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Instructions for use

DHT ELISA

REF **AA E-1900**

2°C – 8°C | Σ 96 | IVD | CE

INTENDED USE

For the direct quantitative determination of dihydrotestosterone (DHT) in human serum by an enzyme immunoassay. For *in vitro* use only.

PRINCIPLE OF THE TEST

The DHT ELISA is a competitive immunoassay. Competition occurs between DHT present in standards, controls and patient samples and an enzyme-labelled antigen (conjugate) for a limited number of anti-DHT antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the enzyme substrate is added, and 30 minutes later the enzymatic reaction is terminated by addition of stopping solution. The resulting optical density (OD), measured with a microplate reader, is inversely proportional to the concentration of DHT in the sample. A standard curve is plotted with a provided set of standards to directly calculate the concentration of DHT in patient samples and controls.

CLINICAL APPLICATIONS

Dihydrotestosterone (DHT) is the most active natural androgen in humans with a principal role in the development of primary and secondary sexual characteristics and potential participation in a myriad of other physiological processes. 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) from peripheral conversion of testosterone, while in females most of the DHT is derived from androstenedione.
Some of the main clinical indications of the DHT measurement in serum are investigations of delayed puberty in men and evaluation of the presence of active testicular tissue[1].

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for professional use only and for in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
   - Do not pipette by mouth.
   - Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
   - Wear protective clothing and disposable gloves.
   - Wash hands thoroughly after performing the test.
   - Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. 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.
4. Avoid microbial contamination of reagents.
5. A standard curve must be established for every run.
6. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
7. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing or improper reagent storage.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
10. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
11. 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 colour, in which case it should not be used.
12. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
13. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
14. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
15. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.

LIMITATIONS

1. The kit is calibrated for the direct determination of DHT in human serum. The kit is not calibrated for the determination of DHT in other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Samples or control sera containing azide or thimerosal are not compatible with this kit, they may lead to false results.
4. Serum samples with a known low concentration (< 50 pg/ml) may be used to dilute serum samples with values higher than the highest standard. Otherwise, results may be reported as "> 2500 pg/ml". The use of any other reagent will lead to false results.
5. The results obtained with this kit shall never be used as the sole basis for a clinical diagnosis. For example, some drugs and the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products have the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should comprise all aspects of a patient's background including the frequency of exposure to animals/products.

SAFETY CAUTIONS AND WARNINGS
BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to blood specimens.
All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.
The standards and controls provided with the kit contain processed human plasma that has been tested by approved methods and found to be negative for the presence of HBsAg and antibodies to HCV, HIV 1/2 and HIV NAT. However, no test method can offer complete assurance that any viable pathogens are absent. Therefore, these components should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen, following good laboratory practices.

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

Approximately 0.1 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 room temperature for up to seven days, at 2–8 °C for up to fourteen days or freeze at or below -20 °C for up to 1 month.
Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRE-TREATMENT

No specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Calibrated single-channel pipette to dispense 50 µl.
2. Calibrated multi-channel pipettes to dispense 50 µl, 100 µl and 150 µl.
3. Calibrated multi-channel pipette to dispense 350 µl (for manual washing only).
4. Disposable pipette tips.
5. Distilled or deionized water.
6. Absorbance microplate reader with a 450 nm filter and an upper OD limit of 3.0 or greater. Automatic microplate washer (recommended).

REAGENTS PROVIDED

1. AA E-0030 WASH-CONC 10x **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: 2–8 °C

Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks at 2–8 °C. Following Preparation: The working wash buffer is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. In order to prevent microbial growth, the container in which the working wash buffer solution is prepared in and stored in should be clean and it is recommended to store the working wash buffer solution under refrigerated conditions (2–8 °C) when not in use.

Preparation of working wash buffer: To prepare the working wash buffer that is used for washing the microplate, dilute the wash buffer concentrate 1:10 in distilled or deionized water before use. If the whole microplate is to be used, add 50 ml of the wash buffer concentrate to 450 ml of deionized or distilled water.

2. AA E-0055 SUBSTRATE **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: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks at 2–8 °C.

3. AA E-1980 STOP-SOLN **Stopping Solution –** Ready To Use

Contents: One bottle containing 1M sulfuric acid.

Volume: 8 ml/bottle

Storage: 2–8 °C

Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks at 2–8 °C.

