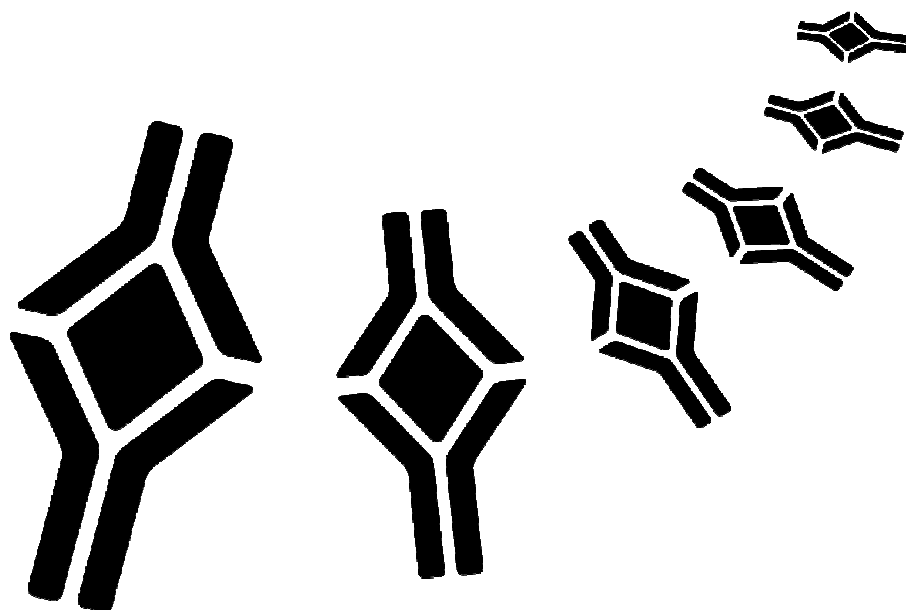


BioVendor

Research
and Diagnostic Products



DIHYDROTESTOSTERONE (DHT) ELISA

Product Data Sheet

Cat. No.: RCAN-DHT-280R

For Research Use Only

CONTENTS

1.	INTENDED USE	3
2.	PRINCIPLE OF THE TEST	3
3.	CLINICAL APPLICATIONS	3
4.	PROCEDURAL CAUTIONS AND WARNINGS	5
5.	LIMITATIONS	6
6.	SAFETY CAUTIONS AND WARNINGS	7
7.	CHEMICAL HAZARDS	7
8.	SPECIMEN COLLECTION AND STORAGE	7
9.	SPECIMEN PRETREATMENT	8
10.	REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED	8
11.	REAGENTS PROVIDED	8
12.	ASSAY PROCEDURE	11
13.	CALCULATIONS	12
14.	TYPICAL TABULATED DATA	12
15.	TYPICAL CALIBRATOR CURVE	13
16.	PERFORMANCE CHARACTERISTICS	13
17.	EXPECTED NORMAL VALUES	17
18.	REFERENCES	17

**»» This kit is manufactured by:
BioVendor – Laboratorní medicína a.s.**

»» Use only the current version of Product Data Sheet enclosed with the kit!

1. INTENDED USE

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

For research use only.

2. 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-labelled antigen (conjugate) 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. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour 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.

3. CLINICAL APPLICATIONS

5 α -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.

4. 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 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 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.

5. 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. 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 include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

6. SAFETY CAUTIONS AND WARNINGS

6.1 Potential biohazardous material

Human serum that may be used in the preparation of the standards and controls 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.

7. 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.

8. 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.

9. SPECIMEN PRETREATMENT

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

10. 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. Plate shaker
5. Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10).

11. REAGENTS PROVIDED

11.1 Rabbit Anti-DHT Antibody Coated Microwell Plate-Break Apart Wells –

Ready To Use.

Contents: One 96 well (12x8) polyclonal antibody-coated microwell plate in a resealable pouch with desiccant.

