Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Aflatoxin B1 (AFB1) ELISA Kit

rev 10/18

(Catalog # K4208-100, 100 assays, Store at 4°C)

I. Introduction:

Aflatoxins are toxic metabolites of the fungi species, such as Aspergillus flavus and Aspergillus parasiticusspergillus. Aflatoxins have a strong carcinogenicity, commonly found in cereal, nuts, cotton seed, human blood and animal feed. Aflatoxin B1 is mainly natural Aflatoxins contamination. Aflatoxins are listed as Category I carcinogen with strong carcinogenicity by the International Agency for Research on Cancer in 1993. Contamination can occur in the field, during growing, harvest, or in processing, storage and transport, detect timely pollution sources is the best way to prevent aflatoxin contamination. This kit is a detection product developed based on competitive ELISA technology, with operation time as short as 50 min and a sensitivity of 0.05 ppb, and linear range from 0.05 ppb to 4.05 ppb.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Aflatoxin B1
Detection Range: 0.15 – 4.05 ppb
Sensitivity: 0.05 ppb
Detection limitation: 0.5 ppb for edible oil, rice, peanuts and corn.

III. Sample Type:

Edible oil, rice, peanuts, corn

IV. Kit Contents:

Components	K4208-100	Part No.
Micro ELISA Plate	8 X 12 Strips	K4208-100-1
Standard	1 ml X 5	K4208-100-2
HRP-conjugate	7 ml	K4208-100-3
Antibody	7 ml	K4208-100-4
TMB substrate	12 ml	K4208-100-5
Stop Solution	10 ml	K4208-100-6
Sample Diluent	50 ml	K4208-100-7
Wash Buffer (10X)	30 ml	K4208-100-8
Plate sealers	4	K4208-100-9

V. User Supplied Reagents and Equipment:

- Chemicals: Methanol, NaCl, N-hexane, Alumina-A
- Microplate reader capable of measuring absorbance at 450 nm
- 25°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes and graduated cylinders for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment. Opened kit may be stable for 1 month at 4°C.

VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Extraction Solution 1:** Add 10 ml of **Sample Diluent** into 190 ml deionized **water**, shake well.
2. **Extraction Solution 2:** Add 30 ml of **Methanol** into 70 ml of **Extraction solution 1**, shake well.
3. **Extraction Solution 3:** Add 70 ml of **Methanol** into 30 mil deionized **water**, shake well.
4. **Extraction Solution 4**: Add 30 ml of **Methanol** into deionized **water**, shake well.
5. **Dilute Solution 1**: Weigh 2 g NaCl to 100 ml deionized water, shake well.
6. **Dilute Solution 2**: Weigh 0.8 g NaCl to 10 ml of Sample Diluent, shake well
7. **Wash Buffer (1X)**: If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals are completely dissolved. Dilute 10 ml of Wash Buffer (10X) into 90 ml deionized water to prepare 100 ml of Wash Buffer (1X). Can be stored at 4°C for one month.
8. **Standards Concentration:**

Standards	S1	S2	S3	S4	S5
Concentration (ppb)	0	0.15	0.45	1.35	4.05

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9. **Sample Preparation:**

Note: The prepared sample maybe stored for up to one day at 2-8°C.

A. Edible oil

1. Take 500 µl of edible oil and add 1 ml of **N-hexane**, shake well.
2. Add 2 ml of **Extraction Solution 2**. Shake well and let it stand for 1 min.
3. Centrifuge at 4000 rpm for 10 min.
4. Transfer 200 µl of under layer into a new centrifugal tube, add 300 µl of **Dilute Solution 1**, and shake well.
5. Take 50 µl of sample for further analysis. (Dilution factor of the samples: 10)

B. Rice and peanuts

1. Weigh 2 g of the homogenized sample
2. Add 10 ml of **Extraction Solution 3** and shake properly for 5 min.
3. Centrifuge at 4000 rpm for 10 min.
4. Transfer 100 µl of supernatant into a new centrifugal tube, add 100 µl of **Sample Diluent**, and shake well.
5. Take 50 µl of sample for further analysis. (Dilution factor of the samples: 10)

C. Corn

1. Weigh 2 g of the homogenized sample and add 1g **Alumina-A**.
2. Add 10 ml of **Extraction Solution 4** and shake properly for 5 min.
3. Centrifuge at 4000 rpm for 10 min.
4. Transfer 100 µl of supernatant into a new centrifugal tube, add 100 µl of **Dilute Solution 2**, and shake well.
5. Take 50 µl of sample for further analysis. (Dilution factor of the samples: 10)

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Add 50 µl of **Standard** or **Sample** per well. Then add 50 µl of **HRP-conjugate** to each well and 50 µl of **Antibody** to each well. Cover the microtiter plate with a new adhesive strip and mix well, then incubate for 30 min at 25°C.
3. Aspirate each well and wash, repeating the process 4 times. Wash by filling each well with 250 µl of **Wash Buffer** 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.
4. Add 100 µl of **TMB Substrate** to each well, mix well. Incubate for 15 minutes at 25°C. Protect from light.
5. Add 50 µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
6. Read result at 450 nm within 10 minutes.

IX. CALCULATION:

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.

$$\textbf{Absorbance Value (\%)} = B/B_0 \times 100\%$$

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 absorbency value of standards as y-axis, logarithmic of the concentration of the Fluoroquinolones standards solution (ppb) as x-axis. The Fluoroquinolones 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.

X. RELATED PRODUCTS:

- AKR7A2 Antibody (NT) (Cat. No. K6741-100)
- Human Recombinant AKR7A2 (Cat. No. 6335-100)
- Human Recombinant AKR7A3 (Cat. No. 6334-50)
- Fluoroquinolones ELISA kit (Cat. No. K4205-100)
- Gentamicin ELISA kit (Cat. No. K4206-100)
- Sulfonamides residue ELISA kit (Cat. No. K4207-100)