UC Biodevices

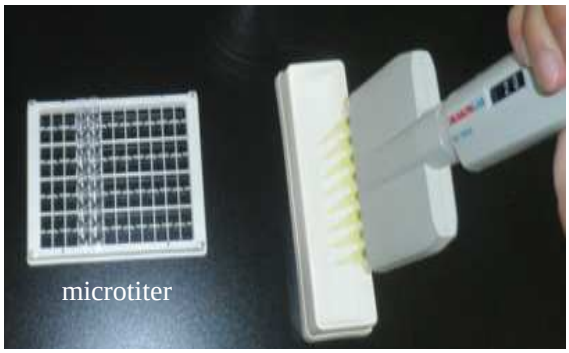
# ELISA Kit for Detection of Aflatoxin M1

## (Product Number: 5501E102)

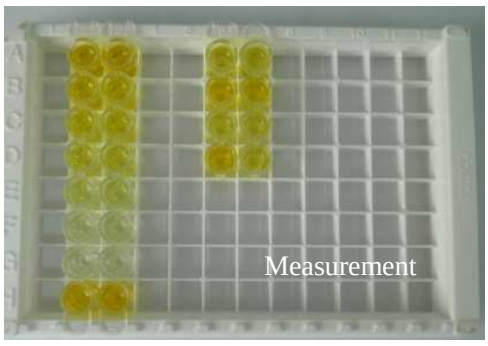
（Aflatoxin is a class Ⅰ carcinogenic substance）

## 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 milk, milk powder and cheese products. The test is a competitive indirect ELISA that provides exact concentrations in parts per billion (ppb). The basis of the test is the antigen-antibody reaction. The wells in the microtiter are coated with specific antibodies against aflatoxin M1. Aflatoxin M1 standards or the sample solutions and after a washing step the enzyme conjugate are added. Free aflatoxin M1 and aflatoxin M1 enzyme conjugate compete the aflatoxin M1 antibody binding sites. Any unbound enzyme conjugate is then removed in the washing steps. Subsequently, substrate is added to react with the bound enzyme conjugate to produce blue color.

**Assay Sensitivity, Precision, Accuracy**

1) Sensitivity: 0.05ppb
2) Linearity:
   a) Milk, yogurt: 0.1-2 ppb
   b) Milk powder: 0.5-10 ppb
   c) Cheese: 0.5-10 ppb
3) Precision：<20%
4) Accuracy：70％-110％ recovery rate

## 2. Kit Contents

1) Microtiter plate ( 8wells×12) precoated with antibodies.
2) 6×aflatoxin standard solutions (1ml/each):0ng/ml, 0.05ng/ml, 0.1ng/ml, 0.25ng/ml, 0.5ng/ml, 1.0ng/ml,

Controls provided: 0, 0.05, 0.1, 0.25, 0.5 and 1.0ppb

3) Enzyme Conjugate(50×) (0.2ml)
4) washing buffer(10×) (50ml)
5) PBS-buffer (30ml)
6) Diluent buffer (50ml)
7) TMB (12ml)
8) Stop solution： (7ml)
9) 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) Centrifuge
5) Shaker
6) Evaporator
7) methanol
8) n-heptane
9) dichloromethane
10) 100ml graduated cylinder
11) 100ml container
12) Timer

**3. Preparation of Working Solutions**

- - **aflatoxin standard solutions**: ready to use.

- **50× Enzyme Conjugate**: dilute 50× with PBS buffer A(1+49).

- **washing buffer**: dilute 10× with distilled water (1+9).

- **TMB**: ready to use.

- **stopping solution**: ready to use.

**4. Preparation of Samples**

**Milk**

a) centrifuge milk samples for degreasing: 10 min/3500g/10℃(if a refrigerated centrifuge is not available, chill sample to 10℃ prior to centrifugation).

b) after centrifugation, remove upper cream layer completely by aspirating through a pasteur pipette

c) Dilute 500µl of the filtrate with 500µl of Diluent buffer.

**Yogurt**

a) weigh 5g yogurt 10 min/4000g

b) after centrifugation, dilute 100µl of Supernatant with 100 µl buffer 1 (1:1 dilution) and use 100µl per well in the test

**Milk powder**

a) weigh 5g milk powder in a flask and fill up to 50ml with deionised water (50℃).

b) dissolve by stirring for 5 min

c) continue with the preparation of milk as described in milk

**Cheese**

a) weigh 2g of the triturated cheese into a centrifugal glass vial

b) add 40 ml dichloromethane and extract by stirring/shaking the vial for 15 min

c) filter the suspension, evaporate 10ml of the extract at 60℃ under a weak $N_2$-stream

d) redissolve the oily residue in 0.5ml methanol, 0.5ml PBS buffer and 1 ml heptane, mix thoroughly

e) centrifuge: 15 min/3000g

f) remove the upper heptane-layer completely

g) pour off an aliquot of the lower methanolic-aqueous phase carefully using a pasteur pipette

h) dilute 100µl of this aliquot with 400 µl buffer 1 (1:4 dilution) and use 100µl per well in the test

**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 2 for Milk and Yogurt, 10 for Milk power and Cheese.

## 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

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