Diethylstilbestrol ELISA Test Kit

1. Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Diethylstilbestrol(DES) in the feed, urine, liver, meat, shrimp and fish. The conjugate antigen is pre-coated on the micro-well stripes. The Diethylstilbestrol in the sample competes with the conjugate antigen pre-coated on the micro-well stripes, to interact with the antibodies against Diethylstilbestrol. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with the content of Diethylstilbestrol in the sample. This value is compared to the standard curve and the content of the corresponding Diethylstilbestrol is subsequently obtained.

2. Technical specifications

Sensitivity: 0.1 ppb

Detection limit

Tissue(shrimp, fish) ..... 0.2 ppb
Pork/liver, chicken/liver ..... 2 ppb
Urine ..... 0.6 ppb
Feed ..... 20 ppb

Recovery rate

Urine ..... 70 ±10%
Feed ..... 90 ±10%
Tissue ..... 85 ±10%

Cross-reactions rate

DES 100%
Dienestrol 38.5%
Hexestrol 8.5%
Ethinylestradiol ..... < 0.1%
Estriol ..... < 0.1%

3. Components

1） Micro-well strips: 12 strips with 8 removable wells each
2） 6 × standard solution (1 mL each): 0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb
3） Enzyme conjugate (12 mL) ..... red cap
4） Antibody working solution (7 mL) ..... blue cap
5） Substrate A solution (7 mL) ..... gray cap
6） Substrate B solution (7 mL) ..... black cap
7） Stop solution (7 mL) ..... yellow cap
8） 20× concentrated washing buffer (40 mL) ..... white cap
9） 5× concentrated redissolving solution (50 mL) ..... transparent cap

4. Materials required but not provided

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1) **Equipments:** microplate reader, printer, homogenizer, nitrogen-drying device, votex, centrifuge, measuring pipets, balance (a sensibility reciprocal of 0.01 g)
2) **Micropipettors:** single-channel 20-100 µL ,100-1000 µL, and multi-channel 250 µL;
3) **Reagents:** NaOH and $CHCl_3$(for all of sample), Acetonitrile (CH_3CN)(for feed and tissue), Acetone(for tissue), deionized water, H_3PO_4 (85%),

5. Sample pre-treatment

Instructions (The following points must be dealt with before the pre-treatment)

1) Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents;
2) Before the experiment, each experimental utensil must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.

Solution preparation before sample pre-treatment

1) 6 M H_3PO_4 : dissolve 100 mL H_3PO_4 in 150 mL deionized water, mix properly
2) 1 M NaOH : dissolve 4 g NaOH in deionized water to 100 mL
3) 2 M NaOH : dissolve 8 g NaOH in deionized water to 100 mL
4) Acetonitrile- Acetone: add 80 mL Acetonitrile and 20 mL Acetone, mix evenly
5) The 5×concentrated redissolving solution is mixed with deionized water at 1:5 (1 mL concentrated redissolving solution + 4 mL deionized water), used for the treated sample redissolving

5.1 Feed

1. Weigh 2 ± 0.05 g of the homogenized sample, add 8 mL Acetonitrile, shake properly for 10 min, centrifuge at above 3000 r/min at 15 ℃ for 10 min
2. Take 2 mL supernatant into a new centrifuge tube, blow to dry withnitrogen or air at 60 ℃.
3. Add 0.5 mL $CHCl_3$, vortex for 20 sec, add 2 mL 1 M NaOH, vortex for 30 sec, centrifuge at above 3000 r/min for 5 min.
4. Take 1 mL supernatant, add 100 µL 6 M H_3PO_4, vortex for 5 sec
5. Dulition: Compound feed-- take 50 µL sample, add 950 µL of the diluted redissolving solution; Concentrated /Premixed feed-- take 25 µL sample, add 975 µL of the diluted redissolving solution
6. Take 50 µL for analysis

Fold of dilution of the sample:

Compound feed ---------100 Concentrated / Premixed feed ----------200

5.2 Meat, liver, shrimp, fish

1. Weigh 2 ± 0.05 g of the homogenized sample, add 6 mL Acetonitrile- Acetone, shake for 10 min, and centrifuge at above 3000 r/min at 15 ℃ for 10 min.
2. Transfer 3 mL supernatant into a new centriffuge tube, blow to dry with nitrogen or air at 60 ℃. Add 0.5 mL $CHCl_3$, vortex for 20 sec, add 2 mL 1 M NaOH, vortex for 30 sec, centrifuge at above 3000 r /min for 5 min.

