UC Biodevices

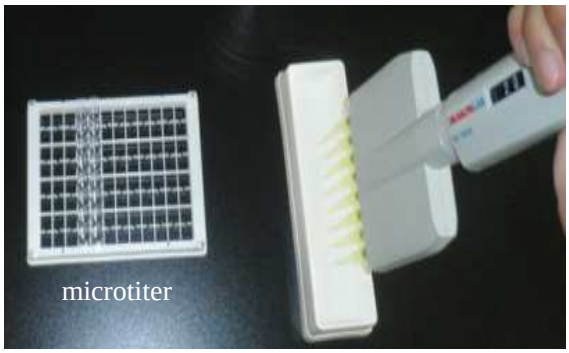
ELISA Kit for Detection of Aflatoxin B1

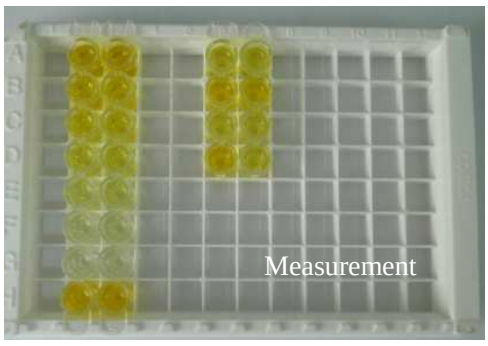
(Product Number: 5501E101)

（Aflatoxin is a class Ⅰ carcinogenic substance）

INSTRUCTION MANUAL

(v. 1.00)





1. Introduction

Aflatoxin is a naturally occurring mycotoxin produced by two types of mold: *Aspergillus flavus* and *Aspergillus parasiticus*. *Aspergillus flavus* is common and widespread in nature and is most often found when certain grains are grown under stressful conditions such as drought. The mold occurs in soil, decaying vegetation, hay, and grains undergoing microbiological deterioration and invades all types of organic substrates whenever and wherever the conditions are favorable for its growth. Favorable conditions include high moisture content and high temperature. While the presence of *Aspergillus flavus* does not always indicate harmful levels of aflatoxin it does mean that the potential for aflatoxin production is present.

This manual establishes procedures for determining aflatoxin in grain and processed grain products. The test is a competitive direct ELISA that provides exact concentrations in parts per billion (ppb). Free toxin in the sample and controls competes with enzyme-labeled toxin (conjugate) for the antibody binding sites. After a wash step, substrate is added to react with the bound enzyme conjugate to produce blue color.

Assay Sensitivity, Precision, Accuracy

1) Sensitivity: 0.25ppb
2) Precision：<20%
3) Accuracy：70％-120％ recovery rate

2. Kit Contents

1) Microtiter plate (8 wells×12) precoated with antibodies to mouse IgG.
2) 6×aflatoxin B1 standard solutions (1ml/each):0ng/ml, 0.25ng/ml, 0.5ng/ml, 1.0ng/ml, 2.0ng/ml, 4.0ng/ml, 8.0ng/ml

   Controls provided: 0ppb, 0.25ppb, 0.5ppb, 1.0ppb, 2.0ppb, 4.0ppb and 8.0ppb
3) Antibody cross-reactivity(80×): Total aflatoxins B1 (0.2ml)
4) Enzyme Conjugate(80×) (0.2ml)
5) washing buffer(10×) (50ml)
6) Diluent buffer A (40ml)
7) Diluent buffer B (40ml)

8) TMB (18ml)

9) Stopping solution (7ml)

10) Instruction Manual

Materials and reagents required but not provided:

1) 50, 100 and 200µl precision micropipette.
2) 50-200µl multichannel micropipette.
3) microtitre plate reader with 450nm filter.
4) methenyl trichloride and n-hexane
5) Methanol - ACS grade or better.
5) 250ml graduated cylinder.
6) 125ml container.
7) Centrifuge（4000r/min）and vortex mixer
8) Timer

3. Preparation of Working Solutions

- **aflatoxin standard solutions**: ready to use.
- **80× Enzyme Conjugate**: dilute 80× with Diluent buffer A (1+79).
- **80×Antibody cross-reactivity**: dilute 80× with Diluent buffer A (1+79).
- **washing buffer**: dilute 10× with distilled water (1+9).
- **TMB**: ready to use.
- **stopping solution**: ready to use.
- **70 percent methanol/water**: Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot. Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed. Store this solution at room temperature in a tightly closed container until needed.