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

11.2 Dihydrotestosterone-Horseradish Peroxidase (HRP) Conjugate Concentrate -

Requires Preparation.

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 12ml of assay buffer. Discard any that is left over.

11.3 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
Calibrator A	0 pg/ml	2.0 ml
Calibrator B	25 pg/ml	0.6 ml
Calibrator C	100 pg/ml	0.6 ml
Calibrator D	500 pg/ml	0.6 ml
Calibrator E	1000 pg/ml	0.6 ml
Calibrator 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.

11.4 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 vial 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.

11.5 Wash Buffer Concentrate - Requires Preparation.

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 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.

11.6 Assay Buffer - Ready To Use.

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

Volume: 15 ml/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

11.7 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.

11.8 Stopping Solution - Ready To Use.

Contents: One vial containing 1M sulfuric acid.

Volume: 6 ml/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label

12. ASSAY PROCEDURE

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. Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
6. Wash the wells 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 timed intervals.
8. Incubate on a plate shaker for 10-15 minutes at room temperature (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 microwell plate 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 450nm 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.

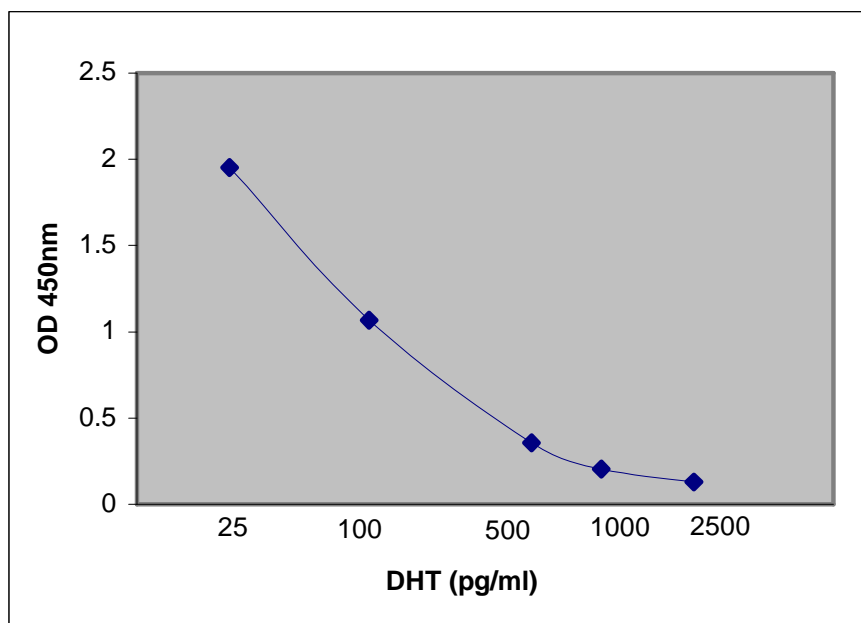
13. CALCULATIONS

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.

14. TYPICAL TABULATED DATA

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

15. TYPICAL CALIBRATOR CURVE



16. PERFORMANCE CHARACTERISTICS

16.1 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 BioVendor Dihydrotestosterone ELISA kit is **6.0 pg/ml**.

16.2 Specificity (cross reactivity)

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

Steroid	%Cross Reactivity
Dihydrotestosterone	100
Testosterone	8.7
5 β Dihydrotestosterone	2.0
Androstenedione	0.2

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

16.3 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

16.4 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

16.5 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

16.6 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

16.7 Comparative studies

The BioVendor 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

17. EXPECTED NORMAL VALUES

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

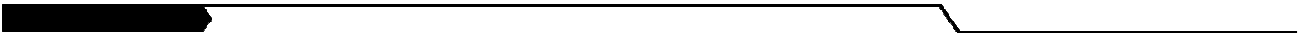
Group	Range (pg/ml)
Females:	
Premenopausal	24-368
Postmenopausal	10-181
Males	250-990