3. Take 1 mL supernatant, add 200 µL 6 M H_3PO_4, vortex for 5 sec.
4. Add 3 mL Acetonitrile (CH_3CN) for extraction, shake properly for 10 min, centrifuge at above 3000 r/min at room temperature (20-25 ℃) for 10 min, take the upper layer, blow to dry with nitrogen or air at 60 ℃.
5. Dissolve dry residues in 1 mL of the diluted redissolving solution.
6. Dilution: shrimp and fish-----directly take 50 µL water phase for detection; Meat and liver-----take 50 µL water phase, add 450 µL of the diluted redissolving solution, shake properly.
7. Take 50 µL for analysis.

Fold of dilution of the sample: shrimp and fish----2 meat and liver-----20

5.3 Urine

1. Take 2 mL urine into centrifuge tube, centrifuge at above 3000 r/min at room temperature (20-25) for 10 min, stop when it is clear.
2. Transfer 1 mL clear urine into centrifuge tube, add 1 mL 1 M NaOH, shake vigorously for 5 min
3. Add 100 µL 6 M H_3PO_4, vortex for 30 sec
4. Add 8 mL $CHCl_3$ for extraction, shake properly for 10 min, centrifuge at above 3000 r/min at 15℃ for 10 min.
5. Remove the upper layer (water phase), take 4 mL of the lower layer, blow to dry with nitrogen or air at 60 ℃.
6. Dissolve dry residues in 3 mL of the diluted redissolving solution。
7. take 50 µL for analysis.

Fold of dilution of the sample:6

6. ELISA procedures

6.1 Instructions

1 Bring all reagents and micro-well strips to the room temperature (20-25 ℃) before use.

2 Return all reagents to 2-8 ℃ immediately after use

3 The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in the procedures of ELISA;

4 For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

6.2 Operation procedures

1. Take out the kit from the refrigerated environment. Take out all the necessary reagents from the kit and place at the room temperature (20-25℃) for at least 30 min. Note that each liquid reagent must be shaken to mix evenly before use
2. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2-8 ℃, not frozen
3. Solution preparation: dilute the 20× concentrated washing buffer with the distilled or deionized water to 800 mL (or just to the required volume) for use
4. Numbering: number the micro-wells according to samples and standard solution; each sample

and standard solution should be performed in duplicate; record their positions.

5. Add 50 μL of the sample or standard solution to separate duplicate wells; add 50 μL of the antibody working solution into each well. Seal the microplate with the cover membrane, and incubate at 37℃ for 30 min
6. Pour the liquid out of the microwells, add 250 μL/well of washing buffer for 10 sec, repeat four to five times, then flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).
7. Add 100 μL enzyme conjugate into every well, seal the microplate with the cover membrane, react at 37℃ for 30 min, continue as described in 6
8. Coloration: add 50 μL of the substrate A solution and then 50 μL of the B solution into each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane and incubate at 37 ℃ for 15 min at dark for coloration
9. Determination: add 50 μL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value (Recommend to read the OD value at the dual-wavelength 450/630 nm within 5 min)

7. Result judgment

There are two methods to judge the results; the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the content of Diethylstilbestrol (DES).

7.1 Qualitative determination

The concentration range (ng/mL) can be obtained from the comparison the average OD value of the sample with that of the standard solutin. Assuming that the OD value of the sample Ⅰ is 0.310, and that of the sample Ⅱ is 0.820, the OD value of standard solutins is: 1.510 for 0 ppb, 1.320 for 0.1 ppb, 1.03 for 0.3 ppb, 0.660 for 0.9 ppb, 0.389 for 2.7 ppb ,0.198 for 8.1 ppb, accordingly the concentration range of the sample Ⅰ is 0.8-1.6 ppb, and that of the sample Ⅱ is 0.2-0.4 ppb. (multiplied by the corresponding dilution fold)

7.2 Quantitative determination

The mean values of the absorbance values is equivalent to the percentage of the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

$$\text{Percentage of absorbance value} = \frac{B}{B_0} \times 100\%$$

B—the average (double wells) OD value of the sample or the standard solutin

B_0—the average OD value of the 0ng/mL standard solutin

Draw the standard curve with the absorption percentages of the standard solutins and the semilogarithm values of the Diethylstilbestrol(DES) standard solutins (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently

multiplied by the corresponding dilution fold, thus finally obtaining the Diethylstilbestrol (DES) concentration in the sample.

Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software)

8. Precautions

1. The room temperature below 20 ℃ or the temperature of the reagents and the samples being not returned to the room temperature (20-25 ℃) will lead to a lower standard OD value
2. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility; So continue to next step immediately after washing
3. Mix evenly, otherwise there will be the undesirable reproducibility
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin;
5. Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use
6. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light
7. Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of the 0 standard solutin of less than 0.5 (A450 nm< 0.5) indicates its degeneration
8. The optimum reaction temperature is 37 ℃, and too high or low temperatures will result in the changes in the detecting sensitivity and OD values.

9. Storage and expiry date

Storage: store at 2-8 ℃, not frozen.

Expiry date: 6 months; date of production is on box.

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