DBC

Diagnostics Biochem Canada

2023
CATALOGUE

Superior Quality Enzyme-linked Immunosorbent Assays

ELISA

Manufacturing Innovative IVD for the World Since 1973

Introduction

DBC was founded in 1973 by a partnership of medical doctors with the initiative to research, develop and market quality radioimmunoassay kits and reagents. As technology in the field progressed, the founders of DBC recognized the need to develop simple and non-isotopic kits for our customers. With a strong research initiative, DBC successfully converted from RIA to the ELISA and LIA technologies of today.

We have been exporting our products to customers around the world for over 48 years. As a leader in the industry, it has always been our goal to offer a wide range of test kits and continually introduce new and unique products to the market place. It is our strong commitment to research and development that has enabled us to offer an everexpanding array of quality products.

This document contains information for all of our ELISA kits currently for sale. Click on a kit name in the Table of Contents to jump directly to that kit. Reference numbers listed on each page link to corresponding kits on our website www.dbc-labs.com.

Please contact us for any additional information about our products, or to learn how to order.

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DBC ELISA Kits

3a-Diol G	CAN-DG-460
17a-Hydroxyprogesterone	CAN-P-400
25-Hydroxyvitamin D	CAN-VD-510
Adiponectin	CAN-APN-5000
Aldosterone	CAN-ALD-500
Androstenedione	CAN-AD-208
Anti-Thyroglobulin (Tg)	CAN-ATG-4150
Anti-Thyroid Peroxidase (TPO)	CAN-ATP-4160
B2-Microglobulin	CAN-B-4300
C-Peptide	CAN-C-P-4380
C-Reactive Protein	CAN-CRP-4360
Chorionic Gonadotropin	CAN-HCG-4120
Cortisol Saliva	CAN-C-240
Cortisol Serum	CAN-C-270
Dehydroepiandrosterone (DHEA)	CAN-DH-490
Dehydroepiandrosterone Sulfate (DHEAS)	CAN-DHS-480
Dihydrotestosterone (DHT)	CAN-DHT-280
Estradiol	CAN-E-430
Estriol, Free	CAN-E-620
Estriol, Free	CAN-E-640
Estrogens, Total	CAN-E-630
Estrone	CAN-E-420

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Ferritin	CAN-F-4280
Follicle Stimulating Hormone (FSH)	CAN-FSH-4060
Growth Hormone (hGH)	CAN-GH-4070
Insulin-Like Growth Factor Binding Protein-1 (IGFBP-1)	CAN-IGF-4140
Leptin	CAN-L-4260
Luteinizing Hormone (hLH)	CAN-LH-4040
Plasma Renin Activity (PRA)	CAN-RA-4600
Pregnenolone	CAN-PRE-4500
Progesterone Saliva	CAN-P-310
Progesterone Serum	CAN-P-305
Prolactin	CAN-PRL-4100
Prostate Specific Antigen, Free	CAN-FPSA-4400
Prostate Specific Antigen, Total	CAN-TPSA-4300
Resistin	CAN-RSN-4000
Sex Hormone Binding Globulin (SHBG)	CAN-SHBG-4010
Testosterone Saliva	CAN-TE-300
Testosterone Serum	CAN-TE-250
Testosterone, Free	CAN-FTE-260
Thyroid Stimulating Hormone	CAN-TSH-4080
Thyroxine, Free (FT4)	CAN-FT4-4340
Thyroxine, Total (T4)	CAN-T4-4240
Triiodothyronine, Free (FT3)	CAN-FT3-4230
Triiodothyronine, Reverse (RT3)	CAN-RT3-100
Triiodothyronine, Total (T3)	CAN-T3-4220

Diagnostics Biochem Canada

5α-Androstane-3α,17β Diol Glucuronide (3α-Diol G) ELISA

REF CAN-DG-460

ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 50 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of the conjugate working solution into each well.
- Incubate on a plate shaker for 30 minutes at room temperature.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

5α-Androstane-3α, 17β-diol glucuronide is a C19 steroid and is either abbreviated as 3α-Diol G, 5α-Diol G or simply, α-Diol G. It is produced mainly as a metabolite of testosterone and dihydrotestosterone (DHT). It is largely produced in target peripheral tissues such as the skin, especially around hair follicles. The stimulation by large amounts of 3α-Diol G leads to excessive hair formation, notably where hair is not normally present in women.

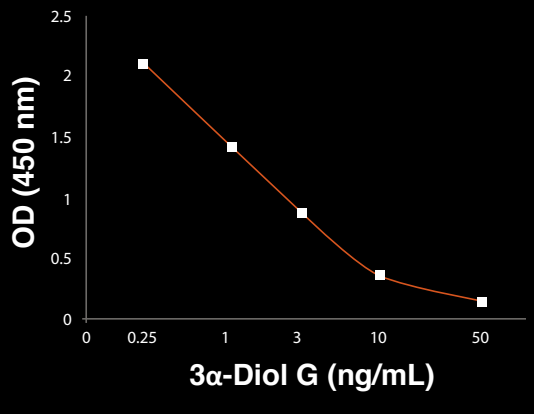
In recent years the interest in the measurement of this steroid has increased among clinical investigators studying women suffering from idiopathic hirsutism.

Among the steroids known to be precursors for 3α-Diol G are dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS), dihydrotestosterone (DHT), androstenedione and testosterone. Only 3α-Diol G has been shown to increase with hirsutism and decrease with treatment. This correlation has also been demonstrated in patients with polycystic ovarian syndrome (PCO). 3α-Diol G determinations have therefore proved to be a useful indicator in a variety of ways including monitoring the progress of treatment of idiopathic hirsutism and women with PCO.

Furthermore, diabetic patients (both men and women) under cyclosporine A therapy have shown increased 3α-Diol G levels, a side effect resulting in the appearance of hair in previously hairless areas.

PRINCIPLE OF THE TEST

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 patient 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 3α-Diol G in the sample. A set of standards is used to plot a standard curve from which the amount of 3α-Diol G in patient samples and controls can be directly read.



Typical calibration curve

PERFORMANCE CHARACTERISTICS

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 DBC Direct 3α-Diol G ELISA kit is **0.1 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct 3α-Diol G ELISA kit with 3α-Diol G cross-reacting at 100%.

Compound	% Cross-Reactivity
3α-Diol G	100
Testosterone	0.2
Progesterone	0.16
Androstenedione	0.14
Cortisol	0.05

The following steroids were tested but cross-reacted at less than 0.01%: Corticosterone, Dehydroepiandrosterone, Dihydrotestosterone, Epi-androsterone, 17β-Estradiol and Estrone.

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.87	0.07	7.8
2	6.86	0.49	7.2
3	21.26	1.29	6.0

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.98	0.10	10.4
2	7.05	0.46	6.5
3	20.92	2.26	10.8

RECOVERY

Spiked samples were prepared by adding defined amounts of 3α-Diol G to three patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Results	Recovery %
1 Unspiked	0.67	-	-
+ 0.5	1.07	1.17	91.4
+ 5.0	4.99	5.67	88.0
+ 15.0	12.66	15.67	80.8
2 Unspiked	1.83	-	-
+ 0.5	2.07	2.33	88.8
+ 5.0	6.18	6.83	90.5
+ 15.0	17.64	16.83	104.8
3 Unspiked	12.76	-	-
+ 0.5	15.32	13.26	115.5
+ 5.0	19.22	17.76	108.2
+ 15.0	22.68	27.76	81.7

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	6.24	-	-
1:2	2.83	3.12	90.7
1:4	1.55	1.56	99.4
1:8	0.74	0.78	94.9
2	13.55	-	-
1:2	6.00	6.77	88.6
1:4	2.71	3.39	80.0
1:8	1.70	1.64	103.6
3	17.05	-	-
1:2	6.93	8.53	81.2
1:4	4.09	4.26	96.0
1:8	2.34	2.13	109.8

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (ng/mL)
Males	1.53–14.82
Females Premenopausal	0.22–4.64
Postmenopausal	0.61–3.71
Puberty	0.51–4.03

Ordering Information: REF CAN-DG-460

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Diagnostics Biochem Canada

17α-Hydroxyprogestone (17α-OHP) ELISA

REF CAN-P-400

ASSAY PROCEDURE

Bring kit components to room temperature. Mix gently by inversion. Prepare working solution.

Pipette 25 µL of each calibrator, control and specimen sample.

Pipette 150 µL of 17α-OHP-HRP conjugate.

Gently shake the microplate by hand for 10 seconds.

Incubate for 1 hour at room temperature (without shaking).

Wash 3 times.
Pipette 150 µL of TMB substrate into each well.

Gently shake the microplate by hand for 10 seconds.

Incubate for 15–20 minutes at room temperature (without shaking).

Pipette 50 µL of stopping solution into each well. Gently shake the microplate for 10 seconds.

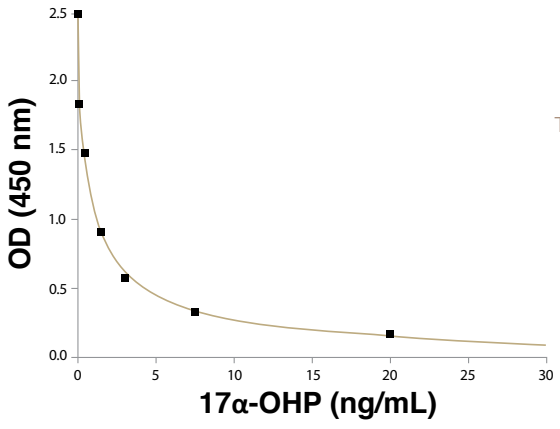
Read the plate on a microplate reader at 450 nm.

17α-hydroxyprogesterone is produced by the adrenal cortex and gonads. 17α-OHP has little progestational activity, but has intense clinical interest because it is the immediate precursor to 11-desoxycortisol, which is produced by the 21-hydroxylation of 17α-OHP. Measurement of 17α-OHP is, consequently, a useful indirect indicator of 21-hydroxlase activity.

In congenital 21-hydroxylase deficiency, the most common variety of congenital adrenal hyperplasia (CAH), 17α-OHP is secreted in abundant excess. Measurement of 17α-OHP is therefore valuable in the initial diagnosis of CAH.

PRINCIPLE OF THE TEST

DBC's 17α-Hydroxyprogesterone (17α-OHP) ELISA is a competitive enzyme immunoassay. Competition occurs between the antigen (present in standards, controls and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate. After samples and conjugate have been incubated for one hour, washing of the microplate removes unbound materials and an enzyme substrate that generates colour is added. The enzymatic reaction is terminated by addition of stopping solution. The optical density, measured with a microplate reader, is inversely proportional to the concentration of 17α-OHP in the sample. A set of standards is used to plot a standard curve from which the concentration of 17α-OHP in patient samples and controls can be directly read.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection (LoD) was determined from the analysis of 60 samples of the blank and a low concentration sample in two independent experiments and it was calculated as follows:

LoD = μB + 1.645σB + 1.645σS

Where σB and σS are the standard deviation of the blank and a low value sample and μB is the mean value of the blank. The LoD was determined to be **0.051 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the 17α-OHP ELISA kit with 17α-OHP cross-reacting at 100%.

Steroid	% Cross-Reactivity
17α-Hydroxyprogesterone	100
Progesterone	1.7
11-Desoxycortisol	< 0.25
DHEA	< 0.25
DHEAS	< 0.25
Cortisol	< 0.25
Cholesterol	< 0.25
Pregnenolone	< 0.25
Pregnenolone-SO4	< 0.25
Prednisone	< 0.25

INTERFERENCE

Serum samples with varying levels of 17α-OHP were tested after spiking with potential interfering substances at levels that exceed the highest found concentration in serum. To calculate the % of interference, results were compared to the same serum samples with no extra substances added. The following substances were tested and did not show significant interference in the assay: hemoglobin up to 2 g/L; bilirubin conjugated and free up to 10 mg/dL; triglycerides up to 5 mg/mL; rheumatoid factor up to 1.2 IU/mL; HAMAS 1.2 μg/mL.

COMPARATIVE STUDIES

The DBC 17α-OHP ELISA kit (y) was compared to a higher level test (RIA) (x). The comparison of 49 serum samples yielded the following linear regression results:

y = 0.83x + 0.13, r = 0.99

PRECISION

Six samples were assayed in duplicate in 40 independent experiments ran by two operators during 10 days. The results (in ng/mL) are tabulated below:

Sample	Mean	Within Run SD	Within Run CV	Total SD	Total CV
1	0.685061	0.026898	3.9%	0.107806	15.7%
2	4.30577	0.19803	4.6%	0.63436	14.7%
3	7.14774	0.27497	3.8%	0.86208	12.1%
4	8.64947	0.42710	4.9%	1.04203	12.0%
5	10.14976	0.39541	3.9%	1.37120	13.5%
6	15.0621	0.6751	4.5%	1.6217	10.8%

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Recovery %
1 1:2 1:5 1:10	15.75 9.14 4.03 1.69	- 116 128 107
2 1:2 1:5 1:10	13.55 6.02 2.61 1.10	- 89 96 81
3 1:2 1:5 1:10	21.88 12.66 5.54 2.55	- 116 127 116

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	N	Median (ng/mL)	95% Confidence Range (ng/mL)
Children 3–12 years old	80	0.31	0.051–2.35
Children 13–17 years old	80	0.72	0.13–1.85
Women < 40 years old	120	0.93	0.27–2.54
Women > 60 years old	120	0.43	0.094–1.02
Men 20–59 years old	240	1.60	0.37–2.87

Ordering Information: **REF CAN-P-400**

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Diagnostics Biochem Canada

25-Hydroxyvitamin D [25(OH)D] ELISA

REF CAN-VD-510

ASSAY PROCEDURE

Bring kit components to room temperature. Prepare working solutions.

Pipette 25 µL of each calibrator, control and specimen sample. Pipette 150 µL of the incubation buffer into each well.

Tap the plate gently by hand for 10 seconds. Incubate at room temperature, in a dark place, for 60 minutes (no shaking).

Wash the wells 3 times with 300 µL/well of diluted wash buffer.

Pipette 150 µL of the working conjugate solution.

Tap the plate gently by hand for 10 seconds. Incubate at room temperature, in a dark place, for 30 minutes (no shaking).

Wash the wells 3 times with 300 µL/well of diluted wash buffer.

Pipette 150 µL of TMB substrate into each well.

Tap the plate gently by hand for 10 seconds. Incubate at room temperature, in a dark place, for 10–15 minutes (no shaking).

Pipette 50 µL of stopping solution to each well. Tap the plate gently by hand for 10 seconds.

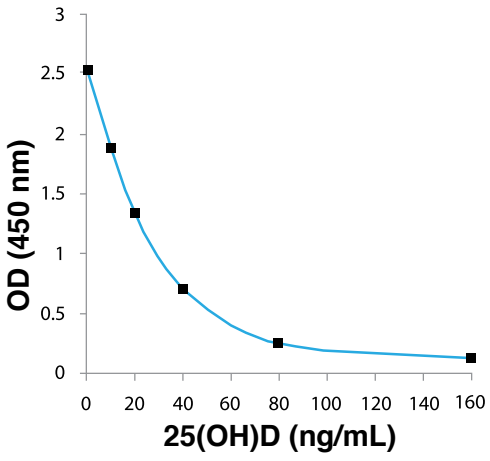
Read the plate on a microplate reader at 450 nm.

Vitamin D concentration in blood should be measured regularly to ensure that satisfactory physiological levels are maintained year round. Vitamin D is assimilated from food sources (both vitamin D2 and vitamin D3) or produced in the skin by sun exposure (vitamin D3). The body stores both vitamin D2 and vitamin D3 mainly in the form of 25-hydroxyvitamin D2 or 25-hydroxyvitamin D3 respectively. Therefore, the ***best approach*** to assess the physiological levels of vitamin D is to analyze the total concentration of ***both*** hydroxylated forms.

This kit measures the total concentration of both **25-hydroxyvitamin D2** and **25-hydroxyvitamin D3** (25(OH)D). The results are expressed in ng/mL.

PRINCIPLE OF THE TEST

DBC's immunoassay of 25(OH)D is a ***sequential competitive assay*** that uses ***two incubations***, with a total assay incubation time of less than two hours. During the first incubation, unlabelled 25(OH)D (present in the standards, controls, serum and plasma samples) is dissociated from binding proteins such as vitamin D binding protein and binds to the anti-25(OH)D antibody immobilized on the microplate wells. A washing step is performed next. During the next incubation, the complex of 25(OH)D-biotin conjugate and streptavidin-HRP conjugate competes with antibody-bound 25(OH)D for antibody binding sites. The washing and decanting procedures remove any unbound materials. The TMB substrate is added next which reacts with HRP to form a coloured product. The intensity of the colour is proportional to the amount of immobilized HRP. Stopping solution is added next which stops the colour development reaction. The optical density of each well is measured in a microplate reader. The absorbance values are inversely proportional to the concentration of 25(OH)D in the sample. A set of calibrators is used to plot a standard curve from which the concentrations of 25(OH)D in the samples and controls can be directly read.



Typical calibration curve

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection (LoD) was determined from the analysis of 64 samples of the blank and a low value sample and it was calculated as follows: LoD = μB + 1.645σB + 1.645σS, where σB and σS are the standard deviation of the blank and low value sample and μB is the mean value of the blank.

LoD = **5.5 ng/mL** of 25(OH)D.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity using the Abraham method with 25(OH)D3 cross reacting at 100%:

Antigen	% Cross-Reactivity
25 (OH)D3	100
25 (OH)D2	100
1,25 $(OH)_2$D3	8.3
3-epi-25 (OH)D3	66
Vitamin D3	< 1.0
Vitamin D2	< 1.0

INTERFERENCE

Interference testing was performed according to the CLSI guideline EP7-A2. Serum samples with varying levels of 25(OH)D were spiked with potential interfering substances at recommended levels and analyzed. Results were compared to the same serum samples with no extra substances added to calculate the % interference. The following substances were tested and did not show significant interference in the assay up to concentrations more elevated than the highest occurring levels: hemoglobin up to 7.5 mg/mL; bilirubin conjugated and free up to 200 μg/mL; triglycerides up to 5.5 mg/mL; cholesterol up to 2.6 mg/mL; ascorbic acid up to 10 mg/mL, biotin up to 40 μg/mL and caffeine up to 10 μg/mL.

