DIHYDROTESTOSTERONE (DHT)

Cat. No.: EKD0012
Version: 5.0

Effective: May 09, 2014

INTENDED USE

For the direct quantitative determination of Dihydrotestosterone by enzyme immunoassay in human serum.

In vitro use only.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, controls and patient samples) and an enzyme-labeled antigen for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added and the appropriate reaction is terminated by addition of the stopping solution. The absorbance is measured at 450nm and the concentration of the analyte in the specimen is determined using a microtiter plate reader.

PROCEDURAL CAUTIONS AND WARNINGS

1. Users should review and understand 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 to ensure precision and accuracy of the results.

3. When the urine is used 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 specimens.

5. Avoid repeated freezing and thawing of reagents and standardization.

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 affect the results.

9. 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.

10. The substrate solution (TMB) is sensitive to light and should be protected from light during storage.

11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with your skin.

12. To prevent contamination of reagents, use a disposable pipette tip for dispensing each reagent, sample, control, standard, and specimen.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of DHT in saliva, plasma or other whole body fluids.

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. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should be based on the patient’s background including the frequency of exposure to animals/products if false results are suspected.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARD MATERIAL

Dihydrotestosterone Human serum that may be used in the preparation of the standards and controls and has been tested and found to be non-reactive 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. However no test method 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

Appropriate anticoagulants are required for duplicate determination. Collect 4-5 ml of blood into an appropriately labeled vacutainer. 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

Precipitating reagent to disperse 50, 100, 150 and 300 μl.
Disposable pipette tips.
Distilled or deionized water.
Microwell plate reader with a filter set at 450nm and an acceptable range.

Equipment:

B) Microwell plate reader with a filter set at 450nm and an acceptable range.

STABILITY

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 buffered concentrate. The entire kit should be used as prepared by spiking buffer with defined quantities of DHT. Refer to vial labels for the acceptance range.

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.

Contents: One bottle containing a non-ionic detergent and a non-mercury preservative.

Stability: 50 ml/bottle
Storage: Refrigerate at 2-8°C

6. Assay Buffer - Ready To Use.

Contents: One vial containing a protein-based buffer with a non-ionic detergent and a non-mercury preservative.

Stability: 15 ml/vial
Storage: Refrigerate at 2-8°C

7. TMB Substrate - Ready To Use.

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide, and found to be non-DMF or DMSO containing buffer.

Stability: 12 months or as indicated on label.

8. Stopping Solution - Ready To Use.

Contents: One vial containing 1 ml sulfuric acid.

Stability: 12 months or as indicated on label.

3. Dihydrotestosterone Calibrators - Ready To Use.

Contents: Six calibrators containing DHT in a protein-based buffered 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.

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Concentration</th>
<th>Volume Vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator A</td>
<td>2.0 μl</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>Calibrator B</td>
<td>25 pg/ml</td>
<td>0.6 ml</td>
</tr>
<tr>
<td>Calibrator C</td>
<td>100 pg/ml</td>
<td>0.6 ml</td>
</tr>
<tr>
<td>Calibrator D</td>
<td>500 pg/ml</td>
<td>0.6 ml</td>
</tr>
<tr>
<td>Calibrator E</td>
<td>1000 pg/ml</td>
<td>0.6 ml</td>
</tr>
<tr>
<td>Calibrator F</td>
<td>2500 pg/ml</td>
<td>0.6 ml</td>
</tr>
</tbody>
</table>

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.

DIHYDROTESTOSTERONE (DHT)

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 regarded as hazardous waste and should be incinerated. In all clinical scenarios the determination of DHT should be regarded as hazardous waste and the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

It has been demonstrated that the human sexual organ is the liver. The major organ to neutralize androgens is the liver.

The intensity of the colour formed is inversely proportional to the concentration of DHT in the sample. A standard is used to plot a standard curve from which the amount of DHT in patient samples and controls can be directly read.

