QUALICHEK™ Diethylstilbestrol ELISA

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Ver 1.0

RUO

Quantitative testing of Diethylstilbestrol in samples, such as tissue, chicken, pork, chicken liver, pork liver etc.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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Introduction:

Diethylstilbestrol (DES), also known as stilbestrol or stilboestrol, is a nonsteroidal estrogen medication which is mostly no longer used. In the past, it was widely used for a variety of indications including pregnancy support for women with a history of recurrent miscarriage, hormone therapy for menopausal symptoms and estrogen deficiency in women, treatment of prostate cancer in men.

Intended Use:

This kit is for quantitative testing for Diethylstilbestrol in sample, such as fish, shrimp, pork, chicken, chicken liver, and pork liver, etc.

Principle:

This kit uses Indirect-Competitive-ELISA as the method. It can detect DES in samples, such as fish, shrimp, pork, chicken, chicken liver, and pork liver, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody, standard and other supplementary reagents. The microplate 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.

Materials Provided:

1. Coated Microtiter plate - 1x 96 wells
2. Standards Liquid -1mL each (0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, 4.05 ppb)
3. High Concentrated Standard (100 ppb)- 1 mL
4. HRP Conjugate - 11 mL
5. Antibody Working Solution - 5.5 mL
6. Substrate A - 6 mL
7. Substrate B - 6 mL
8. Stop Solution - 6 mL
9. Wash Buffer (20X) - 40 mL
10. Plate Sealer - 3 pieces

Material required but not provided:

Instrument: Microtiter plate reader, Printer, Homogenizer, Nitrogen Evaporators/Water bath, Oscillators, centrifuge, Graduated pipette, Balance (sensibility 0.01 g).
High-precision transferpettor: single channel (20-200 µL, 100-1000 µL), Multichannel (300 µL) micropipettes.
Reagents: Methanol, Acetonitrile, Chloroform, Acetone, Sodium hydroxide, Concentrated phosphoric acid (85%).

Storage Information:

1. Store main kit components at 2-8°C Avoid freeze / thaw cycles.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

Sample pretreatment:

Bring all reagents and samples to room temperature before use.

Open the microplate reader in advance, 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/water bath at 60℃;
3. Add 0.5 mL of chloroform and oscillate for 20 sec, then add 2 mL of 2 M NaOH 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 H3PO4 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/water bath 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, duck, 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 for 30 min 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 per well, then add 50 µL of antibody working solution, cover the plate with sealer, oscillate for 5 s gently to mix thoroughly, incubate for 30 min at 25℃.
3. **Wash:** uncover the sealer carefully; remove the liquid in each well. Immediately add 300 µL of wash buffer to each well and wash. Repeat wash procedure for 5 times, 30 s 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 100µl of HRP conjugate to each well, incubate shading light for 30 min at 25^0C
5. **Wash**: repeat step 3.

6. **Color Development:** add 50 µL of Substrate Reagent A to each well, and then add 50 µL of Substrate Reagent B. Gently oscillate for 5 s to mix thoroughly. Incubate shading light for 15 min at 25℃ (The reaction time may be shortened or prolonged according to the depth of the color).
7. **Stop reaction:** add 50 µL of stop solution to each well, oscillate gently to mix thoroughly.
8. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm with a microplate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10 min after stop reaction.

Judgment of result:

Absorbance (%) =A/A0×100%

A: Average absorbance of standard or sample

A0: Average absorbance of 0 ppb Standard

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. The overall OD value will be lower when reagents have not been 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. Operate 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 Microtiter plate should be covered by plate sealer. Avoid the reagents to strong light.
5. Do not use expired kit or reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard (concentration: 0)＜0.5 unit (A450nm＜0.5), it indicates the reagent may be deteriorated.
7. Stop solution is caustic; avoid contact with skin and eyes.

Technical specification:

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
Reactivity: Diethylstilbestrol---100%, Dienestrol---38.5%,
Hexoestrol---8.5%, Alkynediols---＜0.1%, Estriol ---＜0.1%
Sample recovery rate: Tissue---85%±10%

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