PRECISION

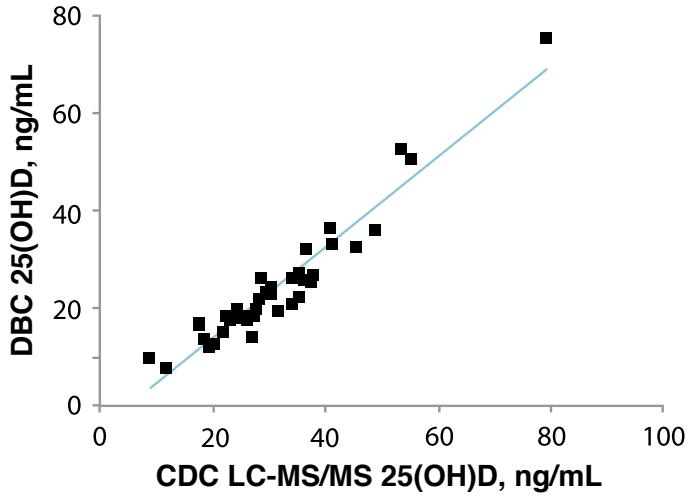
The precision study followed EP5-A3 and used a nested components -of-variance design with 21 testing days, two runs per testing day, and two replicate measurements per run (a 21 x 2 x 2 design) for each sample. Data was analyzed with a two-way nested ANOVA and summarized in the table below:

Sample	Mean (ng/mL)	Repeatability SD	Repeatability CV %	Within Lab SD	Within Lab CV %
1	21.87	1.09	5.0%	1.77	8.1%
2	36.57	1.01	2.8%	3.17	8.7%
3	45.01	1.07	2.4%	4.45	9.9%
4	60.25	2.82	4.7%	6.21	10.3%

COMPARATIVE STUDIES

The DBC 25(OH)D ELISA kit (y) was compared to a higher level test (LC-MS/MS) (x). The comparison of 40 serum samples yielded the following linear regression results:

y = 0.93x – 4.68, r = 0.96



REFERENCE VALUES (SERUM/PLASMA)

As for all clinical assays each laboratory should collect data and establish their own range of reference values. Data presented here are from samples collected in Florida (USA) from putatively healthy Black, White and Hispanic individuals of both genders and between 20 and 60 years old. Population reference ranges for 25(OH)D vary widely depending on age, ethnic background, geographic location and season. Population-based ranges correlate poorly with serum 25(OH)D concentrations that are associated with biologically and clinically relevant vitamin D effects and are therefore of limited clinical value.

N	25(OH)D Mean (ng/mL)	25(OH)D Median (ng/mL)	25(OH)D Range (2.5^{th}–97.5^{th} percentile) (ng/mL)
120	24.6	23.5	12.6–42.3

Results from the NHANES III study (1) yielded a mean of 30 ng/mL among 15,390 individuals.

Ordering Information: REF CAN-VD-510

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Diagnostics Biochem Canada

Adiponectin ELISA

REF CAN-APN-5000

ASSAY PROCEDURE

- Bring kit components to room temperature. Prepare working solutions.
- Pipette 50 µL of each calibrator, control and diluted specimen sample.
- Pipette 100 µL of the monoclonal anti-adiponectin-biotin conjugate into each well.
- Incubate on a plate shaker for 1 hour at room temperature.
- Wash the wells 3 times.
- Pipette 100 µL of the streptavidin-HRP into each well.
- Incubate on a plate shaker for 30 minutes at room temperature.
- Wash the wells 3 times.
- Pipette 150 µL of TMB substrate. Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of stopping solution to each well.
- Read the plate on a microplate reader at 450 nm.

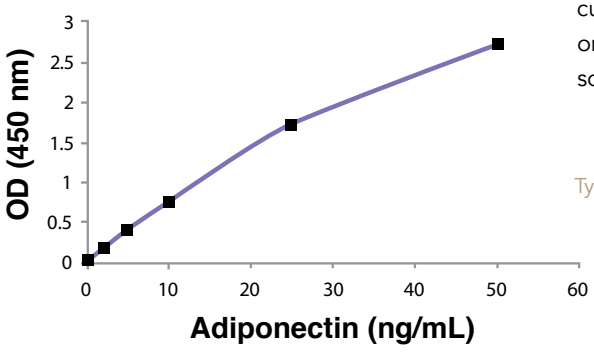
Adiponectin is a hormone that modulates glucose regulation and fatty acid oxidation. It is secreted from adipose tissue and placenta into the bloodstream and represents 0.01% of all plasma protein. Adiponectin increases insulin sensitivity and decreases plasma glucose by increasing tissue fat oxidation.

Adiponectin concentrations correlate negatively with glucose, insulin and triglycerides (TG) concentrations, liver fat content and body mass index and positively with high density lipoprotein cholesterol levels, hepatic insulin sensitivity and insulin stimulated glucose disposal. Adiponectin levels decrease in patients with type 2 diabetes and in patients with coronary heart disease.

Adiponectin could be used as a marker for energy metabolism and body weight regulation, metabolic syndrome, type 2 diabetes, coronary artery disease, or atherosclerosis.

PRINCIPLE OF THE TEST

The principle of the adiponectin ELISA is a two-step sandwich enzyme immunoassay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for adiponectin is immobilized onto the microplate and another monoclonal antibody specific for a different epitope of adiponectin is conjugated to biotin. During the first step, adiponectin present in the samples and standards is bound to the immobilized antibody and to the biotinylated antibody, thus forming a sandwich complex. Unbound biotinylated antibody is removed by a washing. In the second step, streptavidin-HRP is added, which binds specifi cally to bound biotinylated antibody. Unbound streptavidin-HRP is removed by washing. Next, the enzyme substrate (TMB) is added. The colour intensity of the enzymatic reaction is directly proportional to the concentration of adiponectin. The enzymatic reaction is terminated by the addition of stopping solution. The absorbance is measured on a microplate reader at 450 nm. The concentration of adiponectin in samples and controls can be calculated from of a plot of the standard curve, either graphically or by using immunoassay software.



Typical calibration curve

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection (LoD) was determined from the analysis of 60 samples of the blank and a low value sample and it was calculated as follows: LoD = μB + 1.645σB + 1.645σS, where σB and σS are the standard deviation of the blank and low value sample and μB is the mean value of the blank.

LoD = **0.06 ng/mL** of Adiponectin.

SPECIFICITY (CROSS-REACTIVITY)

The blank was spiked separately with 100 ng/mL of human leptin, human resistin, human TNF-α and IL-6 and 10 ng/mL of C-peptide. The signal obtained for each was compared to the signal of adiponectin at 10 ng/mL. % Cross-Reactivity = (Signal of substance tested/Signal of Adiponectin at 10 ng/mL) *100

Analyte	Concentration (ng/mL)	% Cross-Reactivity
Leptin	100	0
TNF-α	100	0
IL-6	100	0.9
Resistin	100	0.1
C-peptide	10	0

INTERFERENCE

Interference testing was performed according to CLSI guideline EP7-A2. Serum samples with varying levels of adiponectin were spiked with potential interfering substances at recommended levels and analyzed. Results were compared to the same serum samples with no extra substances added to calculate the % interference. The following substances were tested and did not show significant interference in the assay: hemoglobin up to 0.25 g/L, bilirubin conjugated and free up to 85 μM, triglycerides up to 5.5 mg/mL and human serum albumin up to 60 g/L.

INTER-ASSAY PRECISION

Three samples were assayed 20 times each on the same calibrator curve. The results are tabulated below.

Sample	Mean (μg/mL)	SD (μg/mL)	CV %
1	6.59	0.36	5.5
2	11.92	0.55	4.6
3	36.82	2.75	7.5

INTRA-ASSAY PRECISION

Three samples were assayed in 20 different tests in the span of 20 days. The results are tabulated below.

Sample	Mean (μg/mL)	SD (μg/mL)	CV %
1	6.16	0.52	8.4
2	12.07	0.81	6.7
3	38.39	2.55	6.6

RECOVERY

Three patient serum samples were spiked by adding defined amounts of adiponectin to samples that were initially diluted 1:1000 in working dilution buffer. The results (in μg/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	3.65	-	-
+ 5 μg/mL	8.74	8.65	101
+ 10 μg/mL	13.65	13.65	100
+ 20 μg/mL	23.58	23.65	99.7
2 Unspiked	8.48	-	-
+ 5 μg/mL	13.25	13.48	98.3
+ 10 μg/mL	18.18	18.48	98.4
+ 20 μg/mL	25.88	28.48	90.9
3 Unspiked	12.65	-	-
+ 5 μg/mL	16.28	17.65	92.2
+ 10 μg/mL	20.57	22.65	90.8
+ 20 μg/mL	28.19	32.65	86.3

LINEARITY

Three patient serum samples that were initially diluted 1:1000 were serially diluted further with working dilution buffer. The results (in μg/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
Sample 1	8.11	-	-
1:2	3.9	4.06	96.1
1:4	1.96	2.03	96.6
1:8	0.92	1.01	91.1
Sample 2	11.23	-	-
1:2	5.45	5.62	97.0
1:4	2.62	2.81	93.2
1:8	1.27	1.40	90.7
Sample 3	36.25	-	-
1:2	15.88	18.13	87.6
1:4	7.53	9.06	83.1
1:8	3.73	4.53	82.3

COMPARATIVE STUDIES

The DBC Adiponectin ELISA kit (y) was compared with a leading competitor ELISA kit (x). The comparison of 40 serum samples yielded the following linear regression results: y = 0.85x + 1.01, r = 0.98

REFERENCE VALUES

As for all clinical assays each laboratory should collect data and establish their own range of reference values.

Group	N	Mean (μg/mL)	95% Confidence Range (μg/mL)
BMI < 25	50	9.7	3.4–19.5
BMI 25–30	50	7.1	2.6–13.7
BMI > 30	50	4.5	1.8–9.4

Ordering Information: REF CAN-APN-5000

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Diagnostics Biochem Canada

Aldosterone ELISA

REF CAN-ALD-500

ASSAY PROCEDURE

- Bring kit components to room temperature. Mix gently by inversion.
- Prepare working solution.
- Pipette 50 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of the aldosterone-HRP conjugate into each well.
- Incubate on a microplate shaker for 60 minutes at room temperature.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a microplate shaker for 20 minutes at room temperature.
- Pipette 50 µL of stopping solution. Gently shake the microplate by hand to mix.
- Read the plate on a microplate reader at 450 nm.

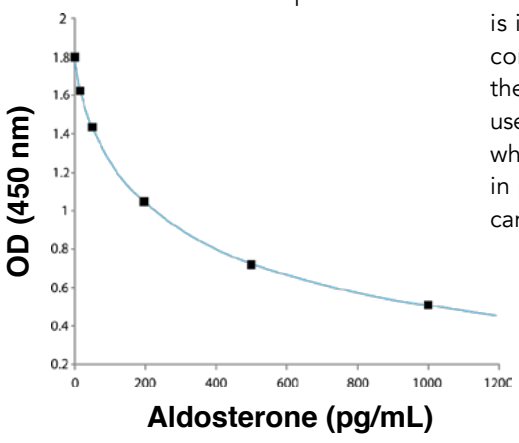
Aldosterone is a potent mineralocorticoid whose synthesis and release are controlled by the renin-angiotensin system of the body. Aldosterone promotes the reabsorption of sodium in the distal tubules of the kidney resulting in potassium secretion along with sodium retention, which controls the circulating blood volume. Chronic overproduction and secretion of aldosterone leads to hypertension.

Measurement of aldosterone levels in serum in conjunction with plasma renin activity levels can be used to differentiate between primary and secondary aldosteronism.

CONDITION	SERUM ALDOSTERONE	PLASMA RENIN
Primary Aldosteronism	High	Low
Secondary Aldosteronism	High	High

PRINCIPLE OF THE TEST

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 patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate wells. 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 with a microplate reader. The intensity of the colour formed is inversely proportional to the concentration of aldosterone in the sample. A set of standards is used to plot a standard curve from which the amount of aldosterone in patient samples and controls can be directly read.



Typical calibration curve

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection (LoD) was determined from the analysis of 60 samples of the blank and a low value sample and it was calculated as follows:

LoD = μB + 1.645σB + 1.645σS, where σB and σS are the standard deviation of the blank and low value sample and μB is the mean value of the blank.

The Limit of Detection (LoD) was determined to be **9.1 pg/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with aldosterone cross-reacting at 100%:

Steroid	% Cross-Reactivity
Aldosterone	100
Androsterone	0.01
Cortisol	0.01
Dihydrotestosterone	0.01
11-Deoxycorticosterone	0.075
Testosterone	0.009

The following compounds were tested and cross-reacted at less than 0.001%: Caffeine, Cholesterol, Cortisone and DHEAS.

INTERFERENCE

The following substances were tested and did not show significant interference in the assay: hemoglobin up to 4 g/L, bilirubin conjugated and free up to 125 mg/L and triglycerides up to 30 mg/mL.

INTRA-ASSAY PRECISION

Four serum and four urine samples were assayed 24 times each on the same calibrator curve. The results are tabulated below:

Serum Sample	Mean (pg/mL)	SD (pg/mL)	CV %
1	81.2	7.6	9.4
2	284.5	25.9	9.1
3	403.0	22.2	5.5
4	529.5	36.5	6.9

Urine Sample	Mean (pg/mL)	SD (pg/mL)	CV %
1	41.2	5.1	12.5
2	335.8	24.9	7.4
3	604.7	36.4	6.0
4	865.8	61.4	7.1

INTER-ASSAY PRECISION

Five serum samples were assayed in 20 different tests in the span of at least ten days. The results are tabulated below:

Sample	Mean (pg/mL)	SD (pg/mL)	CV %
1	80.8	10.4	12.8
2	209.0	22.5	10.7
3	454.5	51.9	11.4
4	677.3	79.1	11.7
5	902.3	68.4	7.6

LINEARITY

Patient serum samples were diluted with Serum and Plasma Diluent. Patient urine samples were diluted with Urine Diluent after an initial dilution of 1:10 in Urine Diluent. The results are tabulated below:

Serum Sample	Obs. Result (pg/mL)	Exp. Result (pg/mL)	Recovery %
1	241.8	-	-
1:2	127.8	120.9	105.7
1:4	65.0	60.5	107.6
1:8	31.6	30.2	104.5
2	840.8	-	-
1:2	456.7	420.4	108.6
1:4	217.1	210.2	103.3
1:8	125.0	105.1	118.9
3	1152	-	-
1:2	624.3	575.9	108.4
1:4	282.3	287.9	98.1
1:8	123.2	144.0	85.6

Urine Sample	Obs. Result (pg/mL)	Exp. Result (pg/mL)	Recovery %
1	320.1	-	-
1:2	178.7	160.1	111.7
1:4	92.8	80.0	116.0
1:8	34.1	40.0	85.1
2	442.3	-	-
1:2	231.9	221.1	104.9
1:4	126.5	110.6	114.9
1:8	48.5	55.3	87.8
3	572.2	-	-
1:2	290.2	286.1	101.4
1:4	149.4	143.0	104.4
1:8	65.2	71.5	91.1

COMPARATIVE STUDIES

The DBC Aldosterone ELISA kit (y) was compared with a leading competitor ELISA kit (x). The comparison of 42 serum samples yielded the following linear regression results: y = 0.84x + 3.50, r = 0.96

REFERENCE RANGES

As for all clinical assays each laboratory should collect data and establish their own range of reference values.

SERUM/PLASMA

Group	N	95% Confidence Range (pg/mL)
Normal Salt Intake, Upright	183	ND–199

URINE

Group	N	95% Confidence Range (μg/24 hr)
Normal Salt Intake	42	2.8–13

Ordering Information: REF CAN-ALD-500

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Diagnostics Biochem Canada

Androstenedione ELISA

REF CAN-AD-208

ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 25 µL of each calibrator, control, and specimen sample.
- Pipette 100 µL of Androstenedione-HRP conjugate.
- Incubate on a microplate shaker for 1 hour at room temperature.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a microplate shaker for 15–20 minutes at room temperature.
- Add 50 µL of stopping solution.
- Read in a microplate reader at 450 nm.

Androstenedione is produced by the *adrenals* and *gonads*. As a result, the determination of the level of androstenedione in serum is important in the evaluation of the functional state of these glands. Androstenedione is a precursor of testosterone and estrone. Besides the adrenals, in females, the ovaries have been shown to be an important source of androstenedione. It has been reported that there is a fluctuation day by day of androstenedione during the ovulatory cycle.

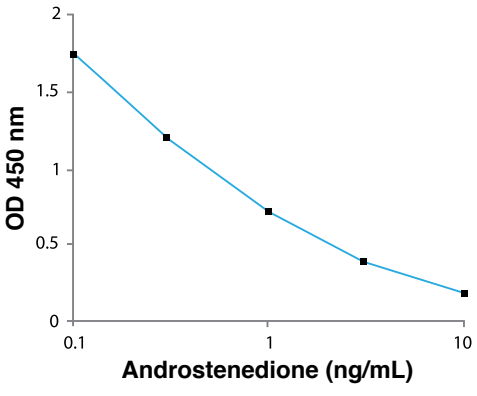
The principle production of testosterone in females is from the conversion of other related androgens, especially androstenedione.

An abnormal testosterone level in women should be accompanied by the estimation of serum androstenedione.

The use of serum testosterone determination in conjunction with the enzyme immunoassay of androstenedione can be used to determine if the source of the excess androgen production is adrenal or ovarian.

PRINCIPLE OF THE TEST

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 patient 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 androstenedione in the sample. A set of standards is used to plot a standard curve from which the amount of androstenedione in patient samples and controls can be directly read.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection (LoD) was determined from the analysis of 64 samples of the blank and a low value sample and it was calculated as follows: LoD = μB + 1.645σB + 1.645σS, where σB and σS are the standard deviation of the blank and low value sample and μB is the mean value of the blank.

The Limit of Detection (LoD) was determined to be **0.04 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with androstenedione cross-reacting at 100%.

