ab285282 – Total Aflatoxin ELISA Kit

For the Quantitative measurement of Total Aflatoxin in grain and feed samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285282

Storage and Stability

Please store the opened kit at 4°C, protect from light and moisture and use within 2 months.

Materials Supplied

Item	Quantity	Storage Condition
ELISA Microplate	96 wells	4°C
Standards (S1 - S6)	1 ml X 6	4°C
HRP Conjugate	5.5 ml	4°C
Antibody Working Solution	5.5 ml	4°C
Substrate Reagent A	6 ml	4°C
Substrate Reagent B	6 ml	4°C
Stop Solution	6 ml	4°C
20X Wash Buffer	40 ml	4°C
Plate Sealer	3	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 70% Methanol.
- Deionized or distilled water.
- Microplate reader with 450 nm wavelength filter

Reagent Preparation

- Restore all reagents and samples to room temperature before use.
- All the reagents should be mixed thoroughly by gentle mixing before pipetting. Avoid bubble formation.

Wash Buffer: Dilute 20x Concentrated Wash Buffer with deionized water. Mix 1 ml of 20x Concentrated wash Buffer in 19 ml Deionized water to prepare 20 ml of 1X wash buffer (1:19).

Standard Concentration

Standards	S1	S2	S3	S4	S5	S6
Concentration ppb)	0	0.02	0.04	0.08	0.16	0.32

Sample Preparation

Pre-treatment of beer: Stir beer thoroughly to remove CO2, take 2 mL of beer sample and add 1 mL of deionized water, then add 7 mL methanol, oscillate for 5 min. Take 0.5 mL of mixed sample liquid and add 0.5 mL of deionized water, mix well. Take 50 µL for detection and analysis. Note: Sample dilution factor: 10, minimum detection dose: 0.2 ppb

Pre-treatment of Sauce/Wheat/Barley feed: Weigh 2 g of crushed homogenate into the 50 mL EP tube, add 10 mL of 70% methanol, oscillate for 5 min, centrifuge at 4000 rpm for 10 min at room temperature. Take 2 mL of supernatant, add 4 mL of trichloromethane or dichloromethane, shake for 5 min, centrifuge at 4000 rpm for 10 min at room temperature. Take the upper phase liquid to another tube; keep the lower phase liquid for use (lower liquid A). Add 4 mL of trichloromethane or dichloromethane to the upper liquid, shake well for 5 min, centrifuge at 4000 rpm for 10 min at room temperature. Discard the upper liquid and keep the lower liquid (lower liquid B) (4) Mix lower liquid A and lower liquid B thoroughly. Take 2 mL of mixed lower liquid and blow-dry with nitrogen evaporators/water bath at 50-60°C. Add 0.5 mL of 70% methanol to dissolve thoroughly, add 0.5 mL of deionized water, mix fully. Take 50 µL for detection and analysis. Note: Sample dilution factor: 10, minimum detection dose: 0.2 ppb.

Pre-treatment of formula feed: Weigh 2 g of crushed homogenate into the 50 mL tube, add 10 mL 70% of methanol, shake for 5 min, centrifuge at 4000 rpm for 10 min at room temperature. Take 0.5 mL of supernatant, add 0.5 mL of deionized water, mix well. Take 50 µL for analysis. Note: Sample dilution factor: 10, minimum detection dose: 0.2 ppb (If aflatoxin content is higher in the sample, take the mixed liquid from step 2, diluted with 35% methanol, the sample dilution multiple is the actual dilution multiple in that case. For example: take the mixed liquid from step 2, diluted 10 times with 35% of methanol, the actual dilution multiple is 10×10=100, detection limit: 2 ppb).

Pre-treatment of grain: Weigh 2 g of crushed homogenate into the 50 ml tube, add 5 mL of 70% methanol, shake for 5 min, centrifuge at 4000 rpm for 10 min at room temperature. Take 0.5 mL of supernatant, add 0.5 mL of deionized water, mix well. Take 50 µL for detection and analysis. Note: Sample dilution factor: 5, minimum detection dose: 0.1 ppb

Pre-treatment of edible oil, peanut, high fat formula feed: Weigh 2 g of crushed homogenate into the 50 mL tube, add 8 mL of N-hexane and 10 mL 70% of methanol, oscillate for 5 min, centrifuge at 4000 rpm for 10min at room temperature. Discard the upper liquid, and take 0.5 mL of lower liquid, add 0.5 mL of deionized water, mix well. Take 50 µL for detection and analysis. Note: Sample dilution factor: 10, minimum detection dose: 0.2 ppb.

Pre-treatment of wine, soy sauce, vinegar: Take 2 mL of sample and add 2 mL of deionized water, then add 10 mL of trichloromethane or dichloromethane, shake for 5 min, centrifuge at 4000 rpm for 10 min at room temperature. Take 1 mL of lower liquid and blow-dry with nitrogen evaporators/water bath at 50-60°C. Add 0.5 mL of 70% methanol to dissolve thoroughly, add 0.5 mL of deionized water, and mix well. Take 50 µL for detection and analysis. Note: Sample dilution factor: 5, minimum detection dose: 0.1 ppb.

Assay Protocol

- Restore all reagents and samples to room temperature before use.
- All the reagents should be mixed thoroughly by gentle mixing before pipetting. Avoid bubble formation.
- It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Add 50 µL of standard or sample per well, then add 50 µL of HRP Conjugate to each well, then add 50 uL of antibody working solution, cover the plate sealer, shake for 5 sec gently to mix thoroughly, incubate in dark for 30 min at 25°C.
2. Remove the sealer carefully. Aspirate the liquid. Immediately add 300 µL of wash buffer (1x) to each well and wash. Repeat wash procedure for 5 times, 30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to remove them).
3. Add 50 µL of substrate reagent A to each well, and then add 50 µL of substrate reagent B. Gently mix for 5 s to mix thoroughly. Incubate at 25°C for 15 min in dark.
4. Add 50 µL of stop solution to each well, shake the plate gently to mix thoroughly.

5. Determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. Read the plate within 5 min after adding stop solution.

Calculation:

1. Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot.
2. Add average absorbance value of sample to standard curve to get corresponding concentration.
3. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor

   Absorbance (%) = A/A0 x 100

A: Average absorbance of standard or samples
A0: Average absorbance of 0 ppb Standard

Technical Support

For all technical or commercial enquiries please go to:

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