Redissolving Solution(1x)**, shake well for 30s.
8. Take 50 μL of sample for further analysis.
   Dilution factor of the samples: 40

J. Milk

(a) Freah milk:

1. Take 50 μL of milk into a new centrifugal tube, add 950 μL of **Redissolving Solution(1x)**, shake well for 30s.
2. Take 50 μL of sample for further analysis.

Dilution factor of the samples: 20

(b) Milk powder, cream

1. Weigh 5.00±0.05g of the sample and 10 mL of **Methanol**, shake strongly for 5 min
2. Centrifuge at more than 4000 rpm for 10 min at room temperature
3. Transfer 2 mL of supernatant and the sample can be dried by blowing nitrogen gas 50°C.
4. And 1 mL of **N-hexane** and shake well, then add 1 ml of **Redissolving Solution(1x)** shake well for 30s.
5. Centrifuge at more than 4000 rpm for 5 min at room temperature
6. Take 50 µL of under layer for further analysis.

Dilution factor of the samples: 1

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 50 µL of **Standard** or **Sample** per well.
4. Then 50 µL of **Antibody** to each well. Mix well and incubate for 30 min at 25°C.
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** (250 µL) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 15~30 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 100 µL of **HRP-conjugate** to each well, incubate for 30 min at 25°C.
7. Repeat the wash process as step 5.
8. Add 50 µL of **Substrate A** and 50 µL of **Substrate B** to each well, mix well. Incubate for 15 minutes at 25°C. **Protect from light.**
9. Add 50 µL of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm (Recommend to read 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 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

There are two methods to judge the results: the first one (A) is the rough judgment, while the second (B) is the quantitative determination. Note that

the OD value of the sample has a negative correlation with Aflatoxins in the sample.

A:

Compare the sample average absorbance values with standards values, the Aflatoxins concentration in the samples can be concluded. For example, the absorbance value of sample 1 is 0.248, the absorbance value of sample 2 is 1.021; absorbance values of standard are:1.821, 1.434, 1.163, 0.767, 0.298, 0.106 and the corresponding concentration are:0 ppb, 0.025 ppb, 0.075 ppb, 0.225 ppb, 0.675 ppb, 2.025 ppb; then the Aflatoxins in sample 1 and sample 2 are 0.675 ppb-2.025 ppb and 0.075 ppb-0.225 ppb; Lastly the reader is multiplied by the corresponding Dilution factor of each sample and the actual concentration of sample is obtained.

B:

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

B_0 ——the average absorbance value of the 0 ppb standard

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

The Aflatoxins 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.

Note:

- Discard the substrate with any color that indicates the degeneration of this solution; when the absorbance value of standard solution 0 of less than 0.5 indicates its degeneration.
- The optimum reaction temperature is 25℃, and too high or too low will result in the changes in the absorbance value and detecting sensitivity.