Steroid	% Cross-Reactivity
Androstenedione	100
DHEA	1.8
Testosterone	0.2
Estrone	< 0.1
Estradiol	< 0.1
Progesterone	< 0.1
17-OH-Progesterone	< 0.1
5α-DHT	< 0.1
Cortisol	< 0.01
DHEAS	< 0.01

INTRA-ASSAY PRECISION

Four samples were assayed 24 times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.083	0.006	7.1
2	0.832	0.051	6.2
3	3.28	0.193	5.9
4	9.36	0.927	9.9

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.528	0.046	8.7
2	1.534	0.149	9.7
3	5.905	0.457	7.7

RECOVERY

Spiked samples were prepared by adding defined amounts of androstenedione to three patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1.Unspiked	0.882	-	-
+ 0.75	1.589	1.632	97
+ 1.5	2.521	2.382	106
+ 3.0	4.522	3.882	116
2.Unspiked	1.527	-	-
+ 0.75	2.466	2.277	108
+ 1.5	3.666	3.627	101
+ 3.0	5.756	6.027	96
3.Unspiked	0.585	-	-
+ 0.75	1.268	1.335	95
+ 1.5	1.878	2.085	90
+ 3.0	3.471	3.585	97

LINEARITY

Three patient serum samples were serially diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	2.317	-	-
1:2	1.220	1.158	105
1:4	0.615	0.579	106
1:8	0.329	0.290	113
2	6.594	-	-
1:2	3.212	3.297	97
1:4	1.594	1.648	97
1:8	0.818	0.824	99
3	7.456	-	-
1:2	3.588	3.728	96
1:4	1.835	1.864	98
1:8	0.963	0.932	103

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	N	Mean (ng/mL)	Range (ng/mL)
Males	20	2.0	0.4–3.5
Females	20	1.4	0.3–2.4

Ordering Information: **REF CAN-AD-208**

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Diagnostics Biochem Canada

Anti-Thyroglobulin ELISA

REF CAN-ATG-4150

ASSAY PROCEDURE

After all kit components have reached room temperature mix gently by inversion. Prepare the Wash Buffer Working Solution.

Prepare all specimen samples that will be tested. Pipette 100 µL of each calibrator, control and pre-treated specimen sample into assigned wells.

Incubate at room temperature for 30 minutes. Do not tap or shake the microplate, and avoid placing in intense light or air currents.

Wash the wells 3 times, each time with 350 µL/well of Wash Buffer Working Solution.

Add 100 µL of the HRP Conjugate. Incubate at room temperature for 30 minutes. Do not tap the microplate and avoid placing in intense light or air currents.

Wash the wells 3 times, each time with 350 µL/well of Wash Buffer Working Solution.

Pipette 100 µL of TMB Substrate into each well at timed intervals (the use of a multichannel pipette is recommended).

Incubate at room temperature for 15–20 minutes (until calibrator F attains dark blue colour for desired OD). Do not tap or shake the microplate, and avoid placing in intense light or air currents.

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 in all wells with a microplate reader, within 20 minutes after addition of the Stopping Solution.

Thyroglobulin represents the major protein found in the thyroid and is a dimeric glycoprotein of 660 kD. Thyroglobulin is produced by the thyroid gland and is involved in the storage and synthesis of thyroid hormones. It is a major autoantigen in autoimmune thyroiditis and is the precursor of T3 and T4. Although anti-Tg autoantibodies are found in conjunction with anti-TPO autoantibodies in the majority of cases of Hashimoto's thyroiditis, Graves' disease and Primary Myxedema, up to 1% of cases of hypothyroidism are associated with anti-Tg autoantibodies alone.

The frequency of thyroglobulin antibodies is approximately 70–80% in subjects with ***autoimmune-thyroiditis***, including ***Hashimoto's disease***, and approximately 30% in individuals with ***Graves' disease***.

Anti-Tg autoantibodies are also associated with cases of mild hypothyroidism or hyperthyroidism, and are frequently found in patients with other autoimmune diseases such as Rheumatoid Arthritis, Pernicious Anaemia and Type I Diabetes.

PRINCIPLE OF THE TEST

The Anti-Tg ELISA is a two-step capture or 'sandwich' type immunoassay. Human thyroglobulin (Tg) antigen is immobilized onto the microplate. In the first incubation step, anti-Tg autoantibodies present in the specimen samples, calibrators and controls is bound to the immobilized antigen. Excess and unbound materials are removed by a washing step. In the second incubation step, anti-human-IgG HRP conjugate is added, which binds specifically to any autoantibodies that are bound to the immobilized antigen. Unbound HRP conjugate is removed by a washing step. Next, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue coloured product that is directly proportional to the amount of anti-Tg autoantibodies present. The enzymatic reaction is terminated by the addition of the stopping solution, converting the blue colour to a yellow colour. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of anti-Tg autoantibodies in specimen samples and controls can be directly read.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The analytical sensitivity study was performed according to the CLSI EP17-A2 guideline. The Limit of Background (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) are summarized in the table below:

Parameter	Anti-Tg (IU/mL)
LoB	1.054
LoD	2.061
LoQ	3.4

RECOVERY

Low value samples and high value samples were mixed at different ratios. The measured values were compared to the expected values and the recovery % for each sample was calculated. The results are summarized in the table below.

	Sample	Measured (IU/mL)	Expected (IU/mL)	Recovery %
Low value: S1	100% S1	40.8	40.8	100.0
	90% S1/10% S2	125.7	101.8	123.5
	70% S1/30% S2	281.1	223.7	125.7
	50% S1/50% S2	427.7	345.7	123.7
High value: S2	30% S1/70% S2	549.2	467.6	117.4
	10% S1/90% S2	612.6	589.6	103.9
	100% S2	650.6	650.6	100.0
Low value: S3	100% S3	38.2	38.2	100.0
	90% S3/10% S4	120.0	117.1	102.5
	70% S3/30% S4	264.9	274.7	96.4
	50% S3/50% S4	396.0	432.4	91.6
High value: S4	30% S3/70% S4	503.6	590.0	85.4
	10% S3/90% S4	687.6	747.7	92.0
	100% S4	826.5	826.5	100.0

COMPARATIVE STUDIES

The DBC Anti-Tg ELISA kit (y) was compared against a competitor's ECLIA method (x). The comparison of 65 serum samples yielded the following linear regression results:

$y = 0.8282x + 10.138$, $r = 0.90$

PRECISION

The precision study was performed according to the CLSI EP5-A3 guideline.

The experimental protocol used a nested components-of-variance design with 12 serum samples, 10 testing days, two lots and two scientists per day. Each scientist ran two tests with two lots 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 (IU/mL)	Within Run SD (IU/mL)	Within Run CV %	Between Run SD (IU/mL)	Between Run CV %	Total SD (IU/mL)	Total CV %
1	45.6	1.5	3.3%	3.13	6.9%	3.5	7.7%
2	100.4	3.7	3.6%	4.02	4.0%	5.4	5.4%
3	335.8	11.8	3.5%	19.70	5.9%	23.0	6.8%
4	894.0	54.4	6.1%	60.64	6.8%	81.4	9.1%
5	44.4	1.8	4.1%	3.09	7.0%	3.6	8.1%
6	175.7	5.9	3.4%	9.96	5.7%	11.6	6.6%
7	347.4	14.4	4.2%	21.00	6.0%	25.5	7.3%
8	855.0	57.5	6.7%	57.40	6.7%	81.3	9.5%
9	378.3	26.9	7.1%	54.07	14.3%	60.4	16.0%
10	943.0	88.1	9.3%	119.89	12.7%	148.8	15.8%
11	436.2	22.1	5.1%	31.41	7.2%	38.4	8.8%
12	811.0	70.6	8.7%	76.19	9.4%	103.9	12.8%

REFERENCE RANGES

Serum samples from healthy individuals of diverse races, were screened for serum TSH levels between 0.5 and 3.0 µIU/mL. After completing the screen, 179 samples were selected and tested. Sample details:

101 x female, 78 x male, age range: 18–62 years old, average age: 34 years old. Results are summarized below.

Each laboratory shall establish their own reference ranges.

N	95% Range	Negative for Anti-Tg Autoantibodies	Positive for Anti-Tg Autoantibodies
179	11.65–61.63 IU/mL	≤ 61.6 IU/mL	> 61.6 IU/mL

Ordering Information: REF CAN-ATG-4150

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Diagnostics Biochem Canada

Anti-Thyroid Peroxidase (TPO) ELISA

REF CAN-ATP-4160

ASSAY PROCEDURE

After all kit components have reached room temperature mix gently by inversion. Prepare the Wash Buffer Woking Solution.

Prepare all specimen samples to be tested. Pipette 100 µL of each calibrator, control and pre-treated specimen sample into assigned wells.

Incubate at room temperature for 30 minutes. Do not tap the microplate and avoid placing in intense light or air currents.

Wash the wells 3 times, each time with 350 µL/ well of Wash Buffer Working Solution. Add 100 µL of the HRP Conjugate into each well.

Incubate at room temperature for 30 minutes. Do not tap the microplate and avoid placing in intense light or air currents.

Wash the wells 3 times, each time with 350 µL/well of Wash Buffer Working Solution.

Pipette 100 µL of the TMB Substrate into each well at timed intervals (the use of a multichannel pipette is recommended).

Incubate at room temperature for 15–20 minutes (until calibrator F attains dark blue colour for desired OD). Do not tap the microplate and avoid placing in intense light or air currents.

Pipette 50 µL of Stopping Solution into each well at the same timed intervals as in step 7. Mix thoroughly by gently tapping the plate.

Measure the absorbance at 450 nm in all wells with a microplate reader, within 20 minutes after addition of the stopping solution.

Thyroid peroxidase (TPO), a glycoprotein with a molecular weight of 100 kD, is identified as the primary antigenic component of microsomes in thyroid cells. TPO catalyses the iodination of tyrosine in thyroglobulin during the biosynthesis of triiodothyronine and thyroxine (T3 and T4 respectively). Disorders of the thyroid gland are frequently caused by autoimmune reactions. TPO and thyroglobulin are the most important targets for autoimmune attacks. The major thyroid autoimmune diseases are Hashimoto's thyroiditis and Graves' disease.

Anti-TPO autoantibodies are detected in 90–95% of **autoimmune thyroid disease** (**AITD**) patients, 80% of **Graves' disease** (**GD**), and 10–15% of **non-AITD** patients.

A high level of anti-TPO helps to confirm the diagnosis of these thyroid autoimmune disorders. The prevalence of TPO antibodies is higher in elderly (mean age 80 years) women (10%) compared to elderly men (2%).

PRINCIPLE OF THE TEST

The Anti-TPO ELISA is a two-step capture or 'sandwich' type immunoassay. Thyroid peroxidase (TPO) antigen is immobilized onto the microplate. In the first incubation step, anti-TPO autoantibodies present in the specimen samples, calibrators and controls is bound to the immobilized antigen. Excess and unbound materials are removed by a washing step. In the second incubation step, anti-human-IgG HRP conjugate is added, which binds specifically to any autoantibodies that are bound to the immobilized antigen. Unbound HRP conjugate is removed by a washing step. Next, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue coloured product that is directly proportional to the amount of anti-TPO autoantibodies present. The enzymatic reaction is terminated by the addition of the stopping solution, converting the blue colour to a yellow colour. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of anti-TPO autoantibodies in specimen samples and controls can be directly read.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The analytical sensitivity study was performed according to the CLSI EP17-A2 guideline. The Limit of Background (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) are summarized in the table below:

Parameter	Anti-TPO (IU/mL)
LoB	0.074
LoD	0.819
LoQ	2.89

RECOVERY

Low value samples and high value samples were mixed at different ratios. The measured values were compared to the expected values and the recovery % for each sample was calculated. The results are summarized in the table below.

	Sample	Measured (IU/mL)	Expected (IU/mL)	Recovery %
Low value: S1	100% S1	25.6	25.6	-
	50% S1/50% S2	214.6	191.7	111.9
	30% S1/70% S2	303.8	258.2	117.7
High value: S2	10% S1/90% S2	283.1	324.6	87.2
	100% S2	357.8	357.8	-
Low value: S3	100% S3	33.7	33.7	-
	50% S3/50% S4	215.3	181.2	118.8
	30% S3/70% S4	255.3	240.2	106.3
High value: S4	10% S3/90% S4	360.6	299.3	120.5
	100% S4	328.8	328.8	-
Low value: S5	100% S5	8.8	8.8	-
	50% S5/50% Cerba S6	436.0	428.0	101.9
	30% S5/70% Cerba S6	543.1	595.6	91.2
High value: S6	10% S5/90% Cerba S6	817.1	763.3	107.0
	100% Cerba S6	847.2	847.2	-

INTERFERENCES

An interference study was performed according to the CLSI EP07-A3 guideline. No significant interference was observed for concentrations of up to 2 g/L haemoglobin, 40 mg/dL Bilirubin (conjugated and unconjugated), 15 mg/mL triglycerides, 2.4 µg/mL HAMAS, 2.4 µg/mL Biotin and 1688 IU/mL Rheumatoid Factor.

PRECISION

The precision study was performed according to the CLSI EP5-A3 guideline.

The experimental protocol used a nested components-of-variance design with 9 serum samples, 10 testing days, two lots and two scientists per day. Each scientist ran two tests with two lots 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 (IU/mL)	Within Run SD (IU/mL)	Within Run CV %	Between Run SD (IU/mL)	Between Run CV %	Total SD (IU/mL)	Total CV %
1	15.4	0.8	5.1	2.3	15.2	2.5	16.1
2	73.8	4.0	5.4	8.0	10.9	9.2	12.5
3	188.1	11.5	6.1	22.5	12.0	25.3	13.4
4	484.8	43.7	9.0	66.1	13.6	79.2	16.3
5	72.7	3.4	4.7	6.9	9.5	7.7	10.6
6	90.7	3.6	3.9	7.7	8.4	8.5	9.3
7	272.3	18.9	6.9	35.3	13.0	40.1	14.7
8	282.5	19.9	7.0	48.5	17.2	52.8	18.7
9	222.0	11.5	5.2	24.1	17.2	27.0	12.2

COMPARATIVE STUDIES

The DBC Anti-TPO ELISA kit (y) was compared against a competitor's ECLIA method (x). The comparison of 101 serum samples yielded the following linear regression results: y = 0.90x - 7.36, r = 0.92

REFERENCE RANGES

Serum samples from healthy individuals of diverse races, were screened for serum TSH levels between 0.5 and 3.0 µIU/mL. After completing the screen, 166 samples were selected and tested. Sample details:

100 x female, 66 x male, age range: 18–62 years old, average age: 34 years old. Results are summarized below.

Each laboratory shall establish their own reference ranges.

N	95% Range	Negative for Anti-TPO Autoantibodies	Positive for Anti-TPO Autoantibodies
166	5.3–58.8 IU/mL	≤ 58.8 IU/mL	> 58.8 IU/mL

Ordering Information: REF CAN-ATP-4160

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Diagnostics Biochem Canada

β_2-Microglobulin ELISA

REF CAN-B-4300

ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 20 µL of each calibrator, control, and specimen sample.
- Pipette 100 µL of conjugate working solution.
- Incubate on a microplate shaker for 1 hour at room temperature.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a microplate shaker for 15–20 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read in a microplate reader at 450 nm.

β_2-Microglobulin (β_2-M) is a single polypeptide chain containing 100 amino acids and is found on the surface of nucleated cells. β_2-M is continuously secreted into the circulatory system and therefore maintains a balanced serum level.

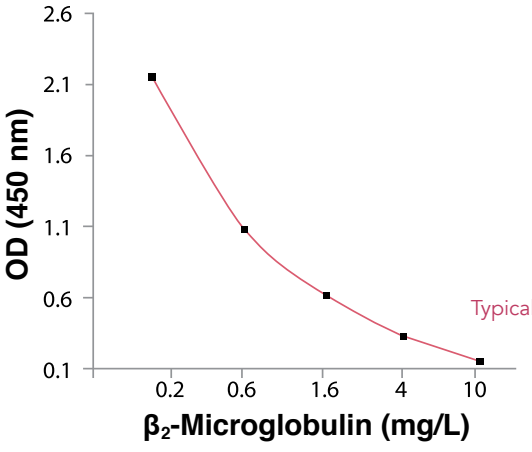
Clinical Trends:

- Decreases in the ***glomerular filtration rate*** gives rise to increases in the level of β_2-M.
- β_2-M is a helpful marker in the diagnosis of ***kidney disease*** and active ***rheumatoid arthritis***.
- Patients with ***acquired immune deficiency syndrome*** (AIDS) show an increased level of β_2-M.

β_2-M has a low concentration in serum. We have found in a normal unselected population that in serum the highest level for β_2-M is 3.8 mg/L. The total number of serum samples tested was 92, showing little differences in the normal level in males, pre- and postmenopausal females. The average for male samples = 1.582 mg/L, for premenopausal female = 1.457 mg/L, for postmenopausal female = 1.608 mg/L and fi nally for young people = 1.13 mg/L.

PRINCIPLE OF THE TEST

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 patient 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 β_2-M in the sample. A set of standards is used to plot a standard curve from which the amount of β_2-M in patient samples and controls can be directly read.



Typical calibration curve

PERFORMANCE CHARACTERISTICS

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 DBC Direct β_2-M ELISA kit is **0.1 mg/L**.

SPECIFICITY

The following compounds were tested for cross-reactivity with the Direct β_2-M ELISA kit with β_2-M cross-reacting at 100%.

