Elabscience®

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DES (Diethylstilbestrol) ELISA Kit

Catalog No: E-FS-E001

96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) 240-252-7376(USA)
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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This kit uses Indirect-Competitive-ELISA as the method. It can detect Diethylstilbestrol (DES) in samples, such as rice, millet, flour and other crops/feed, etc. This kit is composed of Micro ELISA Plate, HRP conjugate, antibody, standard and other supplementary reagents. The micro-plate provided in this kit has been pre-coated with DES. During the reaction, DES in the samples or standard competes with DES on the solid phase supporter for sites of DES antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is for color development. There is a negative correlation between the OD value of samples and the concentration of DES. The concentration of DES in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.05 ppb (ng/mL)

Reaction mode: 25℃, 30 min~30 min~15 min

Detection limit: Tissue (fish, shrimp) ---0.2 ppb, Pork/chicken/liver---0.5 ppb

Cross-reactivity: Diethylstilbestrol---100%, Dienestrol---38.5%, Hexoestrol---8.5%, Alkynediols---＜0.1%, Estriol ---＜0.1%

Sample recovery rate: Tissue---85%±10%

Kit components

Item	Specifications
ELISA Micro-plate	96 wells
Standard Liquid	1 mL each (0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, 4.05 ppb)
High Concentrated Standard (100 ppb)	1 mL
HRP Conjugation (red cap)	11 mL
Antibody Working Solution (blue cap)	5.5 mL
Substrate Reagent A (white cap)	6 mL
Substrate Reagent B (black cap)	6 mL
Stop Solution (yellow cap)	6 mL
20×Concentrated Wash Buffer (white cap)	40 mL
Manual	1 copy

Other supplies required

Instruments: Micro-plate reader, Printer, Homogenizer, Nitrogen Evaporators, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

High-precision transferpettor: single channel (20-200 μL, 100-1000 μL), Multichannel (300 μL).

Reagents: Methanol, Acetonitrile, Chloroform, Acetone, Sodium hydroxide, Concentrated phosphoric acid (85%).

Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the micro-plate reader in advance (30 min), preheat the instrument, and set the testing parameters.

1. **Sample pretreatment Notice:**

   Experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. **Solution preparation**

   Solution 1: 6 M H_3PO_4

   Add 100 mL Concentrated phosphoric acid (85%) to 150 mL with deionized water, mix thoroughly.

   Solution 2: 2 M NaOH

   Dissolve 8 g Sodium hydroxide to 100 mL with deionized water.

   Solution 3: 40% Carbinol

   Carbinol (V): Deionized water (V) =2:3

   Solution 4: Acetonitrile-Acetone mixed liquor

   Acetonitrile (V): Acetone (V) = 4:1

   Solution 5: Wash Buffer

   Dilute 20×Concentrated Wash Buffer with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) = 1:19)

3. **Sample pretreatment procedure**

   Pretreatment of tissue (fish, shrimp, pork/liver, chicken, duck):

(1) Weigh 2±0.05 g of crushed homogenate into the 50 mL EP tube, add 6 mL of Acetonitrile-Acetone mixed liquor, oscillate for 2 min, centrifuge at 4000 r/min for 10 min at 15℃;

(2) Remove 3 mL of the supernatant to another centrifuge tube, dry with Nitrogen Evaporators at 60℃;

(3) Add 0.5 mL of chloroform and oscillate for 20 sec, then add 2 mL of 2 M Na_2OH and oscillate for 30 sec, centrifuge at 4000 r/min for 5 min;

(4) Take 1 mL of the upper liquid to another tube, add 200 μL of 6 M H_3PO_4 and oscillate for 5 sec;

(5) Add 3 mL of acetonitrile for extraction, oscillate for 2 min, centrifuge at 4000 r/min for 10 min at room temperature. Then take 1.5 mL of the upper liquid and dry with Nitrogen Evaporators at 60℃ or air dry;

(6) Redissolve for different samples.

a) Fish, shrimp:

Dissolve the residue with 1 mL of 40% Carbinol, oscillate for 30 sec and take 50 μL for detection and analysis.

Note: Sample dilution factor: 4; minimum detection dose: 0.2 ppb.

b) Pork, chicken, liver:

Dissolved the residue with 2.5 mL of 40% Carbinol, oscillate for 30s and take 50 μL for detection and analysis.

Note: Sample dilution factor: 10; minimum detection dose: 0.5 ppb.

Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells.
2. **Add sample:** add 50 μL of Standard or sample to each well, then add 50 μL antibody working solution, cover the plate with sealer we provided, oscillate for 5 sec gently to mix thoroughly, shading light incubation for 30 min at 25℃.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 250 μL of wash working buffer to each well and wash. Repeat wash procedure for 5 times, 30 sec intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **HRP conjugate:** add 100 μL of HRP Conjugation to each well, shading light incubation for 30 min at 25℃.
5. **Wash:** repeat step 3.
6. **Color Development:** add 50 μL of substrate solution A to each well, and then add 50 μL of substrate solution B. Gently oscillate for 5 sec to mix thoroughly. Incubate shading light for 15 min at 25℃ (The reaction time can be or extended according to the actual color change) .
7. **Stop reaction:** add 50 μL of stop solution to each well, gently oscillate for 5 sec.
8. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm with a micro-plate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10 min after stop reaction.

Result analysis

1. **Absorbance (%)=A/A_0×100%**

   A: Average absorbance of standard or sample

   A_0: Average absorbance of 0 ppb Standard

2. **Drawing and calculation of standard curve**

   Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add average absorbance value of sample to standard curve to get corresponding concentration. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

   For this kit, it is more convenient to use professional analysis software for accurate and fast analysis on a large number of samples.

Notes

1. Overall OD value will be lower when reagents is not brought to room temperature before use or room temperature is below 25℃.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability, move on to the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. ELISA Micro-plate should be covered by plate sealer. Avoid the reagents to strong light.
5. Do not use expired kit and reagents of different batches of kits.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0) is below 0.5 unit($A_{450\ nm}$＜0.5), it indicates the reagent is deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.

Storage and valid period

Storage: Store at 2-8℃. Avoid freeze / thaw cycles.

Valid Period: 1 year, production date is on the packing box.