



Dihydrotestosterone ELISA Kit

Catalog Number KA1886

96 assays

Version: 09

Intended for research use only

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Introduction

Intended Use

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


Principle of the Assay

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, controls and samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate. 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 samples and controls can be directly read.

General Information

Materials Supplied

List of component

Component	Amount
Wash Buffer Concentrate (10x): One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.	50 mL
TMB Substrate: Ready to use. One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.	16 mL
Stopping Solution: Ready to use. One vial containing 1 M sulfuric acid. <div data-bbox="427 712 507 792" data-label="Image">  </div> Hazards identification: H290 May be corrosive to metals. H314 Causes severe skin burns and eye damage.	6 mL
Assay Buffer: Ready to use. One bottle containing a protein-based buffer with a non-mercury preservative.	15 mL
Rabbit Anti-DHT Antibody-Coated Break-Apart Well Microplate: Ready to use. One polyclonal antibody-coated microplate in a resealable pouch with desiccant.	96 (12 x 8) well
Dihydrotestosterone-Horseradish Peroxidase (HRP) Conjugate Concentrate (100x): DHT-HRP Conjugate in a protein-based buffer with a non-mercury preservative.	200 µL

Standards and Controls - Ready to use

Listed below are approximate concentrations, please refer to vial labels for exact concentrations:

Component	Concentration (pg/mL)	Amount
Standard A	0	2.0 mL
Standard B	25	0.6 mL
Standard C	100	0.6 mL
Standard D	500	0.6 mL
Standard E	1000	0.6 mL
Standard F	2500	0.6 mL
Control 1	Refer to vial labels for expected value and acceptable range.	0.6 mL
Control 2		0.6 mL

Contents: DHT in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of DHT.

Storage Instruction

Store the complete kit at 2-8°C. Standards and Controls: Once opened, the standards and controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

Materials Required but Not Supplied

- ✓ Precision pipettes to dispense 50, 100, 150 and 300 µL
- ✓ Disposable pipette tips
- ✓ Distilled or deionized water
- ✓ Microwell plate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater (see Assay Procedure step 10)

Precautions for Use

- ✓ Procedural cautions and warnings
- 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.
- Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
- 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.
- A standard curve must be established for every run.
- The controls should be included in every run and fall within established confidence limits.
- Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
- 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.
- 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.
- When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
- To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.

- 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.
- Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

✓ Limitations

- 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.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
- Only standard A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.

✓ Safety cautions and warnings

- **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. No test method however, 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.

Assay Protocol

Reagent Preparation

- ✓ Wash Buffer Concentrate (10x)
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.
- ✓ Dihydrotestosterone -Horseradish Peroxidase (HRP) Conjugate Concentrate (100x)
Dilute 1:100 in assay buffer before use (eg. 20 µL of Conjugate Concentrate in 2 mL of assay buffer). If the whole plate is to be used dilute 120 µL of Conjugate Concentrate in 12 mL of assay buffer. Discard any that is left over.

Sample Preparation

- ✓ 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.
- ✓ Specimen Pretreatment
This assay is a direct system; no specimen pretreatment is necessary.

Assay Procedure

Specimen Pretreatment: None.

All reagents must reach room temperature before 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. Prepare working solutions of the DHT-HRP conjugate and wash buffer.
2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 50 µL of each standard, 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 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. Gently shake the plate for 10 seconds and incubate for 10-15 minutes at room temperature (no- shaking)

or until Standard A attains dark blue colour for desired OD.

9. Pipette 50 μ L 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.

Note: 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 control samples.

Data Analysis

Calculation of Results

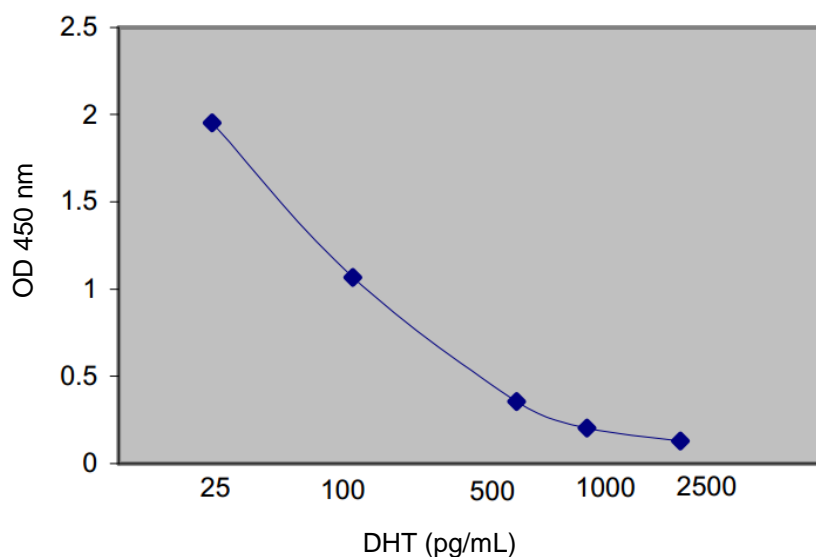
1. Calculate the mean optical density of each standard duplicate.
2. Draw a standard curve on semi-log paper with the mean optical densities on the Y-axis and the standard concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the standard curve.
5. If a sample reads more than 2500 pg/mL then dilute it with standard A at a dilution of no more than 1:8.
The result obtained should be multiplied by the dilution factor.

✓ Typical tabulated data

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	0.128	0.130	2500
Unknown	0.515	0.507	0.511	300

✓ Typical Standard Curve

Sample curve only. Do not use to calculate results.



Performance Characteristics

✓ Sensitivity

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Standard 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
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.

✓ Intra-assay precision

Three samples were assayed ten times each on the same standard 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 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 serum samples were diluted with standard 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

This 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

✓ Expected normal values

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

Resources

References

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Plate Layout

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