Compound	% Cross-Reactivity
β_2-Microglobulin	100
Human IgG	< 0.00001

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in mg/L) are tabulated below:

Sample	Mean	SD	CV %
1	0.78	0.08	5.5
2	3.43	0.03	6.4
3	15.63	0.01	2.9

INTER-ASSAY PRECISION

Two samples were assayed ten times over a period of four weeks. The results (in mg/L) are tabulated below:

Sample	Mean	SD	CV %
1	0.92	0.09	9.5
2	3.64	0.14	3.8

RECOVERY

Spiked samples were prepared by adding defined amounts of β_2-M to two patient serum samples (1:1). The results (in mg/L) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	1.70	-	-
+ 4	3.21	2.85	112.6
+ 12	7.94	6.85	115.9
+ 32	17.05	16.85	101.2
2 Unspiked	2.51	-	-
+ 4	3.25	3.26	99.7
+ 12	8.14	7.26	112.1
+ 32	17.69	17.26	102.5

LINEARITY

Two patient serum samples were diluted with calibrator A. The results (in mg/L) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	15.96	-	-
1:5	3.14	3.19	98.4
1:10	1.57	1.60	98.1
1:20	0.77	0.80	96.3
2	17.71	-	-
1:5	3.63	3.54	102.5
1:10	1.94	1.77	109.6
1:20	1.00	0.89	112.4

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	N	Mean (mg/L)	Range (mg/L)
Males (Age 24–70)	46	1.58	1.15–3.85
Females (Age 19–45)	25	1.46	0.73–3.56
Postmenopausal Females	14	1.61	1.28–2.34
Young Males and Females (Age 3–17)	7	1.13	0.89–1.36

Ordering Information: REF CAN-B-4300

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Diagnostics Biochem Canada

C-Peptide ELISA

REF CAN-C-P-4380

ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 50 µL of each calibrator, control and specimen sample.
- Pipette 50 µL of the conjugate working solution into each well.
- Incubate on a plate shaker for 90 minutes at room temperature.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a plate shaker for 15–20 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

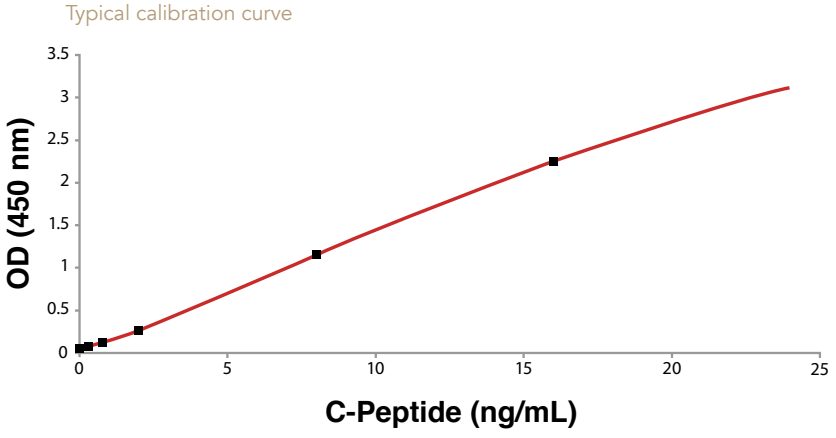
Human C-peptide of insulin is an **amino acid chain** of 3000 molecular weight, joining the A and B chains of insulin. It is secreted from the granules in the islet beta cells at equimolar concentrations with insulin. It is however, not as rapidly degraded by the liver as in the case of insulin, and therefore is more stable in the blood. These characteristics make the determination of C-peptide an advantageous test as an indicator to quantify insulin. It is therefore used in evaluating ***hypoglycemia*** and ***insulnoma***.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical one-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for C-peptide is immobilized onto the microplate and another monoclonal antibody specific for a different region of C-peptide is conjugated to horse radish peroxidase (HRP). C-peptide from the sample and standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. 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 by the enzymatic reaction is directly proportional to the concentration of C-peptide in the sample.

A set of standards is used to plot a standard curve from which the amount of C-peptide in patient samples and controls can be directly read.

Typical calibration curve



PERFORMANCE CHARACTERISTICS

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) plus 2 SD. Therefore, the sensitivity of the DBC Direct C-Peptide ELISA kit is **0.2 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The specificity of the Direct C-Peptide ELISA kit was determined by measuring the apparent C-peptide values of the following compounds:

Substance	Concentration Range	Apparent C-Peptide Value (ng/mL)
Insulin Calibrated against WHO 1st IS 66/304	20–10,000 µIU/mL	Not Detected

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	1.00	0.04	3.5
2	4.14	0.09	2.2
3	12.02	0.55	4.6

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.97	0.03	2.7
2	4.00	0.11	2.7
3	12.30	0.62	5.0

RECOVERY

Spiked samples were prepared by adding defined amounts of C-peptide to three patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Results	Recovery %
1 Unspiked	0.00	-	-
+ 1.0	0.91	1.00	91.0
+ 6.0	5.44	6.00	91.7
+ 8.0	7.82	8.00	97.8
2 Unspiked	0.64	-	-
+ 1.0	1.51	1.64	92.1
+ 2.0	2.27	2.64	86.0
+ 8.0	7.30	8.64	84.5
3 Unspiked	3.10	-	-
+ 0.25	3.10	3.35	92.5
+ 2.0	4.89	5.10	95.9
+ 6.0	9.47	9.10	104.1

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	5.00	-	-
1:2	2.35	2.50	94.0
1:4	1.10	1.25	88.0
1:8	0.51	0.63	81.0
2	6.76	-	-
1:2	3.08	3.38	91.1
1:4	1.42	1.69	84.0
1:8	0.71	0.85	83.5
3	13.36	-	-
1:2	6.02	6.68	90.1
1:4	3.09	3.34	92.5
1:8	1.56	1.67	93.4

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values

Group	N	Mean (ng/mL)	Abs. Range (ng/mL)
Males	26	0.89	0.24–1.98
Females	46	1.13	0.15–5.37

Ordering Information: **REF CAN-C-P-4380**

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Diagnostics Biochem Canada

High-Sensitivity C-Reactive Protein ELISA

REF CAN-CRP-4360

ASSAY PROCEDURE

- Bring kit components to room temperature. Prepare working solutions.
- Pipette 20 µL of each calibrator, control and diluted specimen sample.
- Pipette 200 µL of the assay buffer into each well.
- Incubate on a plate shaker for 30 minutes at room temperature.
- Wash 3 times.
- Pipette 100 µL of conjugate working solution into each well.
- Incubate on a plate shaker for 15 minutes at room temperature.
- Wash 3 times.
- Pipette 100 µL of TMB into each well. Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

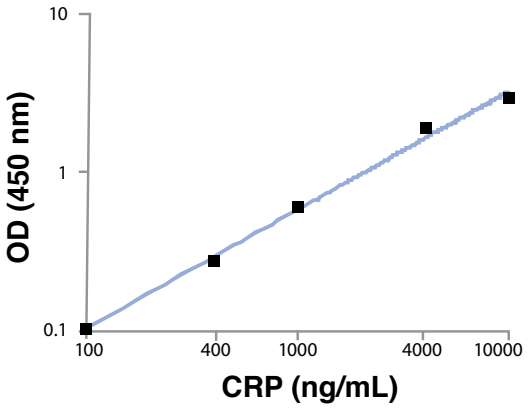
C-reactive protein (CRP) is a pentameric acute phase reactant that is synthesized by the liver. Its production is controlled primarily by interleukin-6. The serum CRP concentration may increase by up to 1000-fold with infection, trauma, surgery, and other acute inflammatory events.

Chronic inflammatory disorders such as auto-immune diseases and malignancy can produce persistent high levels of serum CRP.

Traditionally, CRP has been used clinically for the diagnosis and monitoring of auto-immune and infectous disorders. Recent studies have shown that chronic inflammation is an important component in the development and progression of atherosclerosis. As a result, increased serum CRP concentration are positively associated with the risk of future coronary events.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for CRP is immobilized onto the microplate and another monoclonal antibody specific for a different region of CRP is conjugated to horse radish peroxidase (HRP). CRP from the sample and standards are allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second 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 by the enzymatic reaction is directly proportional to the concentration of CRP in the sample.



Typical calibration curve

A set of standards is used to plot a standard curve from which the amount of CRP in patient samples and controls can be directly read.

PERFORMANCE CHARACTERISTICS

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) plus 2 SD. Therefore, the sensitivity of the DBC hs-CRP ELISA kit is **10 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The specificity of the hs-CRP ELISA kit was determined by measuring the apparent CRP value of samples spiked with the following compounds:

Substance	Apparent CRP Value (ng/mL)
Human Albumin	Not Detected
Human Globulin	Not Detected

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	205.8	31.2	15.2
2	769.2	38.4	5.0
3	8437.8	700.4	8.3

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	227.0	22.4	9.9
2	1022.2	97.2	9.5
3	8791.8	685.8	7.8

HIGH-DOSE HOOK EFFECT

The hs-CRP ELISA kit did not experience a high dose hook effect when it was tested up to a CRP concentration of 160,000 ng/mL.

RECOVERY

Spiked samples were prepared by adding defined amounts of CRP to three patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Results	Recovery %
1 Unspiked	263	-	-
+ 358	760	621	122.4
+ 1430	1820	1693	107.5
+ 5720	6520	5983	109.0
2 Unspiked	1352	-	-
+ 358	1880	1710	109.9
+ 1430	3020	2782	108.6
+ 5720	7720	7072	109.2
3 Unspiked	5546	-	-
+ 358	6107	5904	103.4
+ 1430	6169	6976	88.4
+ 5720	10400	11266	92.3

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	3662	-	-
1:5	894	732.4	122.1
1:25	136	146.5	92.8
1:50	62	73.2	84.7
2	6120	-	-
1:4	1922	1530	125.6
1:16	428	382.5	111.9
1:64	110	95.6	115.0
3	8800	-	-
1:4	2472	2200	112.4
1:16	614	550	111.6
1:64	148	137.5	107.6

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

	Males	Females	Combined
N	43	45	88
Age	17–87	12–79	12–87
Abs. Range	73–63,680	34–39,240	34–63,680
2.5th Percentile	132	139	135
50th Percentile	1197	1033	1104
97.5th Percentile	9710	6578	8910

Ordering Information: **REF CAN-CRP-4360**

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Diagnostics Biochem Canada

Human Chorionic Gonadotropin ELISA

REF CAN-HCG-4120

ASSAY PROCEDURE

(One-step Procedure)

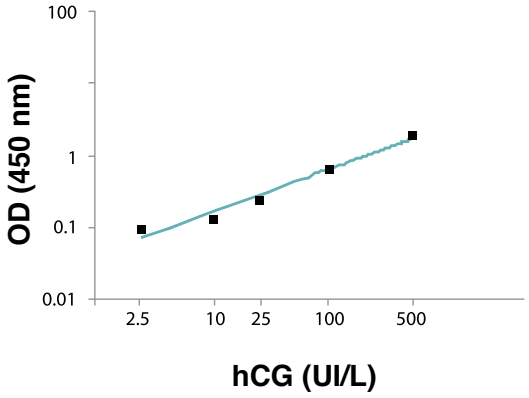
- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 25 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of anti-hGC-HRP conjugate.
- Incubate on a plate shaker for 60 minutes at room temperature.
- Wash 3 times.
- Pipette 100 µL of TMB substrate.
- Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced by the placenta. During normal pregnancy the level of this hormone in serum and urine gradually increases up to about the eighth week of pregnancy. hCG has two subunits, namely α and β. The α subunit is similar to the α subunit of the anterior pituitary gland glycoprotein hormones, namely, LH, TSH and FSH. However, the β subunits of these hormones are different. Therefore, this uniqueness distinguishes one hormone from the other, hence specificity. In raising this monoclonal antibody, the immunogen used was βhCG, which helps to make the DBC assay system very specific and sensitive.

In normal pregnancy the increase in hCG starts at about the 5th day after conception and continues to rise until it reaches a maximum at about the eighth week. In some pathological conditions the level of hCG in serum and/or urine is increased. It is a well known fact that hCG is also a tumour marker which is very important in the diagnosis of choriocarcinoma. In the case of hydatiform mole, hCG is also elevated. In about 50% of patients with testicular teratomas the level of hCG is elevated. It is also relevant to note that hCG is a good indicator, in order to follow the response to treatment.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical one-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for hCG is immobilized onto the microplate and another monoclonal antibody specific for a different region of hCG is conjugated to horse radish peroxidase (HRP). hCG from the sample and standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. 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 by the enzymatic reaction is directly proportional to the concentration of hCG in the sample.



Typical calibration curve

A set of standards is used to plot a standard curve from which the amount of hCG in patient samples and controls can be directly read.

Please note that a two-step procedure is included and is to be used for assaying serum from pregnant women.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based o n 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the DBC Direct hCG ELISA kit is **0.7 IU/L**.

SPECIFICITY (CROSS-REACTIVITY)

The specificity of the Direct hCG ELISA kit was determined by measuring the apparent hCG value of calibrator A spiked with the following compounds:

Substance	Concentration Range	Apparent hCG Value (IU/L)
hFSH Calibrated against WHO 1st IS 83/575	100–4000 IU/L	< 5.0
hLH Calibrated against WHO 2nd IS 80/552	50–200 IU/L	< 5.0
hTSH Calibrated against WHO 2nd IS 80/558	50–750 mIU/L	< 5.0

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in IU/L) are tabulated below:

Sample	Mean	SD	CV %
1	4.52	0.24	5.3
2	18.80	0.60	3.2
3	121.84	5.36	4.4

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in IU/L) are tabulated below:

Sample	Mean	SD	CV %
1	5.24	0.36	6.9
2	14.68	0.72	4.9
3	140.27	11.50	8.2

RECOVERY

Spiked samples were prepared by adding defined amounts of hCG to three patient serum samples (1:1). The results (in IU/L) are tabulated below:

Sample	Observed Result	Expected Results	Recovery %
1 Unspiked + 15 + 50 + 250	3.2 8.4 23.4 145.6	- 9.1 26.6 126.6	- 92.3 88.0 115.0
2 Unspiked + 15 + 50 + 250	3.3 8.7 23.9 142.0	- 9.2 25.0 126.7	- 94.6 95.6 112.1
3 Unspiked + 15 + 50 + 250	3.9 9.2 24.5 148.0	- 9.5 27.0 127.0	- 96.8 90.7 116.5

LINEARITY

Two patient serum samples were diluted with calibrator A. The results (in IU/L) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 1:5 1:10 1:20	67.7 12.1 6.2 3.5	- 13.5 6.8 3.4	- 89.6 91.2 102.9
2 1:5 1:10 1:20	137.7 30.2 14.6 7.6	- 27.5 13.8 6.9	- 109.8 105.8 110.1

REFERENCE VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values:

Group	Range (IU/L)
Males	< 5
Females: Non-Pregnant Pregnant	 < 5 > 250

Ordering Information: **REF CAN-HCG-4120**

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Diagnostics Biochem Canada

Cortisol Saliva ELISA

REF CAN-C-240

ASSAY PROCEDURE

Bring kit components to room temperature. Mix gently by inversion.

Prepare working solutions.

Pipette 25 µL of each calibrator, control and specimen sample.

Pipette 150 µL of the Cortisol-HRP conjugate into each well.

Gently tap the microplate frame for 10 seconds to mix the contents of the wells.

Incubate for 45 minutes at room temperature (no shaking). Wash 3 times.

Pipette 150 µL of TMB substrate. Gently tap the microplate frame for 10 seconds.

Incubate for 15–20 minutes at room temperature (no shaking).

Pipette 50 µL of stopping solution. Gently tap the microplate frame to mix.

Read the plate on a microplate reader at 450 nm.

Cortisol is the most abundant circulating steroid and the major glucocorticoid secreted by the adrenal cortex. Cortisol is physiologically effective in blood pressure maintenance and anti-inflammatory activity. It is also involved in calcium absorption, gluconeogenesis as well as the secretion of gastric acid and pepsin. The levels of cortisol are increased under stress situations, physical exercise and external administration of ACTH.

Most circulating cortisol is bound to corticosteroid-binding globulin (transcortin) and albumin. The amount of unbound or free cortisol (which is considered the active fraction in blood), represents approximately 1–2% of the total amount in blood.

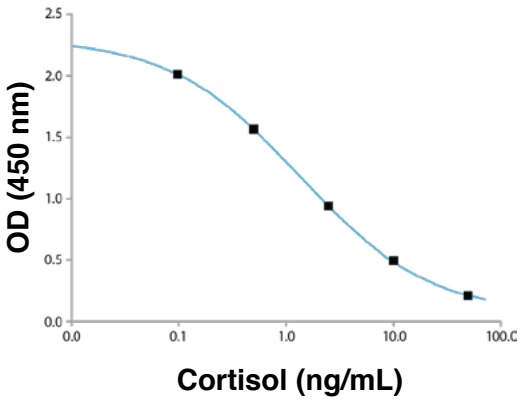
The measurement of salivary cortisol is considered to represent the free fraction, due to the absence of appreciable amounts of cortisol-binding proteins in saliva. The level of salivary cortisol shows a diurnal rhythm with the highest levels in the morning and the lowest levels at night.

Studies consistently report high correlations between serum and salivary cortisol, indicating that salivary cortisol levels reliably estimate serum cortisol levels.

The measurement of salivary cortisol levels can be used as an indicator of adrenal function and the differential diagnosis of Addison's and Cushing's diseases.

PRINCIPLE OF THE TEST

The cortisol saliva ELISA is a competitive immunoassay. Competition occurs between an unlabelled antigen present in calibrators, controls and patient samples and an enzyme-labelled antigen (conjugate) for a limiting number of anti-cortisol antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the enzyme substrate is added and approximately 20 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 cortisol in the sample. A calibrator curve is plotted with a provided set of calibrators to calculate directly the concentration of cortisol in patient samples and controls



Typical calibration curve

PERFORMANCE CHARACTERISTICS

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 Cortisol Saliva ELISA kit is 0.033 ng/mL.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with cortisol cross-reacting at 100%.

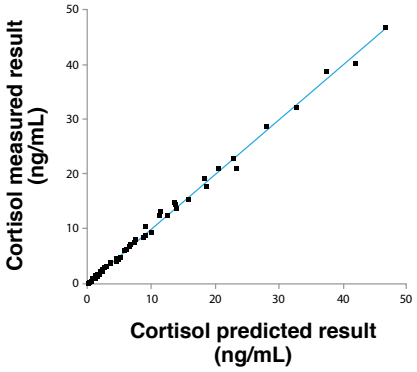
Compound	% Cross-Reactivity
Cortisol	100
Progesterone	1.9
Corticosterone	1.4
11-Deoxycorticosterone	< 0.04
Cortisone	6.3
Prednisone	3.6
DHEAS	< LoD
Prednisolone	17.8

Prednisolone does cross-react with most assays (~30% with most commercially available assays). Prednisolone has a half-life of ~3.5 hours and needs 16–18 hours to be adequately cleared from the circulation.

Since prednisone is converted to prednisolone in vivo, caution must be exercised when assaying the cortisol levels of patients undergoing either therapy. Patients being treated with prednisone or prednisolone should not be tested with this cortisol saliva test until at least 24 hours after the last treatment.