18. REFERENCES

1. Bassett, R.M., A simple chromatographic method for the radioimmunoassay of four androgenic steroids. Medical Laboratory Sciences, 37:31, 1980.
2. Baxendale, P.M., et al., Plasma and salivary androstenedione and dihydrotestosterone in women with hyperandrogenism. Clinical Endocrinology, 18:447, 1983.
3. Brooks, R.V., Androgens. Physiology and Pathology In: Makin, H.L. J., ed., Biochemistry of Steroid Hormones, 2nd ed., Oxford Blackwell Scientific Publications, 565:1984.
4. Cameron, E.H.D., In proceedings of the fifth tenovous workshop, Steroid Immunoassay, ed., Cameron, E.H.D., et al., Alpha Omega Publishing Cordiff, 1975.
5. Dunn, J.F., et al, Transport of Steroid Hormones: Binding of 21 endogenous steroids to both SHBG and CBG in human plasma. J. Clin. Endocr. Metab. 53:58, 1981.
6. Hammond, G.L., et al, The simultaneous radioimmunoassay of seven steroids in human spermatic and peripheral venous blood. J. Clin. Endocr. Metab. 45:16, 1977.
7. Ito, T., et al, Dihydrotestosterone in human peripheral plasma. J.Clin. Endocr. 31:362, 1970.
8. Mean, F., et al, Study of the binding of dihydrotestosterone, testosterone and oestradiol with sex hormone binding globulin. Clinica Cemica Alta 80:171, 1977.

9. Mooradian, A.D., et al, The biological actions of androgens. *Endocr. Rev.* 8:1-28, 1987.
10. Pazzagli, M., et al, Radioimmunoassay of plasma dihydrotestosterone in normal and hypogonadal men. *Clin. Endocr.* 82:380, 1976.
11. Wakelin, K., et al, Relationship of 5 β dihydrotestosterone and 5 α dihydrotestosterone to testosterone in health and disease. *J. Endocrinol.* 87:450, 1980.
12. Wang, C., et al, Solitary androgens in hirsutism: Are they of use in routine evaluation. *Ann. Clin. Biochem.* 23:590, 1986.
13. Kricka, L.J., Human anti-animal antibody interferences in immunological assays. *Clin. Chemistry* 45:7, 1999.
14. Check, J.H., et al, Falsely elevated steroidal assay levels related to heterophile antibodies against various animal species. *Gynecol Obstet Invest* 40:139-140, 1995.

NOTES





HEADQUARTERS: BioVendor - Laboratorní medicína a.s.	Karasek 1767/1	621 00 Brno CZECH REPUBLIC	Phone: +420-549-124-185 Fax: +420-549-211-460	E-mail: info@biovendor.com Web: www.biovendor.com
EUROPEAN UNION: BioVendor GmbH	Im Neuenheimer Feld 583	D-69120 Heidelberg GERMANY	Phone: +49-6221-433-9100 Fax: +49-6221-433-9111	E-mail: infoEU@biovendor.com
USA, CANADA AND MEXICO: BioVendor LLC	1463 Sand Hill Road Suite 227	Candler, NC 28715 USA	Phone: +1-828-670-7807 +1-800-404-7807 Fax: +1-828-670-7809	E-mail: infoUSA@biovendor.com
CHINA - Hong Kong Office: BioVendor Laboratories Ltd	Room 4008 Hong Kong Plaza, No.188	Connaught Road West Hong Kong, CHINA	Phone: +852-2803-0523 Fax: +852-2803-0525	E-mail: infoHK@biovendor.com
CHINA – Mainland Office: BioVendor Laboratories Ltd	Room 2917, 29/F R & F Ying Feng Plaza, No.2 Huaqiang road	Pearl River New Town Guang Zhou, CHINA	Phone: +86-20-38065519 Fax: +86-20-38065529	E-mail: infoCN@biovendor.com