Diethylstilbestrol (DES) ELISA Kit

Catalog No.: abx364814

Size: 96 tests

Storage: Store at 4 °C.

Application: The quantitative detection of Diethylstilbestrol in Muscle and Liver Tissue Homogenates.

Sensitivity: 0.05 ng/ml

Detection Limits: Muscle (Fish, Shrimp) – 0.2 ng/ml, Muscle (Livestock) – 0.5 ng/ml, Liver – 0.5 ng/ml

Principle of the Assay: This kit is based on competitive enzyme-linked immuno-sorbent assay technology. Diethylstilbestrol antigen is precoated onto a 96-well plate. The standards and samples and a biotin-conjugated antibody specific to Diethylstilbestrol are added to the wells and incubated. After washing away the unbound conjugates, avidin conjugated to Horseradish Peroxidase is added to each microplate well. After TMB substrate solution is added only wells that contain bound Diethylstilbestrol antibody will produce a blue color product that changes into yellow after adding the stop solution. The intensity of the yellow color is inversely proportional to the Diethylstilbestrol amount present in the sample. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of Diethylstilbestrol can be calculated.

Kit Components

1. Pre-coated 96-Well Microplate: 12 x 8
2. Standards (1 ml each): 0 ng/ml, 0.05 ng/ml, 0.15 ng/ml, 0.45 ng/ml, 1.35 ng/ml, 4.05 ng/ml
3. Wash Buffer (20X): 40 ml
4. Detection Reagent A: 5.5 ml
5. Detection Reagent B: 11 ml
6. Substrate Reagent A: 6 ml
7. Substrate Reagent B: 6 ml
8. Stop Solution: 6 ml
9. Plate sealer: 1

Materials Required But Not Provided

1. Incubator
2. Microplate reader (450 nm)
3. High-precision pipette and sterile pipette tips
4. Automated plate washer
5. ELISA shaker
6. Vortex mixer
7. Centrifuge
8. 50 ml centrifuge tubes
9. 100 ml and 1 L graduated cylinders
10. Nitrogen evaporator or water bath
11. Homogenizer

Reagents Required But Not Provided

1. Methanol (CH_3OH)
2. Concentrated Phosphoric acid (85 % H_3PO_4)
3. Acetonitrile (CH_3CN)
4. Sodium Hydroxide (NaOH)
5. Acetone (C_3H_6O)
6. Trichloromethane ($ChCl_3$)
7. Deionized water

Protocol

Bring all reagents and samples to room temperature before use.

A. Preparation of Samples and Reagents

1. Reagents

- **Solution 1 – 6 M H_3PO_4 Solution** (for **Fish/Shrimp/Livestock muscle tissue** and **Liver** samples).
  Dilute 100 ml of 85 % H_3PO_4 to 150 ml with deionized water, mix fully.

- **Solution 2 – 2 M NaOH Solution** (for **Fish/Shrimp/Livestock muscle tissue** and **Liver** samples).
  Dissolve 8 g of NaOH in 100 ml deionized water, mix fully.

- **Solution 3 – 40 % Methanol Solution** (for **Fish/Shrimp/Livestock muscle tissue** and **Liver** samples).
  Dilute Methanol with deionized water to a ratio of 2:3 Methanol to Deionized water, mix fully.

- **Solution 4 – Acetonitrile-Acetone Solution** (for **Fish/Shrimp/Livestock muscle tissue** and **Liver** samples).
  Dilute Acetonitrile with Acetone to a ratio of 4:1 Acetonitrile to Acetone, mix fully.

- **Solution 5 – Wash Buffer**.
  Dilute the provided 20X Wash Buffer 20-fold with Deionized Water (i.e. in a 1:19 ratio of 20X Wash Buffer to Deionized water).

