



DHT ELISA



BL-46-E

IN VITRO DIAGNOSTIC USE

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1 INTRODUCTION

The **5 α -Dihydrotestosterone (DHT) Enzyme Immunoassay** Kit provides materials for the quantitative determination of dihydrotestosterone in serum and plasma. This assay is intended for in vitro diagnostic use.

5 α -Dihydrotestosterone (DHT) is a steroid similar to testosterone and androstenedione; which belong to a class called androgens.

5 α -Dihydrotestosterone 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 (free) form in blood.

Dihydrotestosterone has at least 3 times the binding affinity to SHBG than does Testosterone. In males about 70 % of DHT is derived from peripheral conversion of testosterone. However, in females most of the DHT is derived from androstenedione. In the body testosterone is converted to DHT, estradiol and 5 α -androstane-3 α ,17 β -diol.

The major organ to metabolize androgens is the liver. Therefore in the liver the steroid hormones undergo structural modifications which is 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.

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 levels of young people are much higher than those found in normal older people, hence androgens 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.

2 PRINCIPLE OF THE TEST

The **Bio-Line DHT ELISA KIT** procedure is an enzyme immunoassay, which is based on the principle of competitive binding. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a DHT molecule. An aliquot of patient sample containing endogenous DHT is incubated in the coated well with enzyme conjugate, which is an anti-DHT antiserum conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off with aqua dest. The amount of bound peroxidase is proportional to the concentration of DHT in the sample. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of DHT in the patient sample.

3 PRECAUTIONS

- This kit is for in vitro diagnostic use only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Avoid contact with Stop Solution containing 0.3 M H₂SO₄. It may cause skin irritation and burns.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even if the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
- Safety Data Sheets for this product are available upon request.
The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

4 KIT COMPONENTS

4.1 Contents of the Kit

1.

U	U	U
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 12x8 (break apart) strips, 96 wells
Wells coated with anti-Dihydrotestosterone antiserum (rabbit, polyclonal)
2.

CAL	N
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 N=1 to 5, Calibrator B-F, 5 vials, 0.6 ml each, ready to use
See exact values on vial labels
3.

CAL	0
-----	---

 Calibrator A, 1 vial, 1.0 ml, ready to use
0 pg/ml
4.

Ag	HRP	CONC
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 concentrated (100x) 1 vial, 0.2 ml,
Dihydrotestosterone conjugated to horseradish peroxidase
see „Preparation of Reagents“
5.

CHROM	TMB
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 1 vial, 16ml, ready to use
TMB
6.

STOP	SOLN
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 1 vial, 6 ml, ready to use
contains 0.3M H₂SO₄
Avoid contact with the stop solution. It may cause skin irritations and burns.

- | | | | | |
|----|---------|------|---|---|
| 7. | CONTROL | N | 1 vial, 0.5 ml, ready to use
(Concentration is mentioned on the separate QC sheet) | |
| 8. | WASH | SOLN | CONC | concentrate (10x), 1 vial, 50 ml
see „Preparation of Reagents“ |
| 9. | CONJ | BUF | | Conjugate Buffer vial, 11 ml, ready to use |

Note: Additional Zero Calibrator for Sample dilution available on request.

4.2 Equipment and material required but not provided

1. A microtiterplate calibrated reader (450±10 nm).
2. Calibrated variable precision micropipettes (Varipette Eppendorf), Multipette Eppendorf or similar products.
3. Absorbent paper.
4. Distilled water.

4.3 Storage and stability of the Kit

- When stored at 2° to 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
- Enzyme-Conjugate, Substrate Solution, Calibrators and Zero Calibrator must be stored at 2° to 8°C.
- Microtiter wells must be stored at 2° to 8°C. Once the foil bag has been open care should be taken to close it tightly again.
- Assay Buffer: Ready to use buffer is stable for 6 month when stored at 2 - 8 °C.
- Control: Once opened the control should be stored in aliquots at -20°C.
- Enzyme Conjugate: Diluted conjugate is stable up to 60 minutes at room temperature.

4.4 Preparation of Reagents

Allow all reagents and required number of strips to reach room temperature prior to use.

Enzyme Conjugate

Dilute 1 to 100 with conjugate buffer before use (e.g. 10 µl of conjugate in 1 ml of assay buffer). If the whole plate is used, dilute 110 µl of conjugate in 11 ml of assay buffer. Store unused diluted conjugate up to 4 days at 2 - 8 °C.

NOTE: Prior to dilution make sure that no liquid will remain in the stopper.

Wash Buffer

20 ml of the concentrate have to be diluted 1:10 with Aqua dest. up to 200 ml. This gives the ready for use Wash Buffer. Occasionally crystals will form. Warm solution to ensure completely dissolved before use.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national official regulations. Special information for this product are given in the Material Safety Data Sheets.

4.6 Damaged Test Kits

In case of any severe damage of the test kit or components, Bio-Line Europe have to be informed written, latest one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

4.7 Storage

Serum/heparinized plasma

The serum/plasma obtained should be applied in the assay after storing for up to 24 hours at 2 - 8°C. If stored for longer periods the serum should be stored in aliquots at -20°C. Repeated freeze-thaw cycles have to be avoided.

5 SPECIMEN

5.1 Specimen collection

Serum or heparinized plasma should be used. EDTA additives will interfere with the assay! Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not use haemolytic, icteric or lipaemic serum.

5.2 Specimen storage

The serum/plasma obtained should be applied in the assay after storing for up to 24 hours at 2 - 8°C. If stored for longer periods the serum should be stored in aliquots at -20°C. Repeated freeze-thaw cycles have to be avoided.

5.3 Specimen dilution

If in an initial assay, a serum specimen is found to contain more than the highest calibrator, the specimens can be diluted 10-fold with Zero Calibrator and reassayed as described in Assay Procedure.

