Diagnostics Biochem Canada

Dihydrotestosterone (DHT) ELISA

REF CAN-DHT-280

ASSAY PROCEDURE

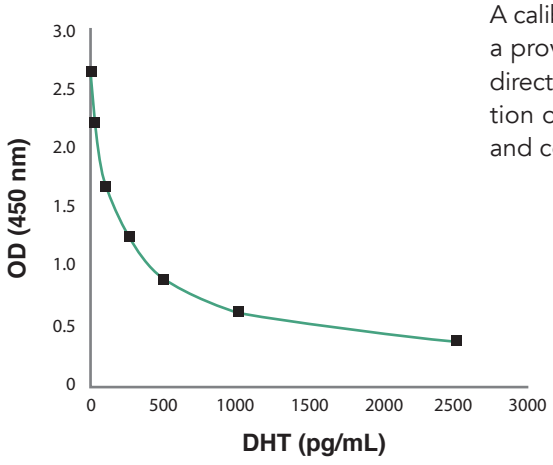
- Bring kit components to room temperature. Mix gently by inversion.
- Prepare working wash buffer.
- Pipette 25 µL of each calibrator, control, and specimen.
- Pipette 50 µL of the DHT-HRP conjugate into each well.
- Gently tap the microplate frame for 10 seconds to mix the contents of the wells.
- Incubate at room temperature (no shaking) for 90 minutes. Wash 3 times.
- Pipette 150 µL of the TMB Substrate into each well.
- Incubate for 30 minutes at room temperature (no shaking).
- Pipette 50 µL of Stopping Solution into each well.
- Gently tap the microplate frame to mix the contents of the wells.
- Measure the absorbance at 450 nm with a microplate reader.

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. In men, the bulk of androgen production takes place mainly in the Leydig cells of the testes. Androgens circulate in the blood bound to proteins, mainly sex hormone-binding globulin (SHBG) to which DHT has the highest binding affinity among all the endogenous steroids. In females, dihydrotestosterone is primarily a peripheral product of testosterone conversion and circulates in the blood in low concentrations.

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.

PRINCIPLE OF THE TEST

The DHT ELISA is a competitive immunoassay. Competition occurs between DHT present in calibrators, 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 approximately 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 calibrator curve is plotted with a provided set of calibrators to directly calculate the concentration of DHT in patient samples and controls.



Typical calibration curve

PERFORMANCE CHARACTERISTICS

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.

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.

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.

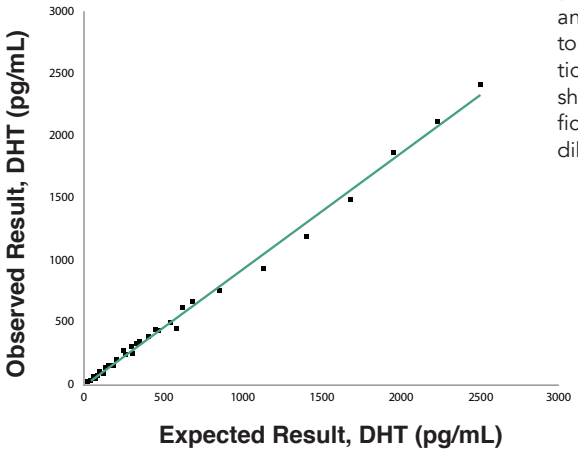
SPECIFICITY (CROSS-REACTIVITY)

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

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.



Ordering Information: REF CAN-DHT-280

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