2. Sample Pretreatment

Fish/Shrimp/Livestock muscle tissue and Liver samples:

1. Remove the fat from samples and homogenize.
2. Weigh 2 g of homogenate into a centrifuge tube, then add 6 ml of Acetonitrile-Acetone Solution and vortex mix for 2 minutes, then centrifuge at 4000 × g for 10 minutes at 15 ºC.
3. Transfer 3 ml of supernatant to a fresh centrifuge tube and dry using nitrogen evaporation or water bath
   Note: Perform this step under ventilation
4. Add 0.5 ml of Trichloromethane and vortex for 20 seconds, then add 2 ml of 2 M NaOH Solution and vortex for 30 seconds.
5. Centrifuge at 4000 × g for 5 minutes, then transfer 1 ml of supernatant to a fresh tube.
6. Add 200 µl of 6 M H_3PO_4 Solution and vortex mix fully.
7. Add 3 ml of Acetonitrile and vortex for 2 minutes, then centrifuge at 4000 × g for 10 minutes at room temperature.
8. Collect 1.5 ml of the upper organic liquid layer and transfer to a fresh tube, then dry using nitrogen evaporation or water bath.
9. Resuspend sample according to sample type:
   a. **Fish and Shrimp muscle tissue samples:** Dissolve the residue with 1 ml of 40 % Methanol Solution and vortex mix for 30 seconds, then collect 50 µl for analysis.
      Note: Sample dilution factor: 4; detection limit: 0.2 ng/ml
   b. **Livestock muscle samples:** Dissolve the residue with 2.5 ml of 40 % Methanol Solution and vortex mix for 30 seconds, then collect 50 µl for analysis.
      Note: Sample dilution factor: 10; detection limit: 0.5 ng/ml
   c. **Liver tissue samples:** Dissolve the residue with 2.5 ml of 40 % Methanol Solution and vortex mix for 30 seconds, then collect 50 µl for analysis.
      Note: Sample dilution factor: 10; detection limit: 0.5 ng/ml

B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Number the sample and ordered standard wells and record their positions. *All samples and standards should be tested in duplicate.*
2. Add 50 µl of standard or sample into the respective standard and sample wells.
3. Add 50 µl of Detection Reagent A to each well.
4. Cover the plate with a plate sealer, shake gently for 5 seconds to mix, and incubate at 25 °C for 30 minutes in the dark.
5. Remove the cover and wash the plate 5 times*. Discard the solution without touching the side walls. Blot the plate on an absorbent material. Add 300 µl of wash buffer to each well and soak for at least 30 sec. Discard the contents and clap the plate on absorbent filter papers or other absorbent material.*
6. Add 100 µl of Detection Reagent B to each well and incubate at 25 °C for 30 minutes in the dark
7. Wash: *Repeat Step 5*
8. Add 50 µl of Substrate Reagent A to each well.
9. Add 50 µl of Substrate Reagent B to each well. Shake gently for 5 seconds to ensure thorough mixing and incubate at 25 °C for 10-20 minutes in the dark.
10. Add 50 µl of Stop Solution to each well and shake gently to ensure thorough mixing.
11. Within 10 minutes of introducing the Stop Solution, determine the optical density of each well with a microplate reader at 450 nm.

C. Analysis of Results

1. Calculating Sample Concentration from Standard Curve

This assay is competitive, therefore there is an inverse correlation between Diethylstilbestrol concentration in the sample and the absorbance measured. The absorbance (%) should be calculated as follows:

$$\%\ \text{Absorbance} = \frac{A_S}{A_0} \times 100$$

where:

A_S	Average absorbance of the sample well
A_0	Average absorbance of the blank (0 µmol/L) well

Plot the Absorbance (%) on the y-axis, and the log concentration (ng/ml) on the x axis. Apply a best fit trendline through the standard points. Use this graph calculate sample concentrations based on their OD values. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

D. Precautions

Bring all reagents to room temperature prior to use.

1. Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.
2. Do not allow the wells to dry fully during the washing procedure. Ensure the Wash Buffer is thoroughly mixed, and the wells are washed completely.
3. Do not use the Substrate Reagent if it has begun to turn blue without being added to the wells.
4. Do not use any reagents that are expired, or use reagents from other kits with this assay.
5. The Stop Solution is caustic. Avoid contact with the skin or eyes, and thoroughly wash any spilled solution with water.
6. It is recommended that the same operator perform the experiment throughout, to avoid inconsistencies between operator procedures.