

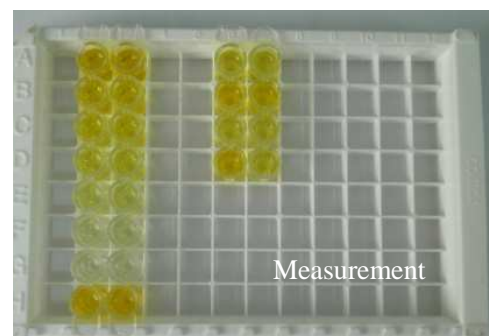
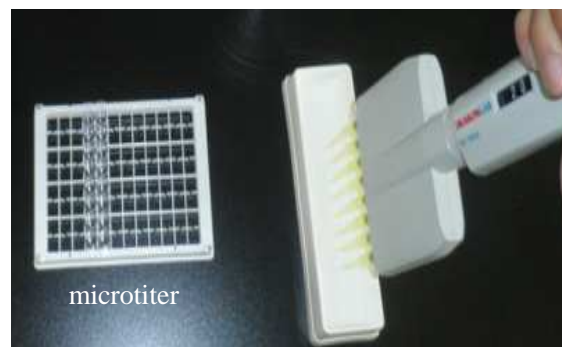
ELISA Kit for Detection of Total Aflatoxin

(Product Number: 5501E103)

(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.

Cross reactivity:

The specificity of the Aflatoxin Total test was established by analyzing the cross-reactivity to the relevant mycotoxins.

Aflatoxin B1.....	100 %
Aflatoxin B2.....	approx.100 %
Aflatoxin G1	approx.100 %
Aflatoxin G2	approx. 80 %
T-2 Toxin.....	0 %
Zearalenon.....	0 %
Ochratoxin.....	0 %
Fumonisin.....	0 %

2. Kit Contents

- 1) Microtiter plate (8wells×12) precoated with antibodies to mouse IgG.
- 2) 5×aflatoxin standard solutions (1ml/each):0ng/ml, 0.5ng/ml, 1ng/ml, 2ng/ml, 5ng/ml
Controls provided: 0, 0.5, 1, 2 and 5ppb
- 3) Aflatoxins antibody (100×): Total aflatoxins (B1, B2, G1, G2) (0.2ml)

- 4) Enzyme Conjugate(100×) (0.2ml)
- 5) Washing buffer(10×) (50ml)
- 6) Antibody and Enzyme diluent buffer (30ml)
- 7) Diluent buffer A (50ml)
- 8) Diluent buffer B (50ml)
- 9) TMB (17ml)
- 10) Stopping solution: (7ml)
- 11) Instruction Manual

Materials and reagents required but not provided:

- 1) 20-200µl and 200-1000µl precision micropipette.
- 2) 50-300µl multichannel micropipette.
- 3) microtitre plate reader with 450nm filter.
- 4) shaker
- 5) 50mL centrifuge tube、funnel and paper filter
- 6) thermostank.
- 7) Methanol - ACS grade or better.
- 8) 100ml graduated cylinder
- 9) 0.22µm Filtering syringe
- 10) Timer

3. Preparation of Working Solutions

- **aflatoxin standard solutions:** ready to use.
- **100× Enzyme Conjugate:** dilute 100× with Antibody and Enzyme diluent buffer (1+99).
- **100×Aflatoxins antibody:** dilute 100× with Antibody and Enzyme diluent buffer (1+99).
- **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 Grains

- a. Transfer 5 grams of ground sample into a 50mL centrifuge tube.
- b. Add 20 ml of the (70/30) methanol/water extraction solvent.
- c. Cover the centrifuge tube and blend on high speed for 3 minutes.
- d. Dilute 200µl of the filtrate with 300µl of Diluent buffer A.

Peanut

- a. Transfer 5 grams of ground sample into a 50mL centrifuge tube.
- b. Add 20 ml of the (70/30) methanol/water extraction solvent.
- c. Cover the centrifuge tube and blend on high speed for 3 minutes.
- d. Filter the extract through a filtering syringe (nylon disc filter: Φ 13mm/0.22µm) .
- e. Dilute 200µl of the filtrate with 300µl of Diluent buffer A.

Feed

- a. Transfer 5 grams of ground sample into a 50mL centrifuge tube.
- b. Add 20 ml of the (70/30) methanol/water extraction solvent.
- c. Cover the centrifuge tube and blend on high speed for 3 minutes.
- d. Filter the extract through a filtering syringe (nylon disc filter: Φ 13mm/0.22µm) .
- e. Dilute 100µl of the filtrate with 500µl of Diluent buffer B.

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°C)
- 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°C). 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:

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

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, Grains and peanut, 24 for feed.

8. Cautions

- 1) If ambient temperature is lower than 20°C or the temperature of the reagents and standard solutions is less than the ambient temperature (e.g., 20- 25°C) 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°C 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

Product specifications and descriptions in this documentation subject to change without notice.

Copyright © 2011 UC BIODEVICES CORP.

August, 2011

50010003 V1.00