UC Biodevices

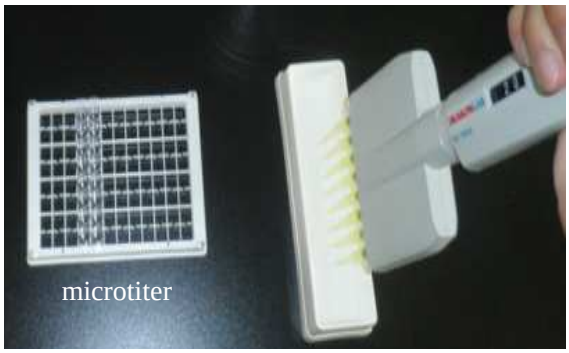
ELISA Kit for Detection of Zearalenone

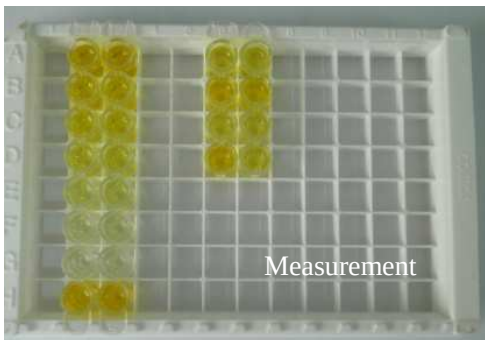
(Product Number: 5501E104)

（Zearalenone has reproductive toxicity and teratogenic effects）

INSTRUCTION MANUAL

(v. 1.00)





1. Introduction

Mycotoxin zearalenone is formed by fungi of the genus Fusarium. Zearalenone is a phytohormone which displays, apart from its anabolic properties, mainly estrogenic effects. Because of its estrogenic properties, zearalenone may induce fertility disorders in animals with clinical signs of hyperestrogenism-anaspect of a disease which although reported mainly in hogs, is described in other species such as cow, horse and sheep, too. The potential health risk for man induced by this mycotoxin, which is taken up with foods of vegetable or animal origin, is extensively discussed.

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. Zearalenone residue in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme labeled anti-antibody, TMB substrate is used to show the color. The addition of the stop solution leads to a color change from blue to yellow. The measurement is performed photometrically at 450 nm. The absorption is inversely proportional to the zearalenone concentration in the sample.

Assay Sensitivity, Precision, Accuracy

1) Sensitivity: 0.5ppb
2) Precision：<20%
3) Accuracy：60-120％ recovery rate

2. Kit Contents

1) Microtiter plate (8 wells×12) precoated with antigen.
2) 6×Zearalenone standard solutions (1ml/each):0ng/ml, 0.5ng/ml, 2.0ng/ml, 5.0ng/ml, 15.0ng/ml, 50.0ng/ml

   Controls provided: 0ppb, 0.5ppb, 2.0ppb, 5.0ppb, 15.0ppb and 50.0ppb
3) Zearalenone antiboby (200×) (0.2ml)
4) Enzyme Conjugate(100×) (0.2ml)
5) Washing buffer(10×) (50ml)
6) Diluent buffer (50ml)
7) TMB (18ml)

8) Stopping solution (7ml)

9) Instruction Manual

Materials and reagents required but not provided:

1) variable 20 µl - 200 µl and 200 - 1000 µl micropipettes

2) microtiter plate reader with 450nm filter.

3) methanol

4) grinder

5) centrifuge and centrifugal vials (50 ml)

6) filter paper

7) Shaker

8) Thermostat

9) Timer

3. Preparation of Working Solutions

-Zearalenone standard solutions: ready to use.

-200×Antibody: dilute 200× with Diluent buffer A(1+199).

- 100× Enzyme Conjugate: dilute 100× with Antibody solution (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 use.

4. Preparation of Samples

Corn and Peanut

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

b. Add 25 ml of the (70/30) methanol/water extraction solvent.

c. Cover the extraction jar and blend on high speed for 10 minutes. Stand for 5 minutes

d. Filter the extract through a filtering syringe.

e. Dilute 100µl of the filtrate with 300µl of Diluent buffer.

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-zearalenone 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 zearalenone concentration in samples expressed in ppb (ng/ml or ng/g). The dilution factors are 20 for Corn.

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

August, 2011

50010004 V1.00