Aflatoxin ELISA kit

Cat#: orb59528 (ELISA Manual)

General description

The ELISA Sreening Kit is a competitive enzyme-labeled immunoassay for the quantitation of aflatoxins in nuts and grain products. The test principle is based on a direct competitive enzyme-linked immunosorbent assay (dc ELISA). In the assay, Aflatoxin in the sample is extracted with methanol/water, and then competes with aflatoxin-HRP enzyme conjugates for a limited amount of antibody which has been coated on the test wells.

Materials and reagents supplied

1. One microtiter plate containing 6 strips of 16 test wells coated with polyclonal antibody specific to Aflatoxins
2. One vial of 【Negativecontrol】, 1.5 mL
3. One vial each of 0.02, 0.1, 0.25, 1.0, and 5.0ppb Aflatoxin B1 standard(calibrator), 1.5 mL
4. One vial of【HRP conjugate】, 6 mL
5. One vial of 【Substrate】, 12mL
6. One vial of 【Stop solution】, 12mL
7. One vial of 【10 x Wash buffer】, 30mL

Additional materials not provided

1. 10 ml syringes
2. 0.45 micron, non-sterile filter units
3. Pipette and centrifuge tubes
4. Marking pen
5. Tape or Parafilm
6. Distilled water or tap
7. Microtiter plate reader or strips reader
8. Absorbent paper towels
9. Timer
10. Extraction buffer: MeOH, ACS grade
11. Calculator (Optional)
12. A multi-channel pipette (Optional)
13. Orbital shaker (Optional)
14. Solid phase extraction devices (Optional)
15. Microtiter plate washer (Optional)

Extraction solution preparation

1. Measure 30 mL of distilled or deionized water for each 100mL being prepared and transfer to a clean glass container with tight-fitting lid.
2. Measure 70 mL of MeOH for each 100 mL being prepared and add to the container.
3. Mix completely. Store tightly sealed to minimize evaporation.

Sample preparation

1. Weigh out 25 g grain and grind it into powder with a food blender or coffee grinder
2. Add 50 mL extraction buffer to 25 g powder (1: 2 dilution)
3. Blend the mixture for 3 minutes in a high speed blender
4. Filter a minimum of 2mL through a filter paper
5. Take 1mL of clear supernatant solution (or filtrate) and add 9 mL of distilled water (1: 10dilution). Mix thoroughly
6. Use this diluted solution in the ELISA (Because of the 1:20 dilution of the grain sample in the extraction solution, the sample toxin concentration calculated from the standard curve should multiple 20 folds to get the toxin concentration in the sample)

Assay Procedure

1. Format the microplate's wells for each standard or sample to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag and stored at 2-8oC.
2. Wash buffer preparation Dilute 10 x washing buffer with distilled water to make 1 x washing buffer by 1:9 ratio. Check if the 10X Wash buffer is at room temperature and the crystal at the bottom is dissolved thoroughly before use.
3. Add 50 ?L of Negative control and standard solution (0.02, 0.1, 0.25, 1.0, and 5.0ppb) or 50 ?Lof each sample into the assigned well.
8. Add 50 ?L of Afla-HRP enzyme conjugate solution (HRP conjugate) to all wells. Swirl the plate gently to mix the content thoroughly. Now the volume of each well is 100 ?L.
9. Incubate 15minutes at room temperature (25-37oC) under dark.
10. Remove liquid from all wells.
11. Flood the wells with at least 300 –350 ?L of 1 x washing buffer, and then decant the liquid from all wells.
12. Repeat the step 7 at least three times.
13. Invert and gently pat the plate on absorbent paper towels to remove remaining solution in wells .
14. Add 100 ?L of substrate solution to each well and shake gently.
15. Incubate 5minutes at room temperature (25-37oC) under dark. Blue color develops in the wells with Negative control.
16. Add 100 ?L of stop solution to each well and mix gently (Now the volume of each well is 200 ?L). Solution will turn from blue into yellow immediately.
17. Read color at OD 450 nm in an ELISA reader within 3-15 minutes after adding the stop solution.

Example of typical plate setup.

