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. 1.0

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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. 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.

2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of DHT in human serum. The kit is not calibrated for the determination of DHT in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only Calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. This kit is intended for research use only and should not be used as a diagnostic tool.

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 300 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-Horseradish Peroxidase (HRP) Conjugate Concentrate**— Requires Preparation X100
   - Contents: DHT-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   - Volume: 200 µL/vial
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.
   - Preparation: Dilute 1:100 in assay buffer before use (eg. 20 µL of HRP in 2 mL of assay buffer). If the whole plate is to be used dilute 120 µL of HRP in 12 mL of assay buffer. Discard any that is left over.

3. **Dihydrotestosterone Calibrators** — Ready To Use
   - Contents: Six 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	2.0 mL
B	25 pg/mL	0.6 mL
C	100 pg/mL	0.6 mL
D	500 pg/mL	0.6 mL
E	1000 pg/mL	0.6 mL
F	2500 pg/mL	0.6 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: 0.6 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. **Assay Buffer** – Ready to Use
   Contents: One bottle containing 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.

7. **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.

8. **Stopping Solution** — Ready To Use
   Contents: One bottle containing 1M sulfuric acid.
   Volume: 6 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 conjugate working solution into each well. (We recommend using a multichannel pipette.)
5. Gently shake the plate for 10 seconds and incubate for 1 hour at room temperature (no shaking).
6. Wash the wells 3 times each time with 300 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. Gently shake the plate for 10 seconds and incubate for 10–15 minutes at room temperature (no shaking), or until calibrator A attains dark blue colour for desired OD.
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

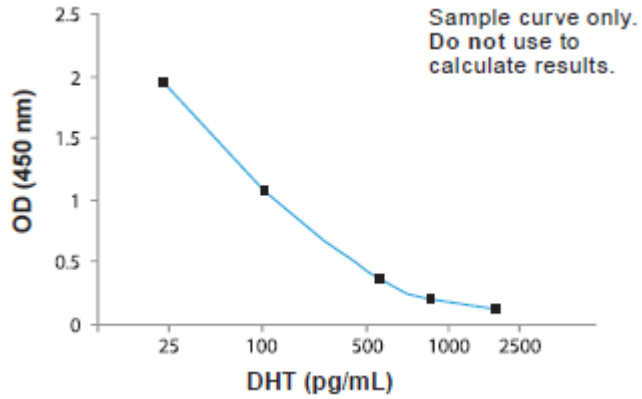
1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. 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	OD 1	OD 2	Mean OD	Value (pg/mL)
A	2.320	2.279	2.300	0
B	1.976	1.928	1.952	25
C	1.058	1.077	1.068	100
D	0.359	0.354	0.357	500
E	0.222	0.205	0.214	1000
F	0.131	0.128	0.130	2500
Unknown	0.515	0.507	0.511	300

TYPICAL CALIBRATOR CURVE



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the DBC Direct Dihydrotestosterone ELISA kit is 6.0 pg/mL.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct Dihydrotestosterone ELISA kit with dihydrotestosterone cross-reacting at 100%.

Steroid	% Cross Reactivity
Dihydrotestosterone	100
Testosterone	8.7
5ß Dihydrotestosterone	2.0
Androstendione	0.2

The following steroids were tested but cross-reacted at less than 0.01%: Dehydroepiandrosterone Sulfate, 17β-Estradiol, Estriol, Estrone, Progesterone, 17-OH Progesterone, Cortisol, and Pregnenolone.

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in pg/mL) are tabulated below:

Sample	Mean	SD	CV %
1	236.74	26.89	11.4
2	869.03	47.41	5.46
3	1008.14	39.36	3.90

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in pg/mL) are tabulated below:

Sample	Mean	SD	CV %
1	280.88	34.07	12.1
2	721.40	54.20	7.5
3	1025.41	60.45	5.9

RECOVERY
Spiked samples were prepared by adding defined amounts of DHT to three patient serum samples. The results (in pg/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1 Unspiked	290.54	-	-
+ 117.53	361.51	408.07	88.6
+ 235.06	501.66	525.60	95.4
+ 470.13	744.81	760.67	97.9
2 Unspiked	324.75	-	-
+ 117.53	389.43	442.29	88.0
+ 235.06	505.23	559.81	90.3
+ 470.13	712.44	794.88	89.6
3 Unspiked	720.11	-	-
+ 117.53	758.13	837.64	90.5
+ 235.06	856.46	955.17	89.7
+ 470.13	1013.61	1190.24	85.1

LINEARITY
Three patient serum samples were diluted with calibrator A. The results (in pg/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1	340.67	-	-
1:2	165.35	170.34	97.1
1:4	95.39	85.17	112.0
1:8	48.47	42.58	113.8
2	1086.01	-	-
1:2	508.58	543.00	93.7
1:4	232.11	271.50	85.5
1:8	114.95	135.75	84.7
3	1313.21	-	-
1:2	612.98	656.61	93.4
1:4	318.63	328.30	97.1
1:8	134.98	164.15	82.2

COMPARATIVE STUDIES
The Direct Dihydrotestosterone ELISA kit (Kit A) was compared with a competitors coated tube RIA kit (Kit B). The results (in pg/mL) are tabulated below:

Group	N	Kit A Mean	Kit B Mean
Females	10	95.5	99.0
Males	10	280.0	252.0

EXPECTED NORMAL VALUES
As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (µg/mL)
Females:	
Premenopausal	24-368
Postmenopausal	10-181
Males	250-990

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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.