LINEARITY

The linearity study was performed with five human saliva samples with concentrations covering the range of the assay and following CLSI guideline EP06-A. The samples were diluted in calibrator A up to ten-fold (1:10), tested in duplicate, and the results (y) compared to the predicted concentration. The statistical analysis shows that the assay is sufficiently linear throughout the dynamic range of the kit up to a 1:10 dilution, when using calibrator A as the diluent.



y = 0.9949x + 0.0888
R² = 0.9965

PRECISION

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 with two lots 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	Within Run SD	Within Run CV	Total SD	Total CV
1	1.870	0.213	11.4%	0.218	11.7%
2	6.637	0.462	7.0%	0.589	8.9%
3	10.196	0.834	8.2%	1.061	10.4%
4	18.756	1.383	7.4%	1.763	9.4%
5	3.035	0.287	9.4%	0.291	9.6%
6	0.679	0.114	16.9%	0.118	17.4%
7	24.800	2.103	8.5%	2.567	10.4%
8	0.356	0.056	15.8%	0.058	16.4%
9	2.309	0.214	9.2%	0.228	9.9%
10	12.939	0.991	7.7%	1.090	8.4%

COMPARATIVE STUDIES

The DBC Cortisol Saliva ELISA kit assay (y) was compared to a commercial Cortisol Saliva ELISA assay (x). The comparison of 47 saliva samples yielded the following linear regression results:

$y = 0.844x + 0.634$, $R^2 = 0.929$

REFERENCE RANGES

Reference ranges (95%) were established using samples obtained from individuals of diverse races. Each laboratory shall establish their own range of reference values.

Group	N	Median (ng/mL)	95% Range (ng/mL)	95% Range (ng/mL)	Total Range (ng/mL)	Total Range (ng/mL)
Males AM	82	1.638	0.21–6.33	AM range (ng/mL) 0.21–7.27	0.07–7.63	AM range (ng/mL) 0.07–7.90
Females AM	78	2.265	0.23–7.27		0.113–7.90	
Males PM	76	0.897	0.16–3.42	PM range (ng/mL) 0.074–3.42	0.045–4.34	PM range (ng/mL) 0.02–4.34
Females PM	77	0.396	0.074–2.38		0.02–3.78	

Ordering Information: REF CAN-C-240

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Diagnostics Biochem Canada

Cortisol Serum ELISA

REF CAN-C-270

ASSAY PROCEDURE


Bring kit components to room temperature. Mix gently by inversion.


Prepare Wash Buffer Working Solution.


Pipette 20 µL of each calibrator, control and specimen sample.


Pipette 150 µL of the HRP Conjugate into each well.


Gently tap the mircoplate frame for 10 seconds to mix the contents.



Incubate at room temperature (no shaking) for 45 minutes. Wash 3 times.



Pipette 150 µL of TMB Substrate. Gently tap the frame to mix contents.


Incubate at room temperature (no shaking) for 15 minutes.


Pipette 50 µL of Stopping Solution.


Read the plate on a microplate reader at 450 nm.

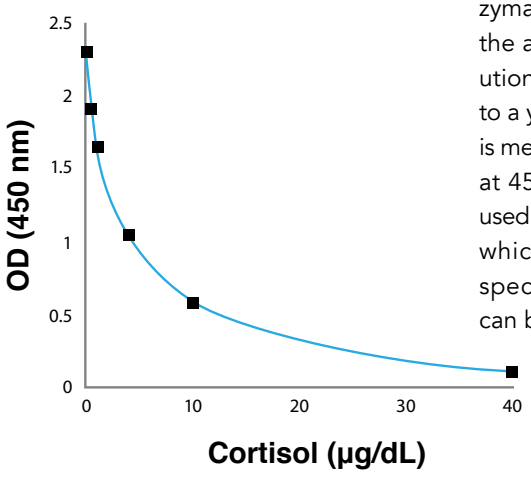
Cortisol is the most abundant circulating steroid and the major glucocorticoid secreted by the adrenal cortex. Cortisol is physiologically effective in blood pressure maintenance and anti-inflammatory activity. It is also involved in calcium absorption, gluconeogenesis as well as the secretion of gastric acid and pepsin.

Measurement of blood cortisol levels can be used as an indicator of adrenal function and the differential diagnosis of Addison's and Cushing's diseases as well as adrenal hyperplasia.

Most circulating cortisol is bound to cortisol binding globulin or transcortin. Therefore, the free cortisol concentration excreted in the urine is very small, and the 24-hour collection of urine is a must in order to obtain an accurate measurement of urinary cortisol. Cortisol in blood shows a diurnal rhythm with the highest levels in the morning and the lowest levels at night.

PRINCIPLE OF THE TEST

The Cortisol ELISA is a competitive immunoassay. Competition occurs between cortisol present in calibrators, controls, specimen samples and an enzyme-labelled antigen (HRP conjugate) for a limited number of anti-cortisol antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue-coloured product that is inversely proportional to the amount of cortisol present. Following an incubation, the enzymatic reaction is terminated by the addition of the stopping solution, converting the blue colour to a yellow colour. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of cortisol in specimen samples and controls can be directly read.



Typical calibration curve

PERFORMANCE CHARACTERISTICS

PRECISION

The precision study was performed according to the CLSI EP5-A3 guideline. The experimental protocol used a nested components-of-variance design with 8 serum samples, 10 testing days, two lots and two scientists per day. Each scientist ran two tests with two lots 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 (µg/dL)	Within Run SD (µg/dL)	Within Run CV%	Between Run SD (µg/dL)	Between Run CV%	Total SD (µg/dL)	Total CV %
1	3.520	0.097	2.7	0.188	5.3	0.220	6.3
2	12.640	0.206	1.6	0.389	3.1	0.440	3.5
3	32.347	0.349	1.1	0.551	1.7	0.652	2.0
4	0.829	0.072	8.7	0.079	9.5	0.107	12.8
5	15.358	0.341	2.2	0.350	2.3	0.502	3.3
6	32.274	0.493	1.5	0.546	1.7	0.736	2.3
7	9.461	0.179	1.9	0.295	3.1	0.361	3.8
8	4.568	0.208	4.5	0.162	3.5	0.274	6.0

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with cortisol cross-reacting at 100%. ND=Not detected.

Compound	% Cross-Reactivity
Cortisol	100
11-Deoxycorticosterone	3.8
11-Deoxycortisol	8.7
17-OH-progesterone	10.2
21-Deoxycortisol	ND
Aldosterone	0.8
Allopregnanolone	2.0
Androstenedione	1.0
Androsterone	ND
Corticosterone	14.2
Cortisone	8.8
Dehydroepiandrosterone	ND
Dehydroepiandrosterone Sulfate	ND
Dihydrotestosterone	ND
Estradiol	ND
Estriol	ND
Estrone	ND
Prednisolone	21.6
Prednisone	16.3
Pregnenolone	0.5
Testosterone	3.3

LINEARITY

The linearity study was according to the CLSI EP06-A guideline using three human serum samples covering the range of the assay.

The samples were diluted in calibrator A at several equidistant concentration levels and up to a 1:10 dilution. Samples were tested in duplicate, and the regression equation of the results (y) compared to the concentration (x) predicted from the dilution factor was:

$y = 0.98x + 0.17$, $r = 0.99$.

The relative non-linearity was ≤ 4.8% at a dilution ratio up to 1:10 for all samples. The statistical analysis demonstrates that the assay is sufficiently linear throughout the dynamic range of the kit.

COMPARATIVE STUDIES

The DBC Cortisol ELISA kit (y) was compared to a competitor's commercial Cortisol ELISA kit (x). The comparison of 71 human serum samples yielded the following linear regression results: $y = 1.17x - 0.73$, $r = 0.99$

REFERENCE RANGES

Reference ranges (95%) were estimated using male and female human serum samples collected during the AM and PM. Samples were obtained from adult individuals of diverse races. Results are summarized below. Each laboratory shall establish their own reference ranges.

Group	N	Mean (µg/dL)	95% Reference Range (µg/dL)
AM	52	11.46	4.94–18.32
PM	52	6.82	3.23–13.82

Ordering Information: REF CAN-C-270

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Diagnostics Biochem Canada

Dehydroepiandrosterone (DHEA) ELISA

REF CAN-DH-490

ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solution of the wash buffer.
- Pipette 25 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of the DHEA-HRP conjugate into each well.
- Gently tap the microplate frame for 10 seconds to mix contents.
- Incubate at room temperature (no shaking) for 90 minutes. Wash 3 times.
- Pipette 150 µL of TMB substrate. Gently tap the microplate frame for 10 seconds.
- Incubate at room temperature (no shaking) for 15–20 minutes
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

Dehyroepiandrosterone (DHEA) is a C19 steroid produced in the adrenal cortex and to a lesser extent in the gonads. DHEA serves as a precursor in testosterone and estrogen synthesis. Due to the presence of a 17-oxo-group, DHEA has relatively weak androgenic activity, which has been estimated at ~10% that of testosterone. However, in neonates, peripubertal children and in adult women, circulating DHEA levels may be several-fold higher than testosterone concentrations, and rapid peripheral tissue conversion to more potent androgens (androstenedione and testosterone) and estrogens may occur. Moreover, DHEA has a relatively low affinity for sex hormone-binding globulin—a factor that may enhance the physiological biopotency of DHEA.

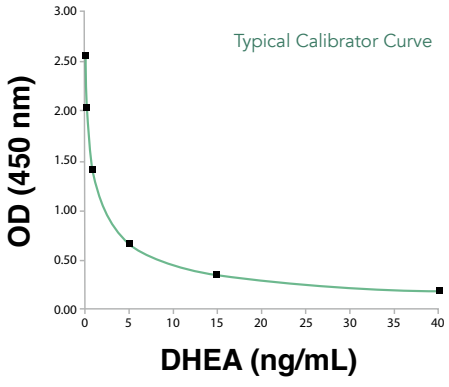
The physiological functions of DHEA are still the subject of investigation. DHEA reportedly plays a role in immune function, lipid metabolism, cholesterol, the nervous system, ageing and protection against viral infection. Serum DHEA levels are relatively high in the fetus and neonates, low during childhood, and increase during puberty until the third decade of life. No consistent change in serum DHEA levels occurs during the menstrual cycle or pregnancy. DHEA has a rapid metabolic clearance rate as compared to its sulfated conjugate. Because of this, serum DHEA levels are 100–1000 fold lower than DHEA-Sulfate levels. In addition, serum DHEA levels show significant diurnal variation which is dependent on adrenocorticotropic hormone (ACTH).

Abnormally low levels may occur in hypoadrenalism, and elevated levels may occur in several conditions, such as 21-hydroxylase and 3b-hydroxysteroid dehydrogenase deficiencies and some cases of female hirsutism.

Measurement of **serum DHEA** is a useful marker of adrenal androgen synthesis.

PRINCIPLE OF THE TEST

The DHEA ELISA is a competitive immunoassay. Competition occurs between DHEA (present in standards, controls and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of anti-DHEA antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the enzyme substrate is added, and approximately 15–20 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 DHEA in the sample. A calibrator curve is plotted with a provided set of calibrators to calculate directly the concentration of DHEA in patient samples and controls.



PERFORMANCE CHARACTERISTICS

PRECISION

The precision experimental protocol was conducted according to the CLSI EP5-A3 guideline using a nested components-of-variance design with 10 testing days, two lots and two operators per day. Each operator ran two tests with two lots per day and two replicate measurements per run (a 10 x 2 x 2 x 2 design) using human serum samples. The results were analyzed with a two-way nested ANOVA and summarized in the table below.

Sample	Mean (ng/mL)	Within Run SD	Within Run CV %	Between Run SD	Between Run CV %	Total SD	Total CV %
1	1.20	0.05	3.8%	0.12	10.2%	0.13	10.9%
2	3.50	0.09	2.7%	0.29	8.3%	0.31	8.7%
3	8.88	0.25	2.8%	0.54	6.1%	0.64	7.2%
4	3.26	0.10	3.0%	0.27	8.4%	0.29	9.0%
5	2.81	0.10	3.5%	0.25	8.7%	0.26	9.4%
6	1.38	0.04	3.2%	0.14	10.1%	0.16	11.5%
7	13.28	0.36	2.7%	0.95	7.1%	1.08	8.1%
8	20.20	0.51	2.5%	1.65	8.2%	1.73	8.6%

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with DHEA cross-reacting at 100%.

Steroid	% Cross-Reactivity
DHEA	100
11-Deoxycortisol	0.08
17-Hydroxypregnenolone	2.08
17α-Hydroxyprogesterone	0.18
Aldosterone	0.12
Androstenedione	0.41
Androsterone	0.14
Cholesterol	< 0.01
Cortisol	0.11
Corticosterone	0.11
DHEAS	< 0.008
DHT	0.37
Epiandrosterone	1.30
Estradiol	0.31
Estrone	0.16
Pregnenolone	9
Progesterone	0.22
Testosterone	0.32

REFERENCE RANGES

Reference ranges were established using serum samples from 264 female donors between 18–63 years old and 130 male donors between 18–65 years old. The reference ranges were determined using a non-parametric method and are summarized in the table below.

As for all clinical assays each laboratory should collect data and establish their own range of reference values.

Adults	Age (years)	n	Median (ng/mL)	Mean (ng/mL)	95% Reference Range (ng/mL)
Males	18–65	130	2.80	3.04	1.33–6.48
Females	18–63	264	2.35	2.61	1.00–5.86

Children	Age (years)	n	Total Range (ng/mL)
Males	1–9	28	0.20–1.5
	10–14	23	0.58–3.7
	15–18	14	1.50–3.6
Females	2–9	27	0.36–3.6
	10–14	21	0.47–5.5
	15–18	19	0.41–5.7

* Since the number of pediatric samples is insufficient to establish a 95% reference range, the total range is provided which shows the lowest to the highest value obtained in each age group.

Ordering Information: REF CAN-DH-490

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Diagnostics Biochem Canada

Dehydroepiandrosterone Sulfate (DHEAS) ELISA

REF CAN-DHS-480

ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 25 µL of each calibrator, control and specimen sample.
- Pipette 200 µL of conjugate working solution into each well.
- Incubate on a plate shaker for 45 minutes at room temperature.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a plate shaker for 15–20 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

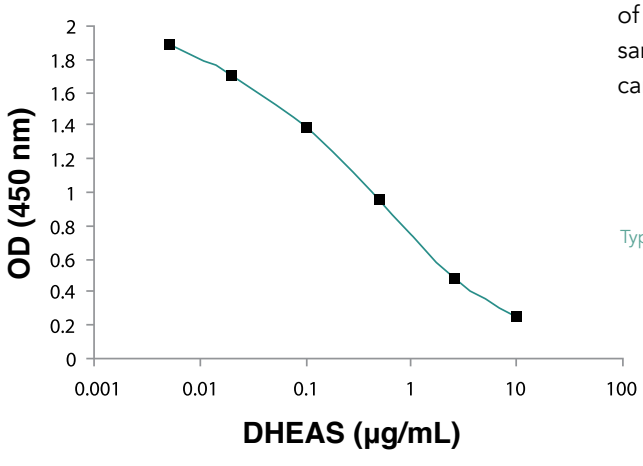
Dehydroepiandrosterone sulfate (DHEAS) is produced by the adrenals and gonads. As a result, the determination of the level of DHEA-S in serum is important in the evaluation of the functional state of these glands. DHEAS is a precursor of testosterone and estrone. Besides the adrenals in females, the ovaries have been shown to be an important source of DHEAS. It has been reported that there is a fluctuation day by day of DHEAS in women during the ovulatory cycle.

The principle production of testosterone in females is from conversion of other related androgens, especially DHEAS. An abnormal testosterone level in women should be accompanied by the estimation of serum DHEAS.

The use of serum testosterone determination in conjunction with Elisa of DHEAS can be used to determine if the source of excess androgen production is ovarian or adrenal.

PRINCIPLE OF THE TEST

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 patient 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 DHEAS in the sample. A set of standards is used to plot a standard curve from which the amount of DHEAS in patient samples and controls can be directly read.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

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 DBC Direct DHEAS ELISA kit is **0.005 µg/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct DHEAS ELISA kit with DHEAS cross-reacting at 100%.

Steroid	% Cross-Reactivity
DHEAS	100
Androsterone	16.0
Androstenedione	1.7
Testosterone	0.9
Progesterone	0.6
DHT	0.6
Cortisol	0.5

The following steroids were tested but cross-reacted at less than 0.001%: 17β-Estradiol, Estrone, Estrone-Sulfate and Pregnenolone.

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in µg/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.24	0.02	7.5
2	2.02	0.18	8.9
3	9.54	0.11	11.5

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in µg/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.13	0.02	15.3
2	1.11	0.09	8.1
3	6.38	0.27	4.2

RECOVERY

Spiked samples were prepared by adding defined amounts of DHEAS to three patient serum samples. The results (in µg/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	0.67	-	-
+ 0.1	0.84	0.77	109.1
+ 1.0	1.97	1.67	118.0
+ 5.0	5.80	5.67	102.3
2 Unspiked	1.21	-	-
+ 0.1	1.41	1.31	107.6
+ 1.0	2.01	2.21	91.0
+ 5.0	4.95	6.21	79.7
3 Unspiked	1.72	-	-
+ 0.1	1.93	1.82	106.0
+ 1.0	2.65	2.72	97.4
+ 5.0	5.45	6.72	81.1

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in µg/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	2.88	-	-
1:2	1.74	1.44	120.8
1:4	0.88	0.72	122.2
1:8	0.43	0.36	119.4
2	6.32	-	-
1:2	3.17	3.16	100.3
1:4	1.63	1.58	103.2
1:8	0.78	0.79	98.7
3	7.12	-	-
1:2	3.09	3.56	86.8
1:4	1.54	1.78	86.5
1:8	0.80	0.89	89.9

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (µg/mL)
Males	0.39–4.63
Females	0.46–2.75
Postmenopausal Females	0.48–2.08

Ordering Information: **REF CAN-DHS-480**

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Diagnostics Biochem Canada

Dihydrotestosterone (DHT) ELISA

REF CAN-DHT-280

ASSAY PROCEDURE

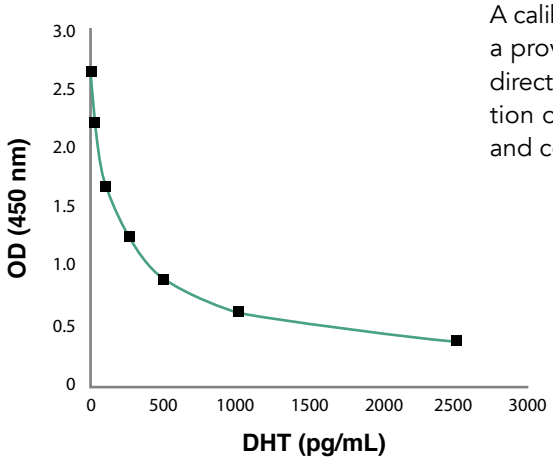
- Bring kit components to room temperature. Mix gently by inversion.
- Prepare working wash buffer.
- Pipette 25 µL of each calibrator, control, and specimen.
- Pipette 50 µL of the DHT-HRP conjugate into each well.
- Gently tap the microplate frame for 10 seconds to mix the contents of the wells.
- 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.

