UC Biodevices

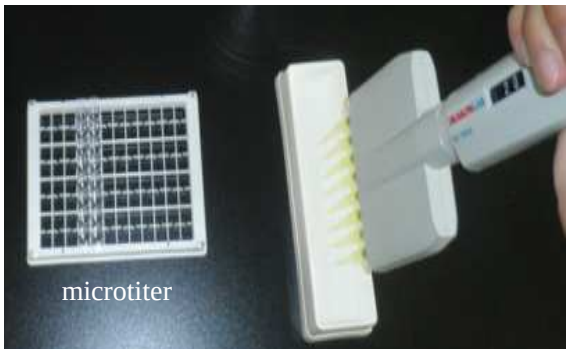
ELISA Kit for Detection of Zeranol

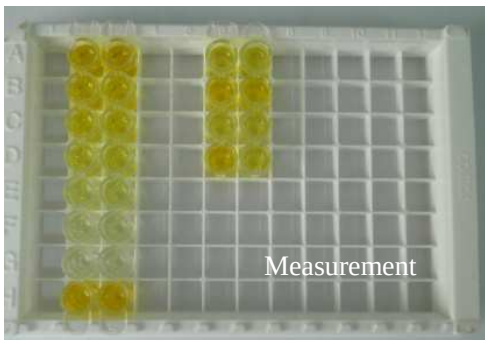
(Product Number: 5501E208)

（Zeranol causes human sexual function disorders and is potentially carcinogenic）

INSTRUCTION MANUAL

(v. 1.00)





1. Introduction

Zearalenone is produced by natural fusarium, and the product of reduction of which is zeranol. Zeranol is a female hormone, which maintains secondary sexual characteristics and protein assimilation effect. It can be used for growth promotion of animals besides therapy, especially in cattle. It is now being inhibited for growth promotion in many countries for the obvious carcinogenicity.

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. Zeranol 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 zeranol concentration in the sample.

Assay Sensitivity, Precision, Accuracy

1) Sensitivity: 0.05ppb
2) Precision：<20%
3) Accuracy：60-120％ recovery rate

2. Kit Contents

1) Microtiter plate (8 wells×12) precoated with antigen.
2) 7×Zeranol standard solutions (1ml/each):0ng/ml, 0.05ng/ml, 0.2ng/ml, 0.5ng/ml, 1.0ng/ml, 2.0ng/ml, 5.0ng/ml

Controls provided: 0ppb, 0.05ppb, 0.2ppb, 0.5ppb, 1.0ppb, 2.0ng/ml and 5.0ppb

3) Zeranol antiboby (200×) (0.2ml)
4) Enzyme Conjugate(100×) (0.2ml)
5) Washing buffer(10×) (50ml)
6) Diluent buffer A (50ml)
7) Diluent buffer B (50ml)
8) Diluent buffer C (50ml)
9) Diluent buffer D (30ml)
10) TMB (18ml)

11) Stopping solution (7ml)

12) Instruction Manual

Materials and reagents required but not provided:

1) variable 20 µl - 200 µl and 200 - 1000 µl micropipettes
2) microtitre plate reader with 450nm filter.
3) Acetonitrile
4) Chloroform
5) N-hexane
6) glucuronidase / aryl sulfatase
7) grinder
8) centrifuge and centrifugal vials (50 ml)
9) filter paper
10) Shaker
11) Thermostat
12) Timer

3. Preparation of Working Solutions

- **Zeranol 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.
- **0.1M sodium hydroxide solution:** Dissolve 0.4g sodium hydroxide with deionized water and dilute to 100ml;
- **1M sodium hydroxide solution:** Dissolve 4g sodium hydroxide with deionized water and dilute to 100ml;
- **Acetonitrile -0.1M sodium hydroxide solution:** Take 90 ml acetonitrile and mix with 10ml 0.1M sodium hydroxide solution;
- **6M phosphoric acid buffer solution:** Take 100ml 85% concentrated phosphoric acid, then add

150ml deionized water, mix completely

4. Preparation of Samples

Beef sample

a. Weigh 1.0g±0.1g sample, add 2ml deionized water, then add 8μl Glucuronidase/ Arylsulfatase, mix completely, incubate at 37℃ for 2h

b. Take out ,then add 8ml acetonitrile, shake for 10min, centrifuge for 10min , at least 3000g;

c. Take 5ml of the supernate, add 2ml chloroform and 6ml n-hexane, shake for 10min, centrifuge for 10min , at least 3000g;

d. Remove the supernatant n-hexane, take the middle-level phase and dry ;

e. Dissolve the dry leftover with 1ml Diluent buffer D, take out 100μl and mix with 400μl extraction solution A;

f. Take 50μl of the prepared solution for assay

Milk sample

a. Take 1ml fresh milk sample, then add 8μl Glucuronidase/ Arylsulfatase, incubate at 37℃ for 2h;

b. Take 100μl of the sample solution and dilute with 900μl extraction solution B;

c. Take 50μl of the prepared solution for assay .

Urine sample

a. Take 2ml urine sample into a centrifuge tube, centrifuge at room temperature for 10min, at least 3000g until it is transparent ;

b. Take 1ml of the transparent urine sample into a centrifuge tube, add 10μl Glucuronidase/ Arylsulfatase, keep in 37℃water bath for 3h, then add 5ml chloroform , shake for 10min, centrifuge at room temperature for 10min, at least 3000g, take 2.5ml of the substrate organic phase and dry with 50℃ nitrogen gas flow;

c. Dissolve the dry leftover with 1ml Diluent buffer D;

d. Take 50μl of the dissolved sample solution, and Diluent buffer B in the volume ratio of 1:4 (50μl sample solution +200μl Diluent buffer B);

e. Take 50μl of the prepared solution for assay

Feed sample

a. Homogenize the feed sample with homogenizer.

b. Weigh 2.0g of the homogenate , add 10ml acetonitrile-0.1M NaOH solution, shake for 10min, centrifuge at room temperature for 20min, at least 3000g. Take 1ml of the supernate , and dry with 50℃ nitrogen gas flow;

c. Dissolve the dry leftover with 0.5ml chloroform, add 2ml 1M NaOH solution, shake for 5min , centrifuge at room temperature for 10min, at least 3000g;

d. Take 1ml of the supernate, add 100µl 6M H_3PO_4(regulate the pH to 6.5), then add 5ml chloroform, shake for 10min, centrifuge at room temperature for 10min, at least 3000g, take 2.5ml of the substrate organic phase and dry with 50℃ nitrogen gas flow ;

e. Take 1ml Diluent buffer D to dissolve the dry leftover , then dilute the sample solution with extraction solution in the volume ratio of 1:9 (450µl Diluent buffer C +50µl sample solution);

f. Take 50µl of the prepared solution for assay .

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-zeranol 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 zeranol concentration in samples expressed in ppb (ng/ml or ng/g). The dilution factors are 10 for Beef, 10 for Milk and Urine, 10 for Feed.

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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