Human Dihydrotestosterone / DHT ELISA Kit

Enzyme Immunoassay for the quantification of human Dihydrotestosterone in serum.

Catalog number: ARG80838

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INTRODUCTION

5 alpha-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

This assay employs the competitive enzyme immunoassay technique for the direct determination of DHT in human serum. A highly specific DHT antibody has been pre-coated onto a microtiter plate. DHT containing samples or standards and a DHT-HRP conjugate are given into the wells of the microtiter plate. Enzyme labeled-DHT and free DHT in standards and samples compete for the antibody binding sites. After incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of DHT is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate	4°C
Standard A (0 pg/ml)	2 ml (ready to use)	4°C
Standards B-F (25, 100, 500, 1000, 2500 pg/ml)	5 X 1 ml (ready to use)	4°C
Control high (327.5 pg/ml; Acc. Range: 245.6 – 409.4 pg/ml)	1 ml (ready to use)	4°C
Control low (54 pg/ml; Acc. Range: 32.4 – 75.6 pg/ml)	1 ml (ready to use)	4°C
101X HRP-DHT conjugate	0.25 ml	4°C
Assay buffer	17 ml (ready to use)	4°C
10X Wash buffer	2 X 50 ml	4°C
TMB substrate	18 ml (ready to use)	4°C (Protect from light)
STOP solution	8 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-DHT Conjugate before use.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- 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.
- Ensure complete dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Controls and standards should be included in every run and fall within established confidence limits.
- 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.
- Change pipette tips between the addition of different reagent or samples.
- When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
- The substrate solution (TMB) 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.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note:

- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
- Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. If the whole plate is to be used dilute 50mL of the wash buffer concentrate in 450mL of water. Store the diluted wash buffer at 2°C- 8°C.
- HRP-DHT: Dilute 1:100 with assay buffer before use. (eg. 20 µL of HRP-DHT 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.
- Samples: If a sample reads more than 2500 pg/ml then dilute it with Standard A (0 pg/ml) at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates. Once the procedure has been started, all steps should be completed without interruption.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it. Store the used strips at 2-8°C
2. Add **50 µl** of each **Standard, Control and sample** in duplicate into appropriate wells of the antibody-coated plate.
3. Add **100 µl** of **freshly prepared HRP-DHT conjugate** into each well.
4. Cover wells and **gently shake** the plate **for 10 seconds**. **Incubate** the plate without shaking for **60 minutes at RT**.
5. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add **150 µl** of **TMB Substrate** to each well. Incubate the plate without shaking for **10-15 minutes (or until Standard A attains dark blue color for desired OD) at room temperature** in dark.
7. Add **50 µl** of **Stop Solution** to each well. Add stop solution at the same timed intervals as in step 6.
8. Read the OD with a microplate reader at **450 nm** immediately. It is

recommended read the absorbance within 20 minutes after adding the stopping solution.

Note:

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.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.

2. Using semi-log graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

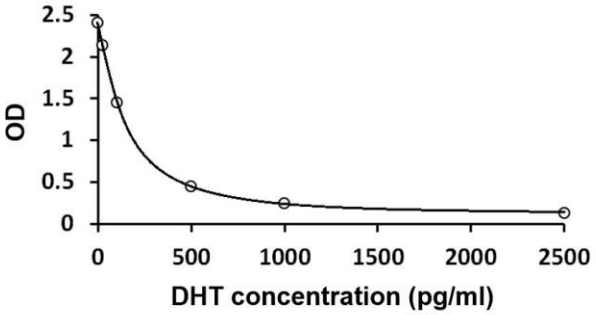
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. If a sample reads more than 2500 pg/ml then dilute it with Standard A (0 pg/ml) at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

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 20 replicate analyses) minus 2 SD. Therefore, the sensitivity of the Dihydrotestosterone ELISA kit is 7.23 pg/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 6.92% and inter-assay precision was 8.5%.

Specificity

The following substances were tested for cross reactivity of the assay:

Steroid	% Cross Reactivity
Dihydrotestosterone	100
Testosterone	8.7
5β Dihydrotestosterone	2.0
Androstenedione	0.2
Dehydroepiandrosterone Sulfate	< 0.01
17 beta-Estradiol	< 0.01
Estriol	< 0.01
Estrone	< 0.01
Progesterone	< 0.01
17-OH Progesterone	< 0.01
Cortisol	< 0.01
Pregnenolone	< 0.01

Recovery

85.1-97.9%

Linearity

82.2 – 113.8

EXPECTED VALUES

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

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