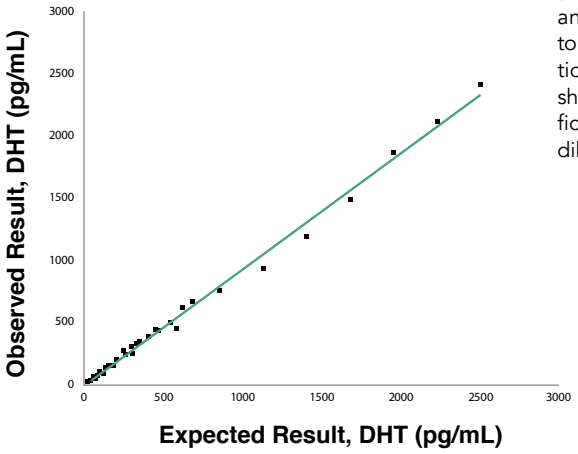
SPECIFICITY (CROSS-REACTIVITY)

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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Diagnostics Biochem Canada

Estradiol ELISA

REF CAN-E-430

ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 50 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of conjugate working solution into each well.
- Incubate on a plate shaker for 1 hour at room temperature.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

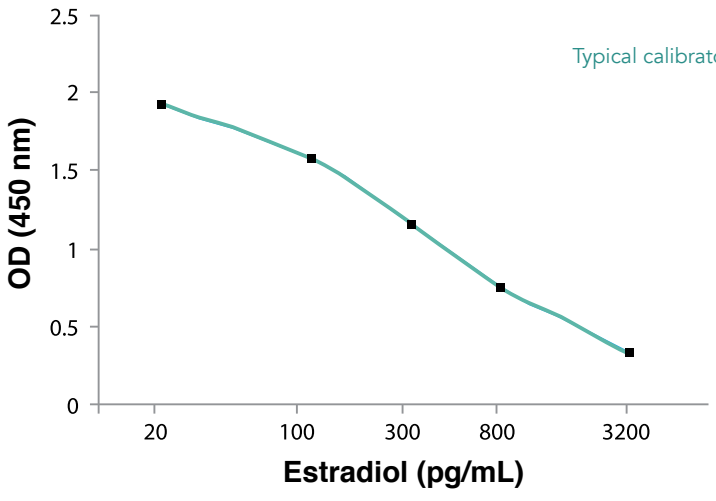
Estradiol is one of the main components of naturally occurring estrogens and is the major estrogen secreted during the menstrual cycle.

The serum levels of estradiol are low during the follicular phase rising gradually until about one day before ovulation when a marked rise in the estradiol level occurs (Ovulatory Peak). The estradiol level falls rapidly at, or right after ovulation and is again within the levels of the follicular phase. There is a second rise of estradiol around day 21 of the cycle (Luteal Peak). The levels then decline gradually to the lowest level at the onset of the next menstrual cycle.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabelled estradiol (present in standards, controls and patient samples) and an enzyme-labelled estradiol (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 unlabelled estradiol in the sample. A set of standards is used to plot a standard curve from which the amount of estradiol in patient samples and controls can be directly read.

Typical calibrator curve



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The detection limit is defined as the concentration of estradiol needed to give a B/B_0 values equivalent to the point where B is equal to B_0 minus 2X the SD of B_0. Based on 20 replicate analyses of standard A, the sensitivity is **10 pg/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct Estradiol ELISA kit with estradiol cross-reacting at 100%.

Steroid	% Cross-Reactivity
Estradiol	100
Estriol	1.6
Estrone	1.3
Progesterone	0.1
Cortisol	0.1

This assay should not be used for patients being treated with the drug fulvestrant (Faslodex®) which cross reacts with estradiol and could lead to a falsely elevated test result.

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	85.624	7.946	9.3
2	355.735	32.372	9.1
3	1104.385	51.243	4.6

INTER-ASSAY PRECISION

Three samples were assayed ten times. The results (in pg/mL) are tabulated below:

Sample	Mean	SD	CV %
1	82.044	8.286	10.1
2	324.623	31.813	9.8
3	1153.301	71.505	6.2

RECOVERY

Three human serum samples were spiked with defined amounts of estradiol. The recovery results (in pg/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	43.312	-	-
+ 800 (20%)	196.874	169.427	116.2
+ 3200 (10%)	360.670	330.284	109.2
+ 3200 (20%)	638.328	569.427	112.1
2 Unspiked	125.661	-	-
+ 800 (20%)	275.461	238.051	115.7
+ 3200 (10%)	415.680	405.146	102.6
+ 3200 (20%)	576.160	638.051	90.3
3 Unspiked	336.297	-	-
+ 800 (20%)	474.791	413.581	114.8
+ 3200 (10%)	600.214	596.634	100.6
+ 3200 (20%)	758.257	813.581	93.2

LINEARITY

Three human serum samples were diluted with calibrator A. The linearity results (in pg/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	638.328	-	-
1:2	272.247	319.164	85.3
1:4	140.592	159.582	88.1
1:8	74.844	79.791	93.8
2	576.160	-	-
1:2	324.092	288.080	112.5
1:4	168.957	144.040	117.3
1:8	73.646	72.020	102.3
3	758.257	-	-
1:2	335.908	379.129	88.6
1:4	186.152	189.564	98.2
1:8	78.103	94.782	82.4

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. The results of an expected range study with apparently normal healthy subjects yielded the following results (all values are reported in pg/mL):

Group	N	Mean	Central 95%
Males	40	22	< 100
Follicular Phase	10	41	15–120
Ovulation	3	289	200–400
Luteal Phase	10	193	175–325
Postmenopausal	30	28	< 90

Ordering Information: **REF CAN-E-430**

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Diagnostics Biochem Canada

Free Estriol
Serum and Saliva
ELISA

REF CAN-E-620

EU: CE IVD CAN: IVD

ASSAY PROCEDURE

Bring kit components to room temperature. Mix gently by inversion.

Prepare working wash buffer.

Serum: pipette 10 µL of each calibrator, control and specimen sample. Saliva: pipette 20 µL of each.

Pipette 150 µL of Estriol-HRP conjugate into each well.

Gently tap the microplate frame for 10 seconds to mix contents.

Incubate for 60 minutes at room temperature (no shaking). Wash 3 times.

Pipette 150 µL of TMB substrate.

Incubate for 10–15 minutes at room temperature (no shaking).

Pipette 50 µL of stopping solution. Gently tap the microplate frame to mix.

Read the plate on a microplate reader at 450 nm.

The production of estriol in pregnant women depends on a healthy maternal-placental-fetal system, the estriol concentration is a marker of both placental and fetal normal development and metabolism.

The determination of serum or saliva estriol concentration is instrumental for the assessment of fetus health in advanced pregnancy[1].

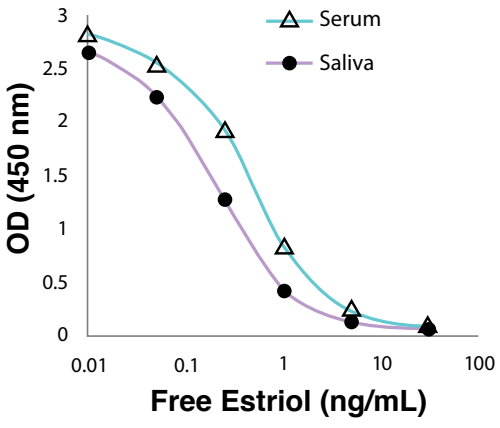
In non-pregnant women and men, estriol levels are low. Notwithstanding, one common application of salivary tests is the monitoring of the estriol levels in women undergoing hormone replacement therapy[2].

Due to the significant temporal fluctuations in the concentrations of this hormone, multiple tests are recommended to obtain reliable results[3].

1. Berkane N, et al. From Pregnancy to Preeclampsia: A Key Role for Estrogens. *Endocr Rev*. 2017;38(2):123–144.
2. Fleck SC, et al. Comparative estrogenicity of endogenous, environmental and dietary estrogens in pregnant women I: Serum levels, variability and the basis for urinary biomonitoring of serum estrogenicity. *Food Chem Toxicol*. 2018;115:511–522
3. Falah N, et al. Estriol review: Clinical applications and potential biomedical importance. *Clin Res Trials*. 2015;1(2):29–33. doi: 10.15761/CRT.1000109

PRINCIPLE OF THE TEST

The Free Estriol (also referred to as unconjugated estriol or uE3 in the literature) ELISA is a competitive immunoassay. Competition occurs between Estriol present in calibrators, controls and patient samples and an enzyme-labelled antigen (conjugate) for a limiting number of anti-Estriol antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the enzyme substrate is added, and approximately 15–20 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 Estriol in the sample. A calibrator curve is plotted with a provided set of calibrators to calculate directly the concentration of estriol in patient samples and controls.



Typical calibrator curve

Serum and salivary assays follow the same procedure except that the volume of the calibrators, controls and samples dispensed into the microplate wells is 10 µL for serum assays and 20 µL for salivary assays.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit was calculated following EP17-A. Sixty replicates of the matrix and a low concentration sample were run in independent tests with two lots of the kit.

Serum: The Limit of Background was determined to be 0.027 ng/mL and the Limit of Detection was determined to be 0.058 ng/mL.

Saliva: The Limit of Background was determined to be 0.017 ng/mL and the Limit of Detection was determined to be 0.034 ng/mL.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with estriol reacting at 100%.

Compound	% Cross-Reactivity
Estriol	100
Estriol-3-Sulfate	0.6
Estriol-3-Glucuronide	1.3
Estradiol	< 0.1
17α-Estradiol	< 0.1
Estradiol Sulfate	< 0.01
Estrone	< 0.1
Estrone Sulfate	< 0.01
Cholesterol	< 0.0001
Corticosterone	< 0.01
DHEAS	< 0.1
Equilin	< 0.1
Prednisone	< 0.001

LINEARITY

The linearity study was performed with four human serum and four human saliva samples covering the range of the assay and following CLSI guideline EP06-A. The samples were diluted in calibrator A at several equidistant concentration levels and up to ten-fold (1:10), tested in duplicate, and the results (y) compared to the predicted concentration (x). The statistical analysis shows that the assay is sufficiently linear up to a 1:10 dilution throughout the dynamic range of the kit when using calibrator A as the diluent.

Serum: $y = 1.04x - 0.71$; $r = 0.99$

Saliva: $y = 0.95x - 0.08$; $r = 0.99$

PRECISION

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 with two lots 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 (ng/mL)	Within Run SD	Within Run CV	Total SD	Total CV
1	0.167	0.023	13.6%	0.026	15.6%
2	0.264	0.032	12.3%	0.036	13.8%
3	0.946	0.062	6.5%	0.066	7.0%
4	4.841	0.326	6.7%	0.366	7.6%
5	11.89	1.107	9.3%	1.148	9.7%
6	16.10	1.621	10.1%	1.639	10.2%
7	3.544	0.232	6.5%	0.256	7.2%
8	1.927	0.110	5.7%	0.119	6.2%
9	5.932	0.403	6.8%	0.448	7.5%
10	9.127	0.606	6.6%	0.619	6.8%

COMPARATIVE STUDIES

The DBC Free Estriol ELISA kit (y) was compared to a commercial Estriol Immunofluorescence assay (x) used for IVD. The comparison of 61 serum samples yielded the following linear regression results:

$y = 0.92x - 0.12$, $r = 0.99$

The DBC Free Estriol ELISA kit (y) was compared to a commercial High Sensitivity Estriol ELISA kit (x). The comparison of 40 saliva samples yielded the following linear regression results:

$y = 1.32x + 0.06$, $r = 0.97$

REFERENCE RANGES

Serum Cohort Group	n	Median (ng/mL)	95% Range (ng/mL)	Total Range (ng/mL)
Adult Males and Non-Pregnant Females	120	< 0.058	ND–0.11	ND–0.12
Pregnant Females First Trimester	30	0.15	—	ND–2.95
Pregnant Females Second Trimester	50	1.20	0.46–3.04	0.45–3.07
Pregnant Females Third Trimester	25	9.5	—	3.6–14.3
Saliva Cohort Group	**n**	**Median (ng/mL)**	**95% Range (ng/mL)**	**Total Range (ng/mL)**
Adult Males and Non-Pregnant Females	80	0.05	ND–0.08	ND–0.08
Pregnant Females Third Trimester	4	0.71	—	0.6–1.3

Ordering Information: **REF CAN-E-620**

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Diagnostics Biochem Canada

Free Estriol Serum ELISA

REF CAN-E-640

ASSAY PROCEDURE

Bring kit components to room temperature. Mix gently by inversion.

Prepare working wash buffer.

Pipette 10 µL of each calibrator, control and specimen sample.

Pipette 150 µL of Estriol-HRP conjugate into each well.

Gently tap the microplate frame for 10 seconds to mix contents.

Incubate for 1 hour at room temperature (no shaking). Wash 3 times.

Pipette 150 µL of TMB substrate.

Incubate for 15–20 minutes at room temperature (no shaking).

Pipette 50 µL of stopping solution. Gently tap the microplate frame to mix.

Read the plate on a microplate reader at 450 nm.

Since the production of estriol in pregnant women depends on a healthy maternal-placental-fetal system, the estriol concentration is a marker of both placental and fetal normal development and metabolism.

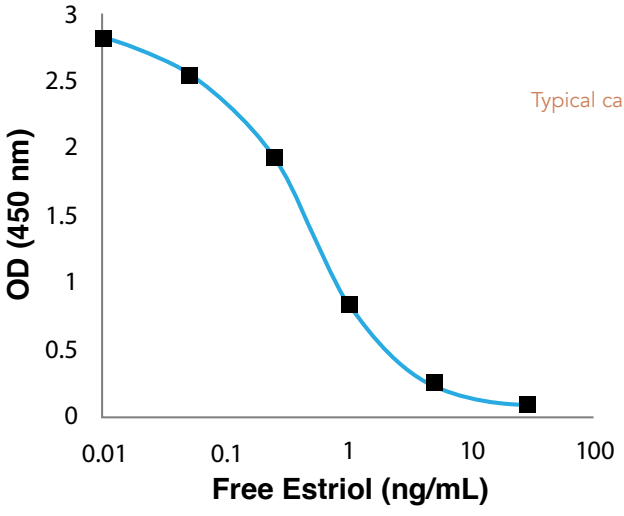
The determination of serum estriol concentration is instrumental for the assessment of fetus health in advanced pregnancy[1].

Due to the significant temporal fluctuations in the concentrations of this hormone, multiple tests are recommended to obtain reliable results[2].

1. Berkane N, et al. From Pregnancy to Preeclampsia: A Key Role for Estrogens. *Endocr Rev.* 2017;38(2):123–144.
2. Fleck SC, et al. Comparative estrogenicity of endogenous, environmental and dietary estrogens in pregnant women I: Serum levels, variability and the basis for urinary biomonitoring of serum estrogenicity. *Food Chem Toxicol.* 2018;115:511–522

PRINCIPLE OF THE TEST

The Free Estriol (also referred to as unconjugated estriol or uE3 in the literature) ELISA is a competitive immunoassay. Competition occurs between Estriol present in calibrators, controls and patient samples and an enzyme-labelled antigen (conjugate) for a limiting number of anti-Estriol antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the enzyme substrate is added, and approximately 15–20 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 Estriol in the sample. A calibrator curve is plotted with a provided set of calibrators to calculate directly the concentration of estriol in patient samples and controls.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit was calculated following EP17-A. Sixty replicates of the matrix and a low concentration sample were run in independent tests with two lots of the kit.

The Limit of Background was determined to be 0.027 ng/mL and the Limit of Detection was determined to be 0.058 ng/mL.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with estriol reacting at 100%.

Compound	% Cross-Reactivity
Estriol	100
Estriol-3-Sulfate	0.6
Estriol-3-Glucuronide	1.3
Estradiol	< 0.1
17α-Estradiol	< 0.1
Estradiol Sulfate	< 0.01
Estrone	< 0.1
Estrone Sulfate	< 0.01
Cholesterol	< 0.0001
Corticosterone	< 0.01
DHEAS	< 0.1
Equilin	< 0.1
Prednisone	< 0.001

INTERFERENCES

Hemoglobin up to 2 g/L, Bilirubin conjugated and unconjugated up to 20 mg/dL, Triglycerides up to 5 mg/mL, Biotin up to 10 µg/mL, Daidzein, Genistein and Resveratrol each up to 200 ng/mL, HAMAS up to 1.2 µg/mL, and Rheumatoid Factor up to 1.2 IU/mL did not interfere with the assay.

COMPARATIVE STUDIES

The DBC Free Estriol ELISA kit (y) was compared to a commercial Estriol Immunofluorescence assay (x) used for IVD. The comparison of 61 serum samples yielded the following linear regression results:
y = 0.92x - 0.12, r = 0.99

PRECISION

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 with two lots 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 (ng/mL)	Within Run SD	Within Run CV%	Total SD	Total CV%
1	0.167	0.023	13.6	0.026	15.6
2	0.264	0.032	12.3	0.036	13.8
3	0.946	0.062	6.5	0.066	7.0
4	4.841	0.326	6.7	0.366	7.6
5	11.89	1.107	9.3	1.148	9.7
6	16.10	1.621	10.1	1.639	10.2
7	3.544	0.232	6.5	0.256	7.2
8	1.927	0.110	5.7	0.119	6.2
9	5.932	0.403	6.8	0.448	7.5
10	9.127	0.606	6.6	0.619	6.8

LINEARITY

The linearity study was performed with four human serum covering the range of the assay and following CLSI guideline EP06-A. The samples were diluted in calibrator A at several equidistant concentration levels and up to ten-fold (1:10), tested in duplicate, and the results (y) compared to the predicted concentration (x). The statistical analysis shows that the assay is sufficiently linear up to a 1:10 dilution throughout the dynamic range of the kit when using calibrator A as the diluent. y = 1.04x - 0.71; r = 0.99

REFERENCE RANGES

Reference ranges (95%) were estimated using samples obtained from individuals of diverse races. Each laboratory shall establish their own range of reference values.