	1	2	3	4	5	6	7	8	9	10	11	12
A			S1	S1	S9	S9						
B	NC	NC	S2	S2	S10	S10						
C	C1	C1	S3	S3								
D	C2	C2	S4	S4								
E	C3	C3	S5	S5								
F	C4	C4	S6	S6								
G	C5	C5	S7	S7								
H			S8	S8								

NC: Negative Control
C1= 0.02ppb Aflatoxin B1standard
C2= 0.1ppb Aflatoxin B1standard
C3= 0.25ppb Aflatoxin B1standard
C4= 1.0 ppb Aflatoxin B1standard
C5= 5.0 ppb Aflatoxin B1standard
S1, S2, S3, S4, S5 etc.=Samples

Calculating Results

1. After reading the wells, average the OD of each set of calibrators and samples, and calculate the B/B0%as follows:

$$B/B_0\% = \frac{\text{average OD of Calibrator or sample}}{\text{average OD of Negative Control}} \times 100$$

2. The %B0calculation is used to equalize different runs of assay. While the raw OD values of Negative Control, Calibrators, and samples may different from run to run, the %B0relationship of calibrators and samples to Negative Control should remain fairly constant.

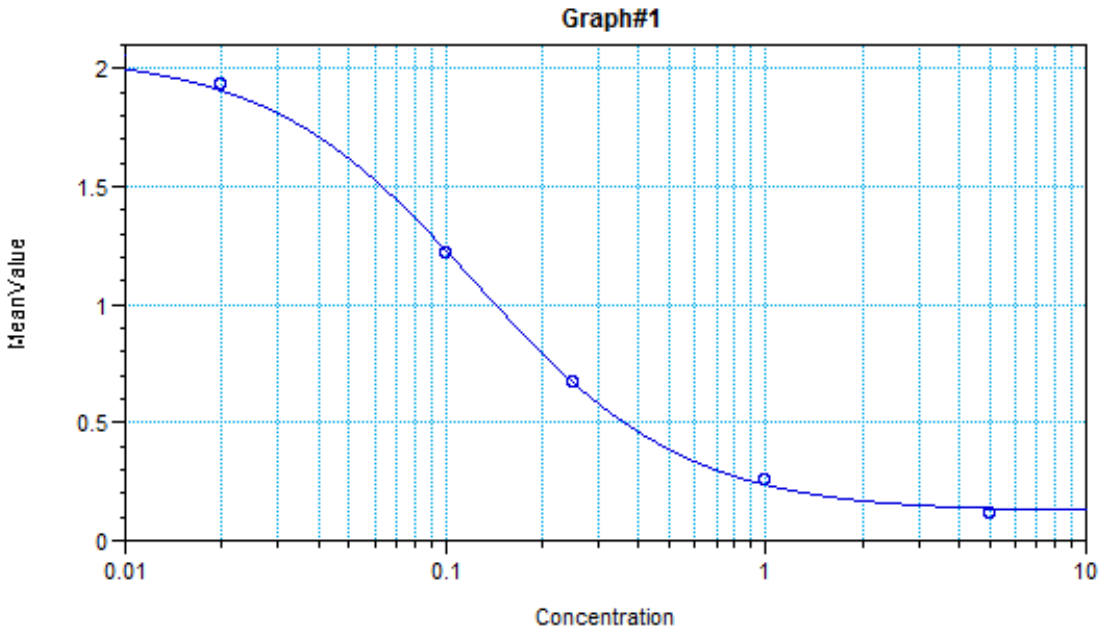
3. 2. Graph the B/B0%of each Calibrator against its Aflatoxin concentration on a semi-log scale
4. Determine the Aflatoxin concentration of each sample by finding its B/B0value and the corresponding concentration level on the graph.
5. Interpolation of sample concentration is only valid if the B/B0%of sample falls within the range of the B/B0%‘s set by calibrators.
6. Because of the 1:20 dilution of the grain sample in the extraction solution, the sample toxin concentration calculated from the standard curve should multiple 20 folds to get the toxin concentration in the sample

Limit of detection

The detection limit for this assay based on Aflatoxin B1is 0.005ppb (ng/mL).

Performance data

1. Aflatoxin B1 standard curve



2. Cross -reactivity

Compound	50% B/Bo (ppb)	Cross-Reactivity (%)
Aflatoxin B1	0.15	100
Aflatoxin B2	0.33	45
AflatoxinG1	0.28	54
AflatoxinG2	1.87	8

Precision

Well Contents	%CV(n=7)
Negative Control	3.2
0.02ppb Calibrator	5.6
0.1ppb Calibrator	0.6
0.25ppb Calibrator	8.1
1.0ppb Calibrator	7.6
5.0 ppb Calibrator	6.1