EAGLE BIOSCIENCES

Dihydrotestosterone (DHT) ELISA Assay Kit

Catalog Number:
DHT31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 3 (14 MAY 24)

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INTENDED USE

The Eagle Biosciences Dihydrotestosterone ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of Dihydrotestosterone in human serum. The Eagle Biosciences Dihydrotestosterone ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

5α-dihydrotestosterone (DHT) is a steroid similar to testosterone and androstenedione, which belong to a class called androgens. DHT is a C19 steroid and possesses androgenic activity. The bulk of androgen production takes place mainly in the Leydig cells of the testes. Androgens circulate in the blood bound to proteins, especially sex hormone binding globulin (SHBG) and albumin. A trace amount of these steroids circulate in the unbound form in the blood and are referred to as the free fractions. DHT has at least three times the binding affinity for SHBG than testosterone. In males about 70% of DHT is derived from peripheral conversion of testosterone, while in females most of the DHT is derived from androstenedione. The major organ to neutralize androgens is the liver. Therefore, in the liver the steroid hormones undergo structural modifications that are generally regarded as prerequisites for their biological inactivation. Some metabolites are formed and some are returned to the circulation before renal excretion. Therefore, elimination of steroids from the body is done through the urine.

Clinical Trends:

- In Klinefelter's syndrome the DHT level is much lower than that found in normal men.
- In idiopathic hirsutism about 40% of the patients have an increased level of DHT.
- In polycystic ovaries (PCO) about 35% of the patients have an increased DHT level.
- The DHT level in young people is much higher than those found in normal older people, hence androgen production increases at puberty which gives rise to masculinizing characteristics. It has been demonstrated that the human testes produce DHT, which appears to originate in the seminiferous tubules. Therefore, in tubular damage the production of DHT is impaired, which causes a decrease in the levels of plasma DHT (patients with germinal cell aplasia and azoospermia).
- There is a very low level of plasma DHT in patients with anorchia.
- It has been reported that in some prostate cancer (especially in stage D) the determination of DHT could be useful in predicting the response to anti-androgen therapy.

PRINCIPLE OF THE ASSAY

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, controls and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the color formed is inversely proportional to the concentration of DHT in the sample. A set of standards is used to plot a standard curve from which the amount of DHT in patient samples and controls can be directly read.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
    a. Do not pipette by mouth.
    b. Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
    c. Wear protective clothing and disposable gloves. d Wash hands thoroughly after performing the test. e Avoid contact with eyes, use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Do not use this kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or saliva pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The TMB Substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
15. Do not use blood contaminated saliva samples.
16. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit may be reported as >2500 pg/mL. If further dilution and retesting is required, only serum with a known low DHT concentration (<50 pg/ml) may be used to dilute serum samples.
18. Avoid microbial contamination of reagents.
19. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
20. To prevent contamination of reagents, do not pour reagents back into the original containers.
21. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.

22. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
26. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of saker used can influence the optical densities and test results. If a different type of shake and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse the microplate wells, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
29. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the participant is established.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be nonreactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. No test method however, can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.2 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 50, 100, 150 and 350 µL
2. Disposable pipette tips

3. Distilled or deionized water
4. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)

REAGENTS PROVIDED

1. **Rabbit Anti-DHT Antibody-Coated Break-Apart Well Microplate** — Ready To Use

   Contents: One 96-well (12x8) polyclonal antibody-coated microplate in a resealable pouch with desiccant.
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.

2. **Dihydrotestosterone (HRP) Conjugate Concentrate**— Ready to use

   Contents: DHT-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   Volume: 15 mL/Bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.

3. **Dihydrotestosterone Calibrators** — Ready To Use

   Contents: Seven vials containing DHT in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of DHT.

   * Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
A	0 pg/mL	1.0 mL
B	25 pg/mL	1.0 mL
C	100 pg/mL	1.0 mL
D	250 pg/mL	1.0 mL
E	500 pg/mL	1.0 mL
F	1000 pg/mL	1.0 mL
G	2500 pg/mL	1.0 mL

   Storage: Refrigerate at 2–8°C.
   Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. **Controls** — Ready to Use

   Contents: Two vials containing DHT in a protein-based buffer with a non-mercury preservative. Prepared by spiking serum with defined quantities of DHT. Refer to vial labels for the acceptable range.
   Volume: 1.0 mL/vial
   Storage: Refrigerate at 2–8°C
   Stability: 12 months in unopened vials or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. **Wash Buffer Concentrate** — Requires Preparation x10
   - Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
   - Volume: 50 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.
   - Preparation: Dilute the wash buffer concentrate 1:10 in distilled or deionized water to prepare the working wash buffer. If one whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

6. **TMB Substrate** — Ready To Use
   - Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
   - Volume: 16 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

7. **Stopping Solution** — Ready To Use
   - Contents: One bottle containing 1M sulfuric acid.
   - Volume: 8 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

ASSAY PROCEDURE

Specimen Pretreatment: None

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the DHT-HRP conjugate and wash buffer.
2. Remove the required number of well strips from the microplate and assemble into a plate frame. Reseal the bag and return any unused strips to the refrigerator.
3. **Pipette 50 µL** of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. **Pipette 100 µL** of the HRP conjugate working solution into each well. (We recommend using a multichannel pipette.)
5. Gently **shake** the plate for 10 seconds and incubate for **90 minutes** at room temperature (no shaking).
6. **Wash** the wells 3 times each time with **350 µL/well** of diluted wash buffer per well. After washing tap the plate firmly against absorbent paper to remove any residual liquid (the use of a washer is strongly recommended).
7. **Pipette 150 µL** of the TMB substrate into each well at timed intervals.
8. **Incubate** the microplate at room temperature (no shaking) for **30 minutes**.
9. **Pipette 50 µL** of stopping solution into each well at the same timed intervals as in step 7.

10. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

* If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

CALCULATIONS

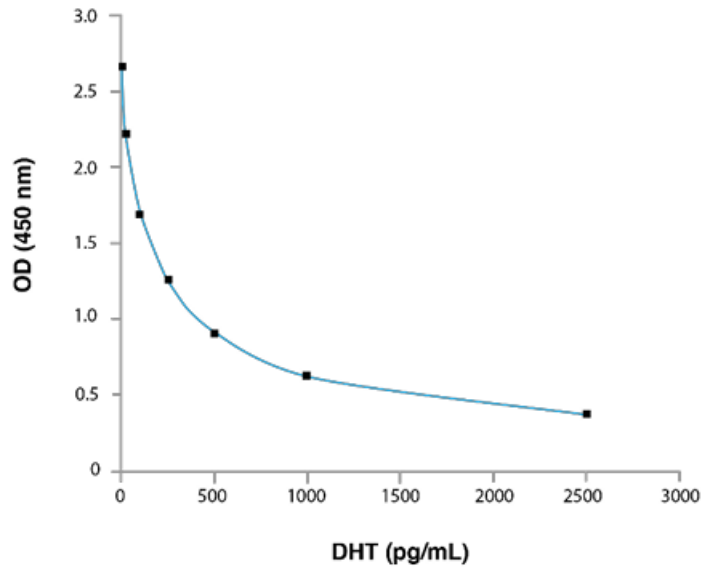
1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 2500 pg/mL then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	Mean OD	% Binding	Value (pg/mL)
A	2.664	100	0
B	2.225	84	25
C	1.695	64	100
D	1.261	47	250
E	0.911	34	500
F	0.622	23	1000
G	0.372	14	2500
Unknown	1.077	-	353

TYPICAL CALIBRATOR CURVE



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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.