Cohort Group	n	Median (ng/mL)	95% Range (ng/mL)	Total Range (ng/mL)
Adult Males and Non-Pregnant Females	120	< 0.058	ND–0.11	ND–0.12
Pregnant Females First Trimester	30	0.15	-	ND–2.95
Pregnant Females Second Trimester	50	1.20	0.46–3.04	0.45–3.07
Pregnant Females Third Trimester	25	9.5	-	3.6–14.3

Ordering Information: REF CAN-E-640

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Diagnostics Biochem Canada

Total Estrogens ELISA

REF CAN-E-630

ASSAY PROCEDURE

Bring kit components to room temperature. Mix gently by inversion. Prepare working wash buffer.

Pipette 50 µL of each calibrator, control and specimen sample.

Incubate on a microplate shaker for 30 minutes at room temperature.

Pipette 150 µL of Estrogen-HRP conjugate.

Incubate on a microplate shaker for 120 minutes at room temperature.

Wash 3 times.

Pipette 150 µL of TMB substrate.

Incubate on a microplate shaker for 30 minutes at room temperature.

Pipette 50 µL of stopping solution. Tap gently on microplate frame to mix.

Read the plate on a microplate reader at 450 nm.

Total estrogens comprise the total quantity of estrone, estradiol, and estriol. The estrogens are involved in the development of female sex organs and secondary sex characteristics. Before the ovum is fertilized, the main action of the estrogens is on the growth and function of the reproductive tract to prepare it for the fertilized ovum.

During the follicular phase of the menstrual cycle, the total estrogens level shows a slight increase. The production of total estrogens then increases markedly to peak at around day 13. The peak is of short duration and by day 16 of the cycle levels will be low. A second peak occurs at around day 21 of the cycle. If fertilization does not occur, the production of total estrogens decreases.

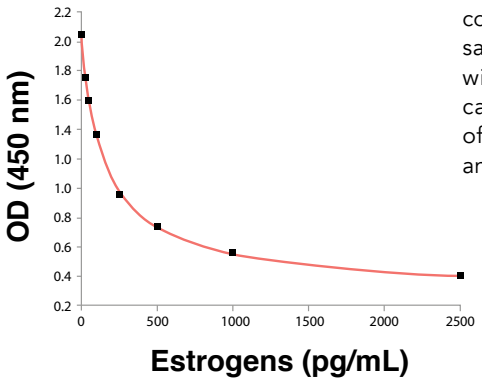
In post-menopausal women, the concentration of all estrogens decreases substantially and estrone becomes the predominant estrogen. In pregnant women, the concentration of all estrogens escalates and estriol becomes the predominant estrogen.

A total estrogens test is commonly indicated to:

- *Aid in diagnosis of sex steroid metabolism related conditions, for example, **premature or delayed puberty**, and **aromatase** and **17 alpha-hydroxylase deficiencies**.*
- *Follow-up female hormone replacement therapy in post-menopausal women.*
- *Prognose antiestrogen therapy, for example, aromatase inhibitor therapy.*

PRINCIPLE OF THE TEST

The total estrogens ELISA is a competitive immunoassay. Competition occurs between total estrogens (estrone, estradiol, and estriol) present in calibrators, controls and patient samples and an enzyme-labelled antigen (conjugate) for a limited number of anti-estrogen 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 total estrogens in the sample. A calibrator curve is plotted with a provided set of calibrators to calculate directly the concentration of total estrogens in patient samples and controls.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit was calculated following EP17-A2. Sixty replicates of the matrix and a low concentration sample were run in independent tests with two lots of the kit. The Limit of Background was determined to be 5.4 pg/mL and the Limit of Detection was determined to be **12.4 pg/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The cross-reactivity was evaluated in relation to estrogens reacting at 100%.

Compound	% Cross-Reactivity	Compound	% Cross-Reactivity
Estrone	100	DHEA	0.3
17β-Estradiol	100	DHEAS	0.004
Estriol	100	DHT	0.5
11-Deoxycorticosterone	0.4	Equilin	6.3
17-Hydroxyprogesterone	0.3	Estradiol sulfate	0.1
17α-Estradiol	5.3	Estrone sulfate	0.07
Aldosterone	0.2	Prednisone	0
Androstenedione	0.2	Pregnenolone	< 0.1
Androsterone	0.2	Pregnenolone sulfate	< 0.1
Cholesterol	0	Progesterone	< 0.1
Corticosterone	< 0.01	Testosterone	0.3
Cortisol	< 0.1		

INTERFERENCES

Hemoglobin up to 2 g/L, Bilirubin conjugated and unconjugated up to 10 mg/dL, Triglycerides up to 5 mg/mL, Biotin up to 2.4 µg/mL, HAMAS up to 1.2 µg/mL, and Rheumatoid Factor up to 1500 IU/mL did not interfere with the assay.

Note on Fulvestran

Estradiol immunoassays have been reported to show interference from the drug Fulvestran (Faslodex®). This cross-reactivity can cause falsely elevated estrogen levels in patients under Fulvestrant treatment.

The following results were obtained with theTotal Estrogens ELISA kit after pooled serum samples from three cohorts were spiked to a concentration of 25 ng/mL of Fulvestran.

Sample	Unspiked Sample (pg/mL)	Sample Spiked to 25 ng/mL Fulvestran (pg/mL)
Pool 1	106.8	128.6
Pool 2	87.8	105.8
Pool 3	326.4	377.6

The Cmax has been reported as 11.4 ng/mL (Robertson and Harrison, 2004) and 25.1 ng/mL (AstraZeneca Canada, 2017).

References

1. Robertson JFR and Harrison M. Fulvestran Pharmacokinetics and pharmacology. *British Journal of Cancer*. 2004; 90:S7–S10.
2. Faslodex® Product Monograph. AstraZeneca Canada, 2017.

PRECISION

The experimental protocol used a nested components-of-variance design with 10 testing days, two runs per scientist 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	Within Run SD	Within Run CV%	Between Run SD	Between Run CV%	Total SD	Total CV%
1	104.6	6.6	6.3	8.3	8.0	11.9	11.4
2	56.5	5.3	9.3	7.0	12.4	8.8	15.5
3	377.2	17.6	4.7	10.8	2.9	24.4	6.5
4	83.3	4.7	5.7	4.2	5.0	7.1	8.5
5	100.2	6.0	6.0	7.5	7.4	9.9	9.9
6	251.8	10.3	4.1	13.3	5.3	17.0	6.8
7	365.9	16.8	4.6	52.2	14.3	54.8	15.0
8	1276.7	78.9	6.2	46.8	3.7	98.0	7.7

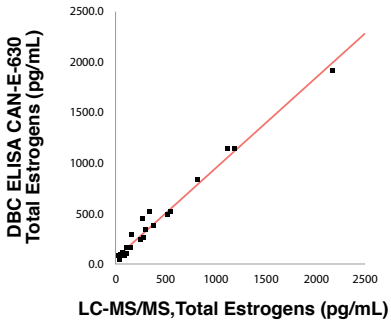
LINEARITY

The linearity study was performed with four human serum samples covering the range of the assay and following CLSI guideline EP06-A. The samples were diluted in calibrator A 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 when using calibrator A as the diluent.

COMPARATIVE STUDIES

The DBC Total Estrogens ELISA kit (y) was compared to Liquid Chromatography-Tandem Mass Spectrometry (x) Estrogens method. The comparison of 27 serum samples yielded the following linear regression results:

y = 0.89x + 62, r = 0.99



REFERENCE RANGES

Reference ranges (95%) were established using samples obtained from individuals of diverse races. Each laboratory shall establish their own range of reference values.

Group	N	Median (pg/mL)	95% Reference Range (pg/mL)
Pre-menopausal Females, cycle 1–10 days 11–20 days 21–30 days	 40 40 40	 120 136 168	 16–328 34–501 48–350
Post-menopausal Females	120	74	40–244
Adult Males	120	104	56–213

Ordering Information: REF CAN-E-630

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Diagnostics Biochem Canada

Estrone ELISA

REF CAN-E-420

ASSAY PROCEDURE

Bring kit components to room temperature. Mix gently by inversion.

Prepare Wash Buffer Working Solution.

Pipette 50 µL of each calibrator, control and specimen sample.

Pipette 100 µL of the HRP Conjugate into each well.

Gently tap the microplate frame for 10 seconds to mix. Incubate for at room temperature (no shaking) for 60 minutes.

Wash 3 times.

Pipette 150 µL of TMB Substrate.

Incubate the microplate at room temperature (no shaking) for 20 minutes Do not tap the microplate and avoid placing in intense light or air currents.

Pipette 50 µL of Stopping Solution. Gently tap on the microplate frame to mix.

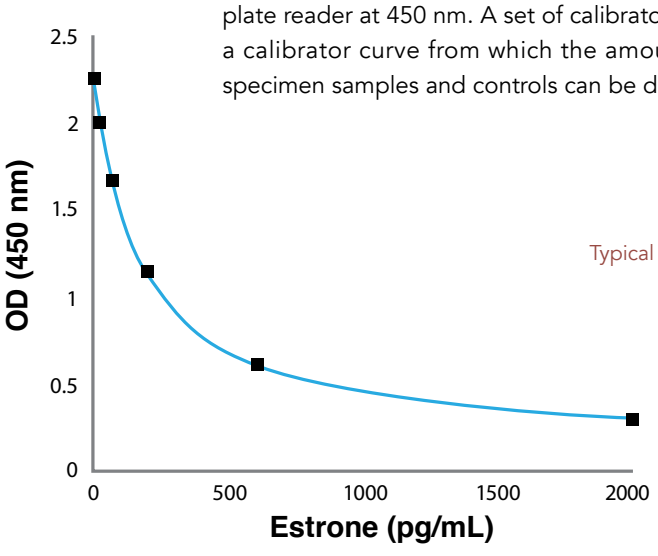
Read the plate on a microplate reader at 450 nm.

Estrone is a steroid, a female sex hormone and, with estradiol and estriol, one of the three most important endogenous estrogens. Estrogens are involved in the development of female sex organs and secondary sex characteristics. Before the ovum is fertilized the main action of the estrogens is on the growth and function of the reproductive tract in order to prepare it for the fertilized ovum.

During the follicular phase of the menstrual cycle the estrone level shows a slight increase. The production of estrone then increases markedly to peak at around day 13. The peak is of short duration and by day 16 of the cycle levels will be low. A second peak occurs at around day 21 of the cycle and if fertilization does not occur, then the production of estrone decreases.

PRINCIPLE OF THE TEST

The Estrone ELISA is a competitive immunoassay. Competition occurs between estrone present in calibrators, controls, specimen samples and an enzyme-labelled antigen (HRP conjugate) for a limited number of anti-estrone antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue-coloured product that is inversely proportional to the amount of estrone present. Following an incubation, the enzymatic reaction is terminated by the addition of the stopping solution, converting the colour from blue to yellow. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of estrone in specimen samples and controls can be directly read.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The analytical sensitivity study was performed according to the CLSI EP17-A2 guideline. The Limit of Background (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) are summarized in the table below:

Parameter	Estrone (pg/mL)
LoB	5.6
LoD	14.8
LoQ	17.7

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct Estrone ELISA kit with estrone cross-reacting at 100%:

Compound	% Cross-Reactivity
Estrone	100
11-Deoxycorticosterone	< 0.1
17-hydroxyprogesterone	< 0.1
17α-estradiol	3.6
17β-Estradiol	7.9
Aldosterone	< 0.1
Androstenedione	< 0.1
Androsterone	< 0.1
Cholesterol	< 0.01
Corticosterone	< 0.1
Cortisol	0.2
Danazol	< 0.1
DHEA	0.1

Compound	% Cross-Reactivity
DHEAS	< 0.1
DHT	< 0.1
Equilin	19.1
Estradiol sulfate	≤ 2.9
Estriol	2.6
Estrone sulfate	2.5
Ethisterone	< 0.1
Prednisone	< 0.1
Pregnenolone	< 0.1
Pregnenolone sulfate	< 0.1
Progesterone	< 0.1
Testosterone	< 0.1

LINEARITY

The linearity study was performed according to the CLSI EP06-Ed2 guideline using six human serum samples covering the range of the assay. The samples were diluted in low estrone value (<60 pg/mL) serum samples up to ten percent (1:10), tested in duplicate, and the regression equation of the results (y) compared to the concentration (x) predicted from the dilution factor was y = 1.001x + 10.2, r = 0.999.

The relative non-linearity ranged between -10.6% and 10.5% across all samples and measurement dilution points. The statistical analysis shows that the assay is sufficiently linear up to a 1:10 dilution when using low estrone value (<60 pg/mL) serum samples as the diluent.

PRECISION

The precision study was performed according to the CLSI EP05-A3 guideline.

The experimental protocol used a nested components-of-variance design with 8 serum samples, 10 testing days, two lots and two scientists per day. Each scientist ran two tests with two lots 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 are summarized in the table below.

Sample	Mean (pg/mL)	Within Run		Between Run		Total	
		SD (pg/mL)	CV %	SD (pg/mL)	CV %	SD (pg/mL)	CV
1	91.5	8.5	9.2	11.7	12.8	14.4	15.8
2	40.7	5.1	12.4	6.2	15.1	8.0	19.6
3	144.8	11.9	8.2	15.2	10.5	20.1	13.9
4	744.4	33.4	4.5	31.7	4.3	46.7	6.3
5	632.8	26.3	4.2	41.9	6.6	56.2	8.9
6	1027.0	55.1	5.4	26.1	2.5	73.5	7.2
7	381.0	18.2	4.8	25.5	6.7	34.1	8.9
8	1211.7	53.0	4.4%	71.2	5.9%	106.2	8.8%

COMPARATIVE STUDIES

This DBC Estrone ELISA kit (y) was compared against a Liquid Chromatography-Mass Spectrometry (LC-MS/MS) method (x) and yielded the following linear regression results:

y = 0.80x + 25.82, 105 samples, r = 0.92, Slope = 0.80.

REFERENCE RANGES

Reference ranges (95%) were estimated using samples obtained from individuals of diverse races (all values are reported in pg/mL). Each laboratory shall establish their own range of reference values.

Cohort	N	Mean	Median	95% Range	
				2.5%	97.5%
Adult Female Premenopausal*	140	93.9	83.3	19.5	231.9
Adult Female, Menstrual cycle					
1–10 days	*40*	*84.4*	*81.5*	*29.8*	*146.7*
11–20 days	*40*	*87.7*	*79.6*	*20.9*	*232.0*
21–30 days	*40*	*82.2*	*73.2*	*27.2*	*173.8*
Adult Female Postmenopausal*	205	31.9	42.5	ND	166.4
Adult Male	202	59.1	52.1	ND	187.2

*The menopausal status was classified according to age.
ND = Non-Detectable; results below the LoD (14.8 pg/mL).

Ordering Information: REF CAN-E-420

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Diagnostics Biochem Canada

Ferritin ELISA

REF CAN-F-4280

ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 20 µL of each calibrator, control and specimen sample.
- Pipette 200 µL of the conjugate working solution into each well.
- Incubate on a plate shaker for 30 minutes at room temperature.
- Wash 5 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

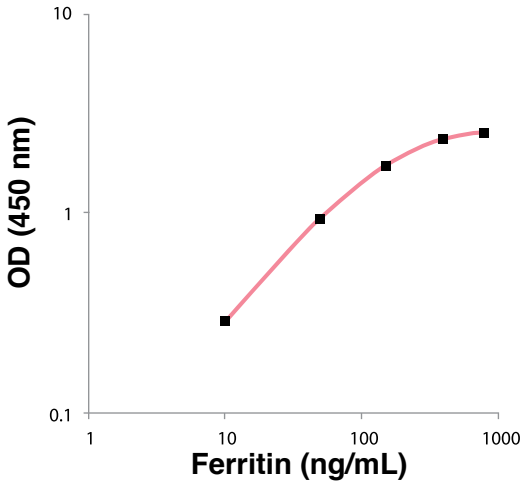
Ferritin is a protein which serves as a storage center for iron. It is found in many tissues but the highest concentrations are in the liver, spleen and bone marrow.

The total body iron stores in normal people correlate well with the concentration of ferritin in serum.

If there is a deficiency in iron, that is the concentration of iron is low in the blood, the ferritin result will be decreased. Likewise, an overload of iron indicates an increase in the level of ferritin. However, in some conditions like liver disease ferritin will be elevated.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical one-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for ferritin is immobilized onto the microplate and another monoclonal antibody specific for a different region of ferritin is conjugated to horse radish peroxidase (HRP). Ferritin from the sample and standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. 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 by the enzymatic reaction is directly proportional to the concentration of ferritin in the sample.



Typical calibrator curve

A set of standards is used to plot a standard curve from which the amount of ferritin in patient samples and controls can be directly read.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection (LoD) was determined from the analysis of 40 samples of the blank and a low value sample.

LoD = **0.44 ng/mL**

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	172.21	6.69	3.9
2	417.23	34.18	8.2
3	923.89	91.20	9.9

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	92.12	6.53	7.1
2	322.73	8.14	2.5
3	1704.63	67.01	3.9

RECOVERY

Spiked samples were prepared by adding defined amounts of ferritin to three patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	63.73	-	-
+ 375	415.25	438.73	94.7
+ 750	780.68	813.73	95.9
+ 1500	1266.70	1563.73	81.0
2 Unspiked	69.00	-	-
+ 375	442.97	444.00	99.8
+ 750	836.26	819.00	102.1
+ 1500	1326.73	1569.00	84.6
3 Unspiked	137.64	-	-
+ 375	484.96	512.64	94.6
+ 750	955.72	887.64	107.7
+ 1500	1463.27	1637.64	89.4

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	348.50	-	-
1:2	185.41	174.25	106.4
1:4	100.96	87.13	115.9
1:8	49.74	43.56	114.2
1:16	23.79	21.78	109.2
2	810.22	-	-
1:2	376.80	405.11	93.0
1:4	196.93	202.56	97.2
1:8	105.08	101.28	103.8
1:16	45.10	50.64	89.1
3	1733.54	-	-
1:2	873.38	866.77	100.8
1:4	409.42	433.38	95.5
1:8	192.05	216.69	88.6
1:16	110.62	108.35	102.1

COMPARATIVE STUDIES

The DBC Direct Ferritin ELISA kit (x) was compared with a competitors coated tube RIA kit (y). The comparison of 29 serum samples yielded the following linear regression results:

$y = 1.03x - 20.12$

$R^2 = 0.97$

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (ng/mL)
Healthy normal males and females	25–283

Ordering Information: REF CAN-F-4280

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Diagnostics Biochem Canada

Follicle Stimulating Hormone (hFSH) ELISA

REF CAN-FSH-4060

ASSAY PROCEDURE

- Bring kit components to room temperature. Prepare working solutions.
- Pipette 25 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of assay buffer into each well.
- Incubate on a plate shaker for 30 minutes at room temperature.
- Wash 3 times.
- Pipette 100 µL of conjugate working solution.
- Incubate on a plate shaker for 30 minutes at room temperature.
- Wash 3 times. Pipette 100 µL of TMB substrate.
- Incubate on a plate shaker for 15–20 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

Human follicle stimulating hormone (FSH) is a glycoprotein hormone produced by the anterior pituitary gland. There are three other glycoprotein hormones, namely Thyroid Stimulating Hormone, Luteinizing Hormone (both produced by anterior pituitary gland) and Human Chorionic Gonadotropin (produced by the placenta) which are structurally similar. Each hormone has an alpha and beta subunit. The α subunits of each hormone are similar while the β subunit is specific to each hormone. The α subunits contain 92 amino acids while the β subunits vary with each hormone. The β subunit of both FSH and LH contain 115 amino acids, TSH 110 amino acids, and hCG 147 amino acids.

