



Technical Manual

DES (Diethylstilbestrol) ELISA Kit

- Catalogue Code: FSES0001
- Competitive ELISA Kit
- Research Use Only

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1. Key features and Sample Types

Sensitivity:

0.05 ppb (ng/mL)

Assay Procedure:

25°C, 30 min~30 min~15 min

Detection Limit:

Muscle (fish, shrimp) - 0.2 ppb;

Muscle (livestock), Liver - 0.5 ppb

Cross Reactivity:

Diethylstilbestrol - 100%, Dienestrol - 38.5%, Hexestrol - 8.5%,

Ethinyl estradiol - < 0.1%, Estriol - < 0.1%

Sample Recovery rate:

85%±10%

Storage:

2-8°C for 6 months.

Expiry:

See Kit Label

2. Storage

Store the kit at 2~8°C. Do not freeze any test kit components.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8°C.

3. Test Principle

This kit uses a Competitive-ELISA method. It can detect Diethylstilbestrol (DES) in samples, such as muscle, liver, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard and other supplementary reagents. The microtiter plate in this kit has been pre-coated with coupled antigen. During the reaction, DES in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti- DES antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and substrate reagent is added for colour 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.

4. Kit Contents

Each kit contains reagents for 96 assays including:

No.	Component	Specification
1	ELISA Microtiter plate	96 wells
2	Standards	1 mL each (0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, 4.05 ppb)
3	HRP Conjugate	11 mL
4	Antibody Working Solution	5.5 mL
5	Substrate Reagent A	6 mL
6	Substrate Reagent B	6 mL
7	Stop Solution	6 mL
8	20×Concentrated Wash Buffer	40 mL
9	Plate Sealer	3 pieces
10	Sealed Bag	1 piece
11	Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution

Additional materials required:

Other materials required but not supplied

- **Instruments:** Microplate reader, Printer, Homogenizer, Nitrogen evaporators, Water bath, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensitivity 0.01 g).
- **Micropipette:** Single channel (20-200 µL, 100-1000 µL), Multichannel (30-300 µL).
- **Reagents:** Methanol, Concentrated phosphoric acid (85% H₃PO₄), Acetonitrile, NaOH, Acetone Trichloromethane (CHCl₃).

5. 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 pre-treatment 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 Solution (*for fish, shrimp, livestock, liver sample*)

Dilute 100 mL of **85% H_3PO_4** to 150 mL with deionized water, mix thoroughly.

Solution 2: 2 M NaOH Solution (*for fish, shrimp, livestock, liver sample*)

Dissolve 8 g of **NaOH** to 100 mL with deionized water.

Solution 3: 40% Methanol Solution (*for fish, shrimp, livestock, liver sample*)

Dilute **Methanol** with deionized water (Methanol (V): Deionized water (V) = 2:3), mix thoroughly.

Solution 4: Acetonitrile-Acetone Solution (*for fish, shrimp, livestock, liver sample*)

Dilute Acetonitrile with deionized Acetone (**Acetonitrile** (V): **Acetone** (V) = 4:1), mix thoroughly.

Solution 5: Wash Buffer

Dilute **20xConcentrated Wash Buffer** with deionized water (20xConcentrated Wash Buffer (V): Deionized water (V) = 1:19), mix thoroughly.

3. Sample pretreatment procedure

Targets may be distributed unevenly, resulting in no detection. To avoid this ensure to take sufficient samples when sampling.

3.1 Pretreatment of muscle (fish, shrimp, livestock), liver sample:

1. Weigh 2 ± 0.05 g of crushed homogenate into the 50 mL centrifuge tube, add 6 mL of **Acetonitrile-Acetone Solution** (Solution 4), vortex for 2 min, centrifuge at 4000 r/min for 10 min at 15°C;
2. Remove 3 mL of the supernatant to another centrifuge tube, dry with nitrogen evaporators/water bath at 60°C
3. Add 0.5 mL of **Trichloromethane** and vortex for 20 s, then add 2 mL of **2 M NaOH Solution** (Solution 2) and vortex for 30s, centrifuge at 4000 r/min for 5 min;
4. Take 1 mL of the supernatant to another tube, add 200 μL of **6 M H_3PO_4 Solution** (Solution 1) and oscillate for 5s;
5. Add 3 mL of **Acetonitrile** for extraction, vortex for 2 min, centrifuge at 4000 r/min for 10 min at room temperature. Then take 1.5 mL of the upper organic liquid and dry with nitrogen evaporators/water bath at 60°C;

6. Other sample types can be redissolved in the following way:

a) Fish, shrimp:

Dissolve the residue with 1 mL of **40% Methanol Solution** (Solution 3), oscillate for 30s and take 50 µL for analysis.

Note: Sample dilution factor: 4; detection limit: 0.2 ppb.

b) Muscle (livestock), liver:

Dissolved the residue with 2.5 mL of **40% Methanol Solution** (Solution 3), oscillate for 30s and take 50 µL for analysis.

Note: Sample dilution factor: 10; detection limit: 0.5 ppb.

6. Assay Procedure

Bring all reagents and samples to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2~8°C.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples must be tested in duplicate.**
2. **Add Sample:** add 50 µL of **Standard or Sample** to each well, then add 50 µL **Antibody Working Solution**, cover the plate with plate sealer. Oscillate for 5s gently to mix thoroughly, incubate at 25°C for 30 min away from direct sunlight.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 300 µL of **Wash Buffer** (Solution 5) to each well and wash. Repeat wash procedure for 5 times, 30 s intervals/time. Invert the plate and pat it against absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **HRP Conjugate:** add 100 µL of **HRP Conjugate** to each well, incubate at 25°C for 30 min away from direct sunlight.
5. **Wash:** repeat step 3.
6. **Colour Development:** add 50 µL of **Substrate Reagent A** to each well, and then add 50 µL of **Substrate Reagent B**. Gently oscillate for 5s to mix thoroughly. Incubate at 25°C for 15 min away from direct sunlight (The reaction time can be extended according to the actual colour change).
7. **Stop Reaction:** add 50 µL of **Stop Solution** to each well. Gently oscillate for 5s to mix thoroughly.
8. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. This step should be finished in 10 min after stop reaction.

7. Data Analysis

1. Absorbance (%) = $A/A_0 \times 100\%$

A: Average absorbance of standard or sample

A₀: 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 form for accurate and fast analysis on a large number of samples.

8. 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°C.
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 kit to strong light.
5. **Each reagent is optimized for use in the FSES0001. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other FSES0001 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue colour. When OD value of standard (concentration: 0) < 0.5 unit (A_{450nm} < 0.5), it indicates the reagents are deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
11. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

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