Hazards identification:

H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.

4. Standards and **Controls –** Ready To Use

Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

Cat. no.	Symbol	Standard	Concentration	Volume/Vial
AA E-1901	STANDARD A	**Standard A**	0 pg/ml	1.0 ml
AA E-1902	STANDARD B	**Standard B**	25 pg/ml	1.0 ml
AA E-1903	STANDARD C	**Standard C**	100 pg/ml	1.0 ml
AA E-1904	STANDARD D	**Standard D**	250 pg/ml	1.0 ml
AA E-1905	STANDARD E	**Standard E**	500 pg/ml	1.0 ml
AA E-1906	STANDARD F	**Standard F**	1000 pg/ml	1.0 ml
AA E-1907	STANDARD G	**Standard G**	2500 pg/ml	1.0 ml
AA E-1951	CONTROL 1	**Control 1**	Refer to vial labels for the acceptable ranges.	1.0 ml
AA E-1952	CONTROL 2	**Control 2**		1.0 ml

Contents: Vials containing DHT in a human serum-based matrix with a non-mercury preservative. Prepared by spiking matrix with defined quantities of DHT.

Storage: 2–8 °C

Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks at 2–8 °C.

5. AA E-1931 96 **Anti-DHT Antibody-Coated Microplate –** Ready To Use

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

Storage: 2–8 °C

Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks at 2–8 °C.

6. AA E-1940 CONJUGATE **DHT-Horseradish Peroxidase (HRP) Conjugate** – Ready To Use

Contents: One bottle containing DHT-HRP conjugate in a protein-based buffer with a non-mercury preservative.

Volume: 15 ml/bottle

Storage: 2–8 °C

Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks at 2–8 °C.

ASSAY PROCEDURE

Specimen Pre-Treatment: **None**.

All kit components, controls and specimen samples must reach room temperature prior to use. Standards, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1.	After all kit components have reached room temperature, mix gently by inversion.
2.	Prepare the working wash buffer (See the *Reagents Provided* section, *1. Wash Buffer Concentrate*).
3.	Plan the microplate wells to be used for standards, controls and samples. *See Recommended Assay Layout* section. Remove the strips that will not be used from the microplate frame and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.
4.	Pipette **50 µl** of each standard, control, and specimen sample into correspondingly labelled wells in duplicate.
5.	Pipette **100 µl** of the DHT-HRP conjugate into each well (the use of a multi-channel pipette is recommended).
6.	Gently tap the microplate frame for 10 seconds to mix the contents of the wells and incubate the microplate at room temperature (no shaking) for **90 minutes**.
7.	Wash the wells either with an automatic microplate washer (preferred) or manually as stated below. Automatic: Using an automatic microplate washer, wash the wells **3 times** with working wash buffer, using **350 µl/well** for each wash. Following washing, tap the plate firmly against absorbent paper to ensure that wells are dry. Manually: Briskly empty the contents of the wells over a waste container. Using a multi-channel pipette, add 350 µl of working wash buffer into each well. Briskly empty the contents of the wells over a waste container. Repeat the same above pipetting and emptying steps 2 more times. After the final time, tap the plate firmly against absorbent paper to ensure that wells are dry.
8.	Pipette **150 µl** of TMB Substrate into each well (the use of a multi-channel pipette is recommended).
9.	Incubate the microplate at room temperature (no shaking) for **30 minutes**.
10.	Pipette **50 µl** of Stopping Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for addition of the TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.
11.	Measure the optical density (absorbance) in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the stopping solution.

CALCULATIONS

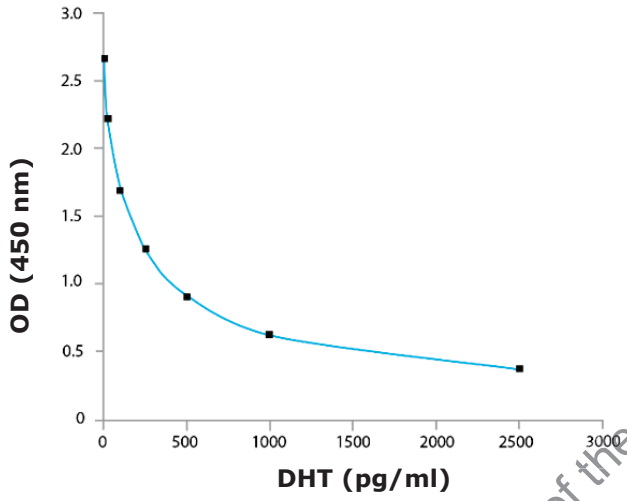
1. Calculate the mean optical density of each standard, control and specimen sample.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a standard curve.
3. Read the values of the unknowns directly off the standard curve.
4. If a sample reads more than 2,500 pg/ml, refer to *Limitations* section, point 4.