CLINICAL APPLICATIONS

Serum DHT is a steroid similar to testosterone and androsterone, which belong to a class of 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 a glycoprotein called 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 unbound form in the blood and are referred to as the free fractions. DHT has at least three unbound form in the blood and are referred to as the free fractions. DHT has at least three unbound form in the blood and are referred to as the free fractions. DHT has at least three unbound form in the blood and are referred to as the free fractions. DHT has at least three unbound form in the blood and are referred to as the free fractions.

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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 microwell strips. 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 washes 3 times with 300 μl of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry (The use of a washer is recommended).
7. Pipette 150 μl of TMB substrate into each well at 25°C.
8. Gently shake the plate for 10 seconds and incubate for 10-15 minutes at room temperature (no-shaking) (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 in as step 7.
10. Read the plate on a microowell plate reader at 450nm within 20 minutes after addition of the stopping solution.

* If the OD exceeds the upper limit of detection or if a 450nm filter is unavailable, a 405 or 415nm 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 immunnoasay 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 1:1. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>OD 1</th>
<th>OD 2</th>
<th>Mean OD</th>
<th>Value (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.300</td>
<td>2.279</td>
<td>2.300</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>1.976</td>
<td>1.938</td>
<td>1.958</td>
<td>100</td>
</tr>
<tr>
<td>C</td>
<td>1.000</td>
<td>0.977</td>
<td>1.000</td>
<td>1000</td>
</tr>
<tr>
<td>D</td>
<td>0.359</td>
<td>0.354</td>
<td>0.357</td>
<td>500</td>
</tr>
<tr>
<td>E</td>
<td>0.222</td>
<td>0.205</td>
<td>0.214</td>
<td>1000</td>
</tr>
<tr>
<td>F</td>
<td>0.139</td>
<td>0.129</td>
<td>0.134</td>
<td>2500</td>
</tr>
</tbody>
</table>

TYPICAL CALIBRATOR CURVE

Sample curve only. Do not use to calculate results.

SPECIFICITY (CROSS ACTIVITY)

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

INTRA-ASSAY PRECISION

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

<table>
<thead>
<tr>
<th>Sample Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>280.88</td>
<td>34.07</td>
</tr>
<tr>
<td>2</td>
<td>721.40</td>
<td>54.20</td>
</tr>
<tr>
<td>3</td>
<td>1025.41</td>
<td>60.45</td>
</tr>
</tbody>
</table>

INTER-ASSAY PRECISION

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

<table>
<thead>
<tr>
<th>Sample Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>280.88</td>
<td>34.07</td>
</tr>
<tr>
<td>2</td>
<td>721.40</td>
<td>54.20</td>
</tr>
<tr>
<td>3</td>
<td>1025.41</td>
<td>60.45</td>
</tr>
</tbody>
</table>

RECOVERY

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Obs.Result</th>
<th>Exp.Result</th>
<th>Recovery%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Unspiked</td>
<td>290.54</td>
<td>115.73</td>
<td>44.47</td>
</tr>
<tr>
<td>2 Unspiked</td>
<td>324.75</td>
<td>115.73</td>
<td>44.29</td>
</tr>
<tr>
<td>3 Unspiked</td>
<td>720.11</td>
<td>115.73</td>
<td>43.64</td>
</tr>
</tbody>
</table>

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 Biomatik Direct Dihydrotestosterone ELISA kit is 0.4 pg/ml.

Specificity

Inhibition of four androgenic steroids:

1. Testosterone
2. 5α-Dihydrotestosterone
3. Androstenedione

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

EXPECTED NORMAL VALUES

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

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:

<table>
<thead>
<tr>
<th>Group</th>
<th>Steroid</th>
<th>N</th>
<th>Kit A Mean</th>
<th>Kit B Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>10</td>
<td>95.5</td>
<td>99.0</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>280.0</td>
<td>252.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

REFERENCES


CONTACT INFORMATION

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