6 TEST PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipet tips for each calibrator, control of sample in order to avoid crosscontamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents be ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature. Therefore, if the Optical Density is too high or too low, the substrate incubation time can be decreased or increased, respectively.

6.2 Procedural Notes

- All calibrators, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
- The concentration of the samples can be read directly from this calibrator curve. Samples with a concentration higher than that of the highest calibrator have to be diluted 1 : 10 with Zero Calibrator. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3 Assay Procedure

1. Secure the desired number of Microtiterwells in the holder.
2. Dispense **50 µl** of Calibrators controls and samples **with new disposable tips** into appropriate wells.
3. Dispense **100 µl** Enzyme Conjugate into each well.
4. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for **60 minutes** at room temperature on a shaker.
6. Briskly shake out the contents of the wells.
Rinse the wells 3 times with wash buffer (dilute concentrate 1 to 10 with distilled water). Strike the wells sharply on absorbent paper to remove residual water droplets.

Important note:

The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

7. Add **150 µl** of Substrate Solution to each well.
8. Incubate for **10-15 minutes** at room temperature on a shaker.
9. Stop the enzymatic reaction by adding **50 µl** of Stop Solution to each well.
10. Read the OD at **450±10 nm** with a microtiterplate reader **within 10 minutes** after adding the Stop Solution.

6.4 Calculation of Results

1. Calculate the average absorbance values for each set of calibrators, controls and patient samples.
2. Construct a calibrator curve by plotting the mean absorbance obtained from each calibrator against its concentration in pg/ml 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 of DHT in pg/ml from the calibrator curve. Depending on experience and/or the availability of computer capability, other methods of data deduction may be employed.
4. Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log can generally give a good fit.
5. The concentration of the samples can be read directly from this calibrator curve. Samples with DHT concentration higher than that of the highest calibrator have to be diluted with zero calibrator. For the calculation of the concentrations this dilution factor has to be taken into account.

Below is listed a typical example of a calibrator curve with the DHT ELISA.

Calibrators (pg/ml)	OD 450 nm
0	2.096
25	1.528
100	1.166
500	0.512
1000	0.347
2500	0.250

7 ASSAY CHARACTERISTICS

7.1 Expected values

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

	pg/ml
Females:	
Premenopausal	24 - 368
Postmenopausal	10 - 181
Males:	250 - 990

7.2 Specificity

STERIOD	% CROSS-REACTIVITY	Steroid	% cross-reactivity
Dihydrotestosterone	100.0	17β-Estradiol	< 0.01
Testosterone	8.7 *	Estriol	< 0.01
5β-Dihydrotestosterone	2	Estrone	< 0.01
Androstenedione	0.2	Progesterone	< 0.01
Dehydroepiandrosteronesulfat	< 0.01	17-OH-Progesterone	< 0.01
Cortisol	< 0.01	Pregnenolone	< 0.01

* This cross-reactivity does not influence the test results of this ELISA due to a specific complexing buffer system which blocks the binding of testosterone to the antibody.

7.3 Sensitivity

The minimum detectable concentration of DHT by this assay is estimated to be 9.7 pg/ml.

7.4 Accuracy

Quality Control

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

7.5 Precision

7.5.1 Intra Assay Variation

The within assay variability is shown below:

Sample	n	Mean	Standard deviation (pg/ml)	C.V. (%)
1	20	236.7	27.0	11.4
2	20	809.0	47.4	5.9
2	20	1008.1	39.4	3.9

7.5.2 Inter Assay Variation

The between assay variability is shown below:

Sample	n	Mean	Standard deviation (pg/ml)	C.V. (%)
1	10	280.9	34.1	12.1
2	10	721.4	54.2	7.5
3	10	1025.4	60.5	5.9

7.6 Recovery

Dihydrotestosterone Calibrators (D, E, F) were used to spike 3 different samples (1 : 3). The results (in pg/ml) are tabulated below:

Sample	Added	Expected	Found	% Recovery
1 (324.8)			324.8	
	117.5	442.3	389.4	88.1
	235.1	559.8	505.2	90.3
	470.1	794.9	712.4	89.6
2 (290.5)			290.5	
	117.5	408.1	361.5	88.6
	235.1	525.6	501.7	95.5
	470.1	760.7	744.8	97.9
3 (720.1)			720.1	
	117.5	837.6	758.1	90.5
	235.1	955.2	856.5	89.7
	470.1	1190.2	1013.6	85.2

7.7 Linearity

Three patient samples were assayed, both undiluted and diluted with calibrator A. The results (in pg/ml) are tabulated below:

Sample	Dilution	Measured conc. (pg/ml)	Expected conc. (pg/ml)	Recovery (%)
A	--	340.7		
	1:2	165.4	170.4	97.0
	1:4	95.4	85.2	112.0
	1:8	48.5	42.6	107.1
B	--	1086.0		
	1:2	508.6	543.0	93.7
	1:4	232.1	271.5	85.5
	1:8	115.0	135.8	84.7
C	--	1313.2		
	1:2	613.0	656.6	93.4
	1:4	318.6	328.3	97.1
	1:8	135.0	164.2	82.2

8 LIMITATIONS OF USE

8.1 Interfering Substances

Any improper handling of samples or modification of this test might influence the results. Interferences caused by improper sample handling are explained in the chapters 'Specimen - Collection'.

8.2 High-Dose-Hook Effect

There exists no High-Dose-Hook effect in a competitive assay.

9 LEGAL ASPECTS

9.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national calibrators and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications.

9.2 Therapeutical Consequences

Therapeutical consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 9.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutical consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutical consequences.

9.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results subject to point 9.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

10 REFERENCES

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