TYPICAL TABULATED DATA

Standard	Mean OD (450 nm)	% Binding	Value (pg/ml)
A	2.664	100	0
B	2.225	84	25
C	1.695	64	100
D	1.261	47	250
E	0.911	34	500
F	0.622	23	1000
G	0.372	14	2500
Unknown	1.077	40	353

TYPICAL STANDARD CURVE

Sample curve only.
Do not use to calculate results.



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit was calculated following EP17-A2. Sixty replicates of the matrix and low concentration samples were run in independent tests with three lots of the kit. The Limit of Background was determined to be 9.4 pg/ml and the Limit of Detection was determined to be 17 pg/ml.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with 5α-DHT cross-reacting at 100 %.

Compound	% Cross-Reactivity
5α-DHT	100
17-hydroxyprogesterone	< 0.01
17β-estradiol	< 0.01
Aldosterone	< 0.01
Androstenedione	0.6
Corticosterone	< 0.01
Cortisol	< 0.01
Danazol	< 0.01
DHEAS	< 0.01
Estriol	< 0.01
Estrone	< 0.01
Ethisterone	0.03
Pregnenolone	< 0.01
Progesterone	<0.01
Testosterone	8.1

INTERFERENCES

Haemoglobin up to 10 g/l, Bilirubin conjugated and unconjugated up to 10 mg/dl, Triglycerides up to 1500 mg/dl, Biotin up to 2.4 µg/ml, HAMAS up to 1.2 µg/ml, and Rheumatoid Factor up to 2531 IU/ml did not interfere with the assay.
Interferences were observed for both bilirubin conjugated and unconjugated at levels of 20 mg/dl or higher.

PRECISION

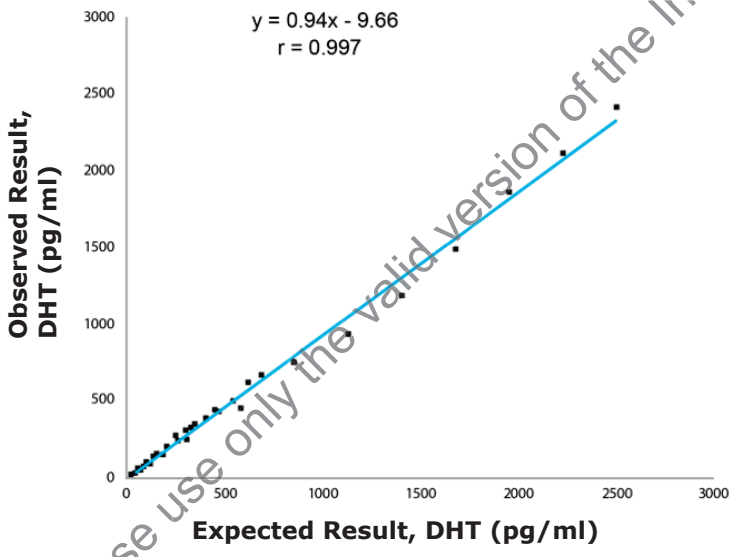
A precision study was conducted according to EP05-A2. The experimental protocol used a nested components-of-variance design with 10 testing days, two lots and two scientists per day. Each scientist ran two tests per day and two replicate measurements per run (a 10 x 2 x 2 x 2 design) for each sample. The results were analyzed with a two-way nested ANOVA and summarized in the table below.