4. Preparation of Samples

Corn and Peanut

a. Transfer 5 grams of ground sample into an extraction mixing jar.

b. Add 20ml of the (70/30) methanol/water extraction solvent.

c. Cover the extraction jar and blend on high speed for 10 minutes.

d. Filter the extract through a filtering syringe.

e. Dilute 500µl of the filtrate with 750µl of Diluent buffer A.

Feed

a. Transfer 5 grams of ground sample into an extraction mixing jar.

b. Add 20 ml of the (70/30) methanol/water extraction solvent.

c. Cover the extraction jar and blend on high speed for 10 minutes.

d. Filter the extract through a filtering syringe.

e. Take 2ml filtrate obtained by adding 3ml chloroform, oscillation 1 min, put it aside for 1min, 3500r/min centrifuge 5 minutes.

f. Suction to the upper and the middle emulsion layer of aqueous solution will be lower chloroform solution at 50°C under a weak N_2-stream.

g. Add 2ml (70/30) methanol/water fixed volume.

h. Dilute 100µl of the filtrate with 500µl of Diluent buffer B.

Oils

a. Transfer 5 grams of ground sample into an extraction mixing jar.

b. Add 10 ml of the (70/30) methanol/water and 10ml n-hexane extraction solvent.

c. Cover the extraction jar and blend on high speed for 10 minutes.

d. Put it aside for layering, 3000r/min centrifuge 2 minutes. Suction to the upper solution and the middle emulsion layer, to retain the lower extract.

e. Dilute 500µl of the filtrate with 750µl of Diluent buffer A.

5. Immunoassay Procedure

a) Allow reagents, microwells, and sample extracts to reach room temperature prior to running the test.

b) Insert a sufficient number of wells into the microwell holder for all standards and samples to be tested.

c) Using a new pipette tip for each standard and sample, pipette 50µl of standards and prepared sample to separate wells.

d) Add 50µl of enzyme conjugate into each well.

e) Add 50µl of anti-aflatoxin antibody into each well.

f) Incubate for 20 minutes at room temperature (37℃)

g) Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.

h) Using a wash bottle, fill each well with washing buffer .Empty the wells again and remove all remaining liquid. Repeat this step 2 times (total of 4 washes).

i) Add 150µl of TMB to each well. Incubate for 10 minutes at room temperature (37℃). Cover the wells with a paper towel to protect them from light sources.

j) Add 50µl of stop solution to each well.

k) Read results using a microwell reader with a 450 nm Thermo Labsystems.

6. Calculation

Divide the mean absorbance value of standards and samples (B) by the mean absorbance value of the Maximum Binding (Bo) and multiply by 100. Maximum binding is thus made equal to 100% and the absorbance values are quoted in percentages:

$$\frac{\text{absorbance standard (or sample)}}{\text{absorbance maximum binding}} \times 100 = B/Bo\ \%$$

7. Results

Since the samples have been diluted prior to assay, the concentrations in ng/ml read from the standard curve must be multiplied by the respective dilution factor to obtain the effective aflatoxin concentration in samples expressed in ppb (ng/ml or ng/g). The dilution factors are 10 for Corn, 20 for feed, 5 for oil.

8. Cautions

1) If ambient temperature is lower than 20℃ or the temperature of the reagents and standard solutions is less than the ambient temperature (e.g., 20- 25℃） when used, the testing results may have negative bias (lower than it should be).

2) If it takes too long time to dry the washed microtiter during the washing steps, the results may

demonstrate nonlinear calibration plot and poor repeatability, thus once the microtiter is dried after the washing steps, the following step should be immediately implemented to prevent the undesirable occurrence.

3) The standard solutions and chromogenic solution contain light-sensitive substances, thus the solutions should not be exposed under direct light.
4) To gain excellent results, any solutions should be thoroughly mixed before added on microtiter and the microtiter should be completely cleaned in between each step.
5) The stopping solution is a very strong acidic solution, so users should avoid the solution directly contacting with skin.
6) Neither use expired reagents, nor dilutes reagents or employs reagents produced by varied companies; otherwise, only gain less sensitive results.
7) Do not interchange individual reagents between kits of different lot numbers.
8) The kit should be stored at 2-8℃ but not be frozen. Place any unused microwells to their original foil bag and reseal them together with the desiccant provided.

9. Contact Information

UC BIODEVICES CORP.
3652 Edison Way
Fremont, CA 94538
USA
Tel: 1-510-730-2598
Fax: 1-510-795-1795
www.ucbiodevices.com

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