DATA SHEET

User´s Manual

5α Dihydrotestosteronihydrotestosterone (DHT) ELISA
Enzyme Immunoassay for the quantitative determination of 5α- Dihydrotestosterone in human serum

INTENDED USE

Enzyme immunoassay for the *in-vitro-diagnostic* quantitative determination of 5α-Dihydrotestosterone in human serum.

DE2330

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SUMMARY AND EXPLANATION

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.

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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.

TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the competition principle. An unknown amount of antigen present in the sample and a fixed amount of enzyme labelled antigen compete for the binding sites of the antibodies coated onto the wells. After incubation the wells are washed to stop the competition reaction. After the substrate reaction the intensity of the developed color is inversely proportional to the amount of the antigen in the sample. Results of samples can be determined directly using the standard curve.

WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See

MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available o upon request.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.
9. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters. The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2–8°C.
Once opened standards and controls should be used within 14 days or aliquoted and stored frozen.

SPECIMEN COLLECTION AND STORAGE

Serum

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Do not use specimens containing NaN3 or Thimerosal, as they may lead to false results. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage	4°C	≤ - 10°C (Aliquots)	Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles.
Stability	24 h	≥ 24 h	

MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. coated with rabbit anti-DHT antibody (polyclonal).
1 x 0.2 mL	ENZCONJ CONC	**Enzyme Conjugate,** Concentrate (100 x) Contains: DHT-HRP Conjugate, in protein-containing buffer, non-mercury preservatives.
1 x 2 mL	CAL A	**Standard A** 0 pg/mL Ready to use. Contains: Dihydrotestosterone, in protein-containing buffer, non-mercury preservatives. Exact concentrations see vial labels or QC certficate.
5 x 0.6 mL	CAL B-F	**Standard B-F** 25; 100; 500; 1000; 2500 pg/mL Ready to use. Contains: Dihydrotestosterone, in protein-containing buffer, non-mercury preservatives. Exact concentrations see vial labels or QC certficate.
1 x 0.6 mL	CONTROL+	**Positive Control** Ready to use. Contains: DHT in protein-containing buffer, non-mercury preservatives. Exact concentrations see vial labels or QC certficate.
1 x 0.6 mL	CONTROL-	**Negative Control** Ready to use. Contains: DHT in protein-containing buffer, non-mercury preservatives. Exact concentrations see vial labels or QC certficate.
1 x 16 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains: TMB and hydrogen peroxide in a non-DMF or DMSO containing buffer.
1 x 6 mL	TMB STOP	**TMB Stop Solution** Ready to use. 2 N acidic solution.
1 x 50 mL	WASHBUF CONC	**Wash Buffer,** Concentrate (10 x) Contains: Buffer with non-ionic detergent and non-mercury preservatives.
1 x 15 mL	ASSAYBUF	**Assay Buffer** Ready to use. Contains: protein-containing buffer, non-mercury preservatives.
1 x	FOIL	**Adhesive Foil**

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volume: 50, 100, 150, 300 µL
2. Vortex mixer
3. Orbital shaker (200-900 rpm) (e.g. EAS 2/4, SLT)
4. 8-Channel Micropipettor with reagent reservoirs
5. Wash bottle, automated or semi-automated microtiter plate washing system
6. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
7. Bidistilled or deionised water
8. Paper towels, pipette tips and timer

PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Some components contain £ 250 µL solution. Take care that the solution is completely on the bottom of the vial before opening.
5. It is advised to determine samples in duplicate to be able to identify potential pipetting nerrors.
6. Use a pipetting scheme to verify an appropriate plate layout.
7. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
8. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
9. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

PRE-TEST SETUP INSTRUCTIONS

Preparation of lyophilized or concentrated components

Dilute / dissolve	Component	with	Diluent	Relation	Storage	Stability
50 mL	WASHBUF CONC	450 mL	bidist. water	1:10	2-8°C	12 month
120 µL	ENZCONJ CONC	12 mL	ASSAYBUF	1:100	Discard any that is left over.	

Samples containing concentrations higher than the highest standard have to be diluted further up to 1:8 with Standard A and reassayed. The result obtained should be multiplied by the dilution factor.

TEST PROCEDURE

1. Pipette **50 µL** of each **Calibrator, Control and sample** into the respective wells of the Microtiter Plate.
2. Pipette **100 µL** of freshly prepared **Enzyme Conjugate (1:100)** into each well.
3. **Incubate 1 h** at **RT (18-25°C)** on an orbital shaker (approx. 200 rpm).
4. Wash plate **3 x** with **300 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
5. For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
6. Pipette **150 µL** of **TMB Substrate Solution** into each well.
7. **Incubate 10 - 15 min** at **RT (18-25°C)** (or until calibrator A attains dark blue colour for desired OD).
8. Stop the substrate reaction by adding **50 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
9. **Measure** optical density with a photometer at **450 nm*** within **20 min.** *) 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.

QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All kit controls must be found within the acceptable ranges as stated on the vial labels. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

CALCULATION OF RESULTS

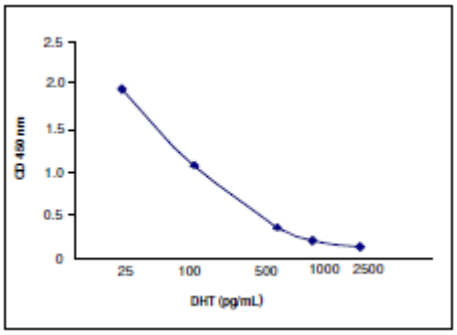
The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logisitcs or Logit-Log. For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used). The concentration of the samples can be read directly from the standard curve. Samples showing concentrations above

the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	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	.0128	0.130	2500
Unknown	0.515	0.507	0.511	300



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

PERFORMANCE

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 Dihydrotestosterone ELISA kit is 6.0 pg/mL.

Specificity (Cross Reactivity)
The following compounds were tested for cross-reactivity with the Dihydrotestosterone ELISA kit with dihydrotestosterone cross-reacting at 100%.

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

LITERATURE 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 bhyperandrogenism. Clinical Endocrinology, 18:447, 1983.
3. Brooks, R.V., Androgens. Physiology and PathologyIn: 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., etal., 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 5ßdihydrotestosterone and 5αdihydrotestosterone 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.

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SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

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