Sample	Mean, (pg/ml)	Within Run SD (pg/ml)	Within Run CV%	Between Run SD (pg/ml)	Between Run CV%	Total SD (pg/ml)	Total CV%
1	31.4	13.7	43.7	3.3	10.5	14.1	44.9*
2	144.2	19.3	13.4	8.5	5.9	21.0	14.6
3	817.5	51.7	6.3	21.1	2.6	55.8	6.8
4	429.5	34.5	8.0	10.8	2.5	36.8	8.6
5	586.2	38.8	6.6	15.5	2.6	41.8	7.1
6	1561	90.0	5.8	24.1	1.5	94.5	6.1
7	1287	71.1	5.5	18.5	1.4	73.4	5.7

* Samples that are close to the limit of quantitation are expected to have a higher imprecision. The allowable total error for samples lower than 145 pg/ml is ± 30 pg/ml.[6]

LINEARITY

The linearity study was performed with four human serum samples covering the range of the assay (between 226 and 2500 pg/ml) and following CLSI guideline EP06-A. The samples were diluted in serum samples with a low concentration of DHT (less than 50 pg/ml) at several equidistant concentration levels and up to ten percent (1:10), tested in duplicate, and the results compared to the predicted concentration. The statistical analysis shows that the assay is sufficiently linear up to a 1:10 dilution.



RECOVERY

Spiked samples were prepared by adding defined amounts of DHT (present in serum samples with a high DHT concentration) to four patient serum samples. The results are tabulated below:

Sample	Concentration Result (pg/ml)	Concentration of Spiking Samples (pg/ml)	Expected Concentration from 9:1 v/v (pg/ml)	Recovery%
1	**91.3**	**-**	**-**	**-**
	177.3	800	162.2	109.3
	230.2	1472	229.4	100.3
	313.8	2672	349.4	89.8
2	**191.6**	**-**	**-**	**-**
	261.0	800	252.5	103.4
	306.2	1472	319.7	95.8
	408.0	2672	439.7	92.8
3	**379.5**	**-**	**-**	**-**
	433.2	800	421.6	102.7
	499.5	1472	488.8	102.2
	573.3	2672	608.8	94.2
4	**360.2**	**-**	**-**	**-**
	383.2	800	404.2	94.8
	461.8	1472	471.4	98.0
	510.8	2672	591.4	86.4

COMPARATIVE STUDIES

The DHT ELISA kit (y) was compared to a Liquid Chromatography-Tandem Mass Spectrometry DHT method (x). The comparison of 90 serum samples yielded the following linear regression results using a Passing-Bablok fit: $y = 0.78x + 73.8$, $r = 0.88$.

REFERENCE RANGES

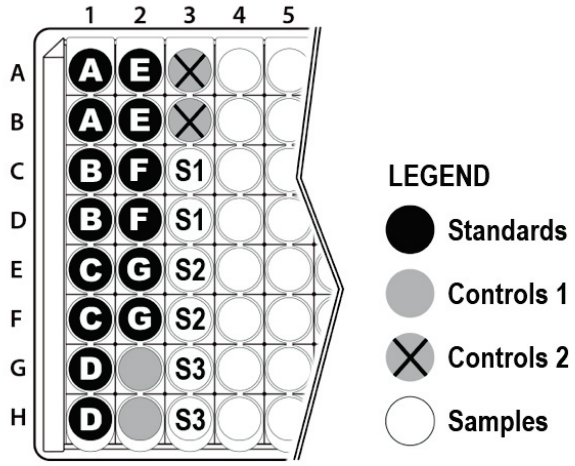
Reference ranges (95%) were estimated using samples obtained from adult individuals of diverse races. Each laboratory shall establish their own range of reference values. ND = Not detectable; lower than the LoD.

Cohort	n	Median (pg/ml)	95% Reference Range (pg/ml)
Adult Males (20–89 years old)	304	380	143 – 842
Adult Females (18–50 years old)	183	91	ND – 596
Adult Females (51–83 years old)	135	53	ND – 431

Reference ranges were estimated using pediatric samples as shown below. Due to the limited sample size, a 95 % reference range could not be established; the total range is provided. Each laboratory shall establish their own range of reference values.

Gender	Age (years)	n	Total Range (pg/ml)
Male	1–9	40	ND – 85.7
	10–14	26	11.1 – 875.6
	15–18	14	70.3 – 1260.9
Female	2–9	40	ND – 88.9
	10–14	21	22.5 – 280.6
	15–18	19	62.6 – 760.3

RECOMMENDED ASSAY LAYOUT



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Symbols:

+2 +8 °C	Storage temperature		Manufacturer	Σ	Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	IVD	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number		