Version 06-05/24

User's Manual

Ethinylestradiol ELISA Kit

REF **DEIASL229**

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

For the quantitative determination of Ethinylestradiol concentrations in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants and other biological fluids.

General Description

Ethinylestradiol is a synthetic estrogen with high oral hormonal potency. It is rapidly reabsorbed from the gastrointestinal tract and the presence of an ethinyl at the 17-position reduces hepatic first-passage metabolism. It is excreted in urine and some is found in faeces. Ethinylestradiol is illegally used in animal husbandry as an anabolic and is excreted as the parent molecule in faeces, and as glucuronide or sulphate tracer in urine.

Principles of Testing

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with Ethinylestradiol(EE) protein. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to Ethinylestradiol(EE). Next,Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of Ethinylestradiol(EE) in the samples is then determined by comparing the OD of the samples to the standard curve.

Reagents And Materials Provided

1. Pre-coated Microplate: 8×12wells
2. Standard (lyophilized): 2
3. Standard Diluent Buffer: 20 mL
4. Biotinylated-Conjugate (100x): 60 µL
5. Biotinylated Conjugate Diluent: 10 mL
6. Streptavidin-HRP(100×): 120 µL
7. HRP Diluent: 12 mL
8. Wash Buffer (25×): 20 mL
9. TMB Substrate Solution: 9 mL
10. Stop reagent: 6 mL
11. Plate Covers: 4
12. Instruction manual: 1

Special Explanation

1. Store kit at 4°C immediately upon receipt.
2. Do not use the kit after the expiration date.
3. Please check whether all components are complete after opening the package. All kit components have been formulated and quality control tested to function successfully as a kit. Do not mix or substitute reagents or materials from other kit, performance cannot be guaranteed if utilized separately or substituted.

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 ± 10 nm.
2. High-speed centrifuge.
3. Electro-heating standing-temperature cultivator.
4. Absorbent paper.
5. Distilled or deionized water.
6. Single or multi-channel pipettes with high precision and disposable tips.
7. Precision pipettes to deliver 2μL to 1mL volumes.

Storage

The unopened kit should be stored at 2 - 8°C. Do not use the kit beyond the expiration date.

Specimen Collection And Preparation

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use .Avoid repeated freeze/thaw cycles

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use .Avoid repeated freeze/thaw cycles.

Tissue homogenates -The preparation of tissue homogenates will vary depending upon tissue type.

1. Tissues were rinsed in ice-cold PBS to remove excess blood thoroughly and weighed before homogenization.
2. Minced the tissues to small pieces and homogenized them in fresh lysis buffer (different lysis buffer needs to be chosen based on subcellular location of the target protein) (w:v=1:9, e.g. 900 μL lysis buffer is added in 100mg tissue sample) with a glass homogenizer on ice (Micro Tissue Grinders woks, too).
3. The resulting suspension was sonicated with an ultrasonic cell disrupter till the solution is clarified.
4. Then, the homogenates were centrifuged for 5 minutes at 10000×g. Collection the supernatant and assay immediately or aliquot and store at ≤-20°C.

Cell Lysates - Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1000×g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells three times in cold PBS.

3. Cells were then resuspended in fresh lysis buffer with concentration of 107 cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified.
4. Centrifuge at 1500×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤-20°C.

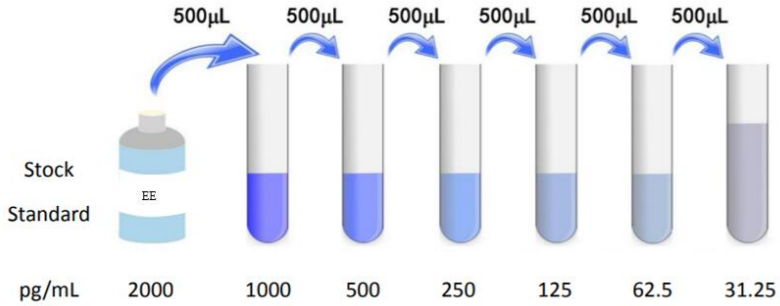
Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Note

1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Avoid repeated freeze/thaw cycles.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.
4. If the concentration of the test material in your sample is higher than that of the standard product, please make the appropriate multiple dilution according to the actual situation (it is recommended to do preliminary experiment to determine the dilution ratio.

Reagent Preparation

1. Bring all kit components and samples to room temperature (18-25°C) before use.
2. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and save the remaining strips and reagents as specified.
3. Dilute the 25x wash buffer into 1x working concentration with double steaming water.
4. Biotinylated-Conjugate (1x) - Centrifuge the vial before opening. Biotinylated-Conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10µL of Biotinylated-Conjugate with 990 µL of Biotinylated-Conjugate Diluent.
5. Standard -Reconstitute the Standard with 1.0mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently(not to foam). The concentration of the standard in the stock solution is 2000 pg/mL. Please prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 2000 pg/mL, 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.25 pg/mL, and the last EP tubes with Standard Diluent is the blank as 0 pg/mL. In order to guarantee the experimental results validity, please use the new standard solution for each experiment.



6. Streptavidin-HRP (1x) - Centrifuge the vial before opening. Streptavidin-HRP requires a 100-fold dilution. A suggested 100-fold dilution is 10 µL of Streptavidin-HRP with 990 µL of HRP Diluent.
7. TMB substrate - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Note

1. Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
2. Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.
3. All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
4. If crystals have formed in the Wash Solution concentrate (25×), warm to room temperature and mix gently until the crystals are completely dissolved.
5. Prepare standards within 15 minutes before assay. This standard can only be used once.
6. The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent color development. Warning TMB is toxic avoid direct contact with hands. Dispose of properly. If a dark blue color develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells. Dispense the TMB solution within 15 minutes following the washing of the microtiter plate.
8. It is highly recommended to use the remaining reagents within 1 month provided this is prior to the expiration date of the kit. For the expiration date of the kit, please refer to the label on the kit box.

Assay Procedure

1. Add the Standard working solution to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side (50 uL for each well). Add the samples to the other wells (50 uL for each well). Immediately add 50µL of Biotinylated Detection Ab working solution to each well. Cover the plate with the sealer provided in the kit. Incubate for 45 min at 37°C. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Aspirate or decant the solution from each well, add 350 uL of wash buffer to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps.
3. Add 100 µL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.
4. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 2.
5. Add 90 µL of Substrate Reagent to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min.
6. Add 50 µL of Stop Solution to each well. Note: Adding the stop solution should be done in the same order

as the substrate solution.

7. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Calculation

This assay employs the competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between EE concentration in the sample and the assay signal intensity. Average the duplicate readings for each standard, control, and samples. Create a standard curve on log-log or semi-log graph paper, with the log of EE concentration on the y-axis and absorbance on the x-axis. Draw the best fit straight line through the standard points and it can be determined by regression analysis. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. Using some plot software, for instance, curve expert.

Detection Range

31.25-2000 pg/mL

Sensitivity

9.34 pg/mL

Specificity

This assay has high sensitivity and excellent specificity for detection of Ethinylestradiol. No significant cross-reactivity or interference between Ethinylestradiol and analogues was observed.

Precautions

1. This kit is sold for lab research and development use only and not for use in humans or animals.
2. Reagents should be treated as hazardous substances and should be handled with care and disposed of properly.
3. Gloves, lab coat, and protective eyewear should always be worn, Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.