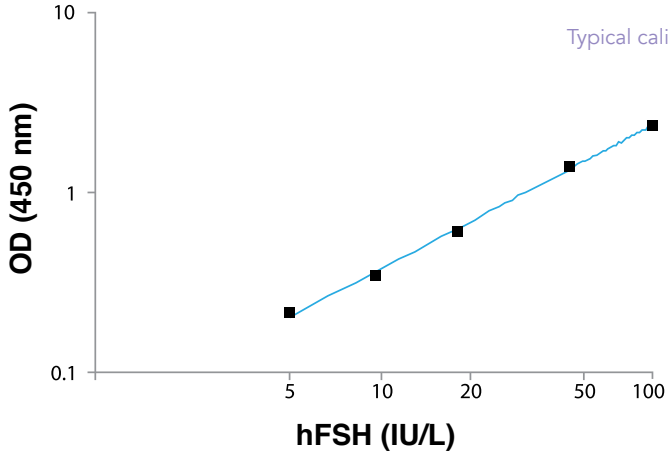
The FSH and LH hormones function differently in females and males. It is to be noted that in women the growth and maturation of the ovarian follicle is dependent on FSH, while in men both LH and FSH act on the testes.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for FSH is immobilized onto the microplate and another monoclonal antibody specific for a different region of FSH is conjugated to horse radish peroxidase (HRP). FSH from the sample and standards are allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second 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 by the enzymatic reaction is directly proportional to the concentration of FSH in the sample.

A set of standards is used to plot a standard curve from which the amount of FSH in patient samples and controls can be directly read.

Typical calibrator curve



PERFORMANCE CHARACTERISTICS

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) plus 2 SD. Therefore, the sensitivity of the DBC Direct FSH ELISA kit is **1 IU/L**.

SPECIFICITY (CROSS-REACTIVITY)

The specificity of the Direct hFSH ELISA kit was determined by measuring the apparent hFSH value of calibrator A spiked with the following compounds:

Substance	Concentration Range	Apparent hFSH Value (IU/L)
hCG Calibrated against WHO 3rd IS 75/537	1000–50,000 IU/L	Not Detected
hLH Calibrated against WHO 2nd IS 80/552	5–250 IU/L	Not Detected
hTSH Calibrated against WHO 2nd IS 80/558	5–250 mIU/L	< 4.0

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in IU/L) are tabulated below:

Sample	Mean	SD	CV %
1	7.41	0.43	5.8
2	48.57	1.70	3.5
3	138.12	4.70	3.4

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in IU/L) are tabulated below:

Sample	Mean	SD	CV %
1	7.11	0.24	3.4
2	44.31	2.01	4.5
3	120.63	7.74	7.7

RECOVERY

Spiked samples were prepared by adding defined amounts of FSH to three patient serum samples. The results (in IU/L) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	5.65	-	-
+ 9.7	14.75	15.35	96.1
+ 53.5	56.68	59.15	95.8
+ 107.0	103.33	112.65	91.7
2 Unspiked	17.52	-	-
+ 9.7	26.21	27.22	96.3
+ 53.5	70.07	71.02	98.7
+ 107.0	116.40	124.52	93.5
3 Unspiked	58.47	-	-
+ 9.7	72.71	68.17	106.7
+ 53.5	114.25	111.97	102.0
+ 107.0	171.05	165.47	103.4

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in IU/L) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	22.56	-	-
1:2	11.89	11.28	105.4
1:4	6.47	5.64	114.7
1:8	3.27	2.82	116.0
2	123.42	-	-
1:2	66.80	61.71	108.2
1:4	31.78	30.86	103.0
1:8	17.05	15.43	110.5
3	162.67	-	-
1:2	77.93	81.34	95.8
1:4	39.35	40.67	96.8
1:8	21.86	20.33	107.5

HIGH DOSE HOOK EFFECT

The Direct hFSH ELISA kit did not experience any high dose hook effect when tested up to a FSH concentration of 50,000 IU/L.

REFERENCE VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (IU/L)
Males	1–18
Females: Follicular Stage	2–10
Midcycle Peak	7–20
Luteal Stage	1–10
Postmenopausal	18–150

Ordering Information: **REF CAN-FSH-4060**

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Diagnostics Biochem Canada

Growth Hormone (hGH) ELISA

REF CAN-GH-4070

ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 25 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of conjugate working solution into each well.
- Incubate on a plate shaker for 1 hour at room temperature.
- Wash 3 times.
- Pipette 100 µL of TMB substrate.
- Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

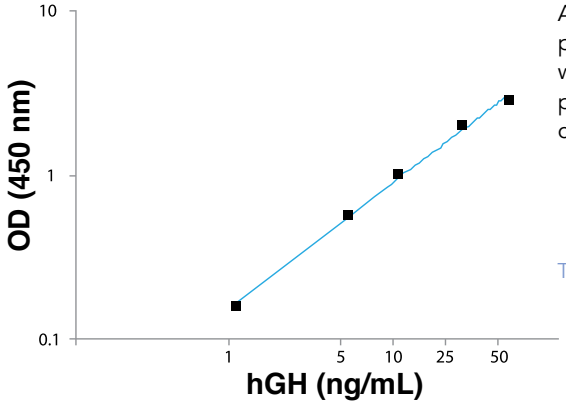
Human growth hormone (hGH) is a polypeptide of 191 amino acids secreted by the somatroph cells of the anterior pituitary. Growth hormone is principally a regulator of body growth and its metabolic effects are primarily anabolic. Some of its effects include promotion of protein conservation through its involvement in a wide range of protein synthesis mechanisms, enhancement of glucose transport and facilitation of glycogen storage. In addition, it induces the release of somatomedins (insulin-like growth factors), which further mediate the cascade of growth promoting actions.

Measurement of hGH is primarily of interest in the diagnosis and treatment of various forms of decreased secretion of hGH. Hyposecretion of hGH in children results in growth retardation and hypersecretion leads to gigantism in children and acromegaly in adults.

The secretion of hGH varies throughout the day under the influence of intricate neurogenic, metabolic and hormonal control. Due to the pulsatile nature of hGH release, it is often inaccurate to define a reference range and status based on single serum measurements. To diagnose disorders of hGH secretion more reliably, dynamic tests are used in which serum hGH levels are measured over a period following suppression or stimulation of hGH secretion.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical one-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for hGH is immobilized onto the microplate and another monoclonal antibody specific for a different region of hGH is conjugated to horse radish peroxidase (HRP). hGH from the sample and standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. 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 by the enzymatic reaction is directly proportional to the concentration of hGH in the sample.



A set of standards is used to plot a standard curve from which the amount of hGH in patient samples and controls can be directly read.

Typical calibrator curve

PERFORMANCE CHARACTERISTICS

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) plus 2 SD. Therefore, the sensitivity of the DBC Direct hGH ELISA kit is **0.2 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The specificity of the Direct hGH ELISA kit was determined by measuring the apparent hGH value of calibrator A spiked with various levels of prolactin.

Substance	Concentration Range (ng/mL)	Apparent hGH Value (ng/mL)
Prolactin Calibrated against WHO 3rd IS 84/500	50 100 500 1000	Not Detected Not Detected Not Detected Not Detected

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	1.46	0.09	5.8
2	12.33	0.68	5.5
3	41.87	0.97	2.3

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	2.95	0.27	9.0
2	19.29	0.86	4.4
3	36.06	1.72	4.7

HIGH DOSE HOOK EFFECT

The Direct hGH ELISA kit did not experience any high dose hook effect.

RECOVERY

Spiked samples were prepared by adding defined amounts of hGH to three patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	ND	-	-
+ 1.0	0.96	1.0	96.0
+ 5.0	5.6	5.0	112.0
+ 50	49	50	98.0
2 Unspiked	0.7	-	-
+ 1.0	1.5	1.7	88.2
+ 5.0	6.6	5.7	115.8
+ 50	53	50.7	104.5
3 Unspiked	1.0	-	-
+ 1.0	1.7	2.0	85.0
+ 5.0	6.8	6.0	113.3
+ 50	48.8	51	95.7

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	6.44	-	-
1:2	3.12	3.22	96.9
1:5	1.15	1.29	89.1
1:10	0.59	0.64	92.2
2	16.60	-	-
1:2	7.97	8.30	96.0
1:5	2.82	3.32	84.9
1:10	1.59	1.66	95.8
3	33.00	-	-
1:2	16	16.5	97.0
1:5	6.4	6.6	97.0
1:10	3.3	3.3	100.0

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	N	95% Confidence Range (ng/mL)
Males	120	ND–3.7
Females		
Premenopausal	120	ND–8.71
Postmenopausal	120	ND–3.09

Ordering Information: **REF CAN-GH-4070**

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Diagnostics Biochem Canada

Insulin-Like Growth Factor Binding Protein-1 (IGFBP-1)

ELISA

REF CAN-IGF-4140

ASSAY PROCEDURE

- Bring kit components to room temperature. Prepare working solutions.
- Pipette 25 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of assay buffer into each well.
- Incubate on a plate shaker for 30 minutes at room temperature.
- Wash 3 times.
- Pipette 100 µL of conjugate working solution.
- Incubate on a plate shaker for 30 minutes at room temperature.
- Wash 3 times. Pipette 100 µL of TMB substrate.
- Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

Insulin-like growth factor binding protein-1 (IGFBP-1) is one of six proteins that specifically bind insulin-like growth factors I and II (IGF-I and IGF-II) in body fluids and tissues.

IFGBP-1 contains 234 amino acids, with a predicted molecular mass of 25 kDa. The major sites of IGFBP-1 synthesis are the fetal/adult liver and decidualized endometrium.

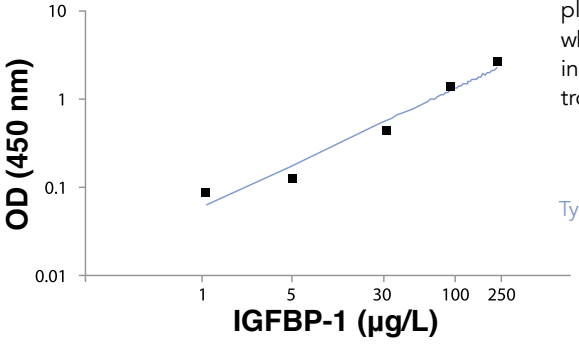
Serum levels of IGFBP-1, which reflect its synthesis by the liver, exhibit considerable diurnal variation. Circulating IGFBP-1 levels are highest early in the morning and lowest in the evening. The levels are high in the fetus and newborn, but decline steadily until puberty. The mean level of IGFBP-1 in healthy adults is 4.4 µg/L (range 0.6–14.4 µg/L). After about 65 years of age, serum IGFBP-1 levels begin to increase. There is also an inverse correlation between body mass index (BMI) and fasting serum IGFBP-1 concentrations.

The most important regulator of circulating IGFBP-1 is insulin. Fasting insulin and IGFBP-1 concentrations are inversely correlated. During a 3-h glucose tolerance test, there is a decrease of about 50% in serum IFGBP-1 levels. Eating a meal also has a decreasing effect.

In insulin-dependent diabetes (IDDM), serum IGFBP-1 levels are elevated. In non-insulin dependent diabetes, in which insulin levels are high, serum IGFBP-1 is decreased. Low levels of IGFBP-1 have also been observed in the following cases: acromegaly, Cushing's syndrome and polysystic ovarian syndrome (PCO).

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for IGFBP-1 is immobilized onto the microplate and another monoclonal antibody specific for a different region of IGFBP-1 is conjugated to horse radish peroxidase (HRP). IGFBP-1 from the sample and standards are allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second 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 by the enzymatic reaction is directly proportional to the concentration of IGFBP-1 in the sample.



Typical calibrator curve

A set of standards is used to plot a standard curve from which the amount of IGFBP-1 in patient samples and controls can be directly read.

PERFORMANCE CHARACTERISTICS

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) plus 2 SD. Therefore, the sensitivity of the DBC Direct IGFBP-1 ELISA kit is **0.5 µg/L**.

SPECIFICITY (CROSS-REACTIVITY)

The specificity of the Direct IGFBP-1 ELISA kit was determined by measuring the apparent IGFBP-1 value of calibrator A spiked with the following compounds.

Substance	Concentration Range	Apparent IGFBP-1 Value (µg/L)
IGFBP-2	Up to 5000 µg/L	Not Detected
IGFBP-3	Up to 10,000 µg/L	Not Detected
IGFBP-4	Up to 5000 µg/L	Not Detected
IGFBP-5	Up to 5000 µg/L	Not Detected

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in µg/L) are tabulated below:

Sample	Mean	SD	CV %
1	5.5	0.14	2.5
2	22	0.75	3.4
3	117	2.8	2.4

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in µg/L) are tabulated below:

Sample	Mean	SD	CV %
1	4.8	0.31	6.4
2	21	1.6	7.4
3	113	5.6	4.9

RECOVERY

Spiked samples were prepared by adding defined amounts of IGFBP-1 to three patient serum samples (1:1). The results (in µg/L) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	5.0	-	-
+ 6.5	5.8	5.75	100.9
+ 35	20	20	100.0
+ 174	90	89.5	100.6
2 Unspiked	20	-	-
+ 6.5	14	13.3	105.3
+ 35	29	24.5	118.4
+ 174	100	97	103.1
3 Unspiked	110	-	-
+ 6.5	62	58.3	106.3
+ 35	80	72.5	110.3
+ 174	155	133	116.5

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in µg/L) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	13.5	-	-
1:2	6.9	6.8	101.5
1:5	3.4	3.4	100.0
1:10	1.6	1.4	114.3
2	38	-	-
1:2	20.9	19	110.0
1:5	8.2	7.6	107.9
1:10	4.2	3.8	110.5
3	120	-	-
1:2	58.2	60	97.0
1:5	22.1	24	92.1
1:10	11.5	12	95.8

HIGH DOSE HOOK EFFECT

The Direct IGFBP-1 ELISA kit did not experience a high dose hook effect when it was tested up to an IGFBP-1 concentration of 200,000 µg/L.

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	N	Mean (µg/L)	Abs. Range (µg/L)
Adults	55	4.4	0.6–14.4

Ordering Information: **REF CAN-IGF-4140**

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Diagnostics Biochem Canada

Leptin ELISA

REF CAN-L-4260

ASSAY PROCEDURE


Bring kit components to room temperature. Prepare working solutions.

Pipette 20 µL of each calibrator, control and specimen sample.

Pipette 80 µL of the monoclonal anti-leptin-biotin conjugate into each well.

Incubate on a plate shaker for 1 hour at room temperature.

Wash 3 times.

Pipette 100 µL of streptavidin-HRP conjugate into each well.

Incubate on a plate shaker for 30 minutes at room temperature.


Wash 3 times. Pipette 100 µL of TMB substrate.

Incubate on a plate shaker for 10–15 minutes at room temperature.

Pipette 50 µL of stopping solution.

Read the plate on a microplate reader at 450 nm.

Human Leptin is a 16 kDa, 146 amino acid residue, non-glycosylated polypeptide. Leptin is encoded by the OB gene. Its major source is the adipose tissue, and its circulating concentrations indirectly reflect body fat stores.

Plasma or serum concentrations of leptin are increased in obese humans and strongly correlate with the degree of adiposity as expressed by percentage of body fat or body mass index.

The recently discovered hormone leptin contributes to the regulation of energy balance by informing the brain of the amount of adipose tissue in the body. The brain may then make the appropriate adjustments in either energy intake or expenditure.

Many areas of leptin physiology remain to be investigated. The roles of leptin in metabolism, insulin sensitivity, as a potential therapeutic modality for weight loss as well as involvement in endocrine function are active areas of research. While the future for leptin as a therapeutic agent is not clear, its involvement in many areas of physiology undoubtedly makes this a new hormone which requires extensive study in the future to understand its physiology.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for leptin is immobilized onto the microplate and another monoclonal antibody specific for a different epitope of leptin is conjugated to biotin. During the first step, leptin present in the samples and standards is bound to the immobilized antibody and to the biotinylated antibody, thus forming a sandwich complex. Excess and unbound biotinylated antibody is removed by a washing step. In the second step, streptavidin-HRP is added, which binds specifically to any bound biotinylated antibody. Again, unbound streptavidin-HRP is removed by a washing step. Next, the enzyme substrate is added (TMB), forming a blue coloured product that is directly proportional to the amount of leptin present. The enzymatic reaction is terminated by the addition of the stopping solution, converting the blue colour to a yellow colour. The absorbance is measured on a microtiter plate reader at 450 nm. A set of standards is used to plot a standard curve from which the amount of leptin in patient samples and controls can be directly read.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection (LoD) for Leptin is 0.50 ng/mL, as determined by use of a NCCLS protcol and with proportions of false positives (α) less than 5% and false negatives (β) less than 5%; based on 82 blank determinations; LoB=0.42 ng/mL.

SPECIFICITY (CROSS-REACTIVITY)

The following substances were tested at 1000 ng/mL and exhibited no cross-reactivity: Mouse Leptin, TNF-α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12, IL-16, GM-CSF, CSF and EGF.

INTRA-ASSAY PRECISION

Four serum samples were assayed twenty times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	2.45	0.09	3.7
2	7.94	0.34	4.3
3	11.67	0.64	5.5
4	27.51	1.37	5.0

INTER-ASSAY PRECISION

Four samples were assayed ten times over a period of ten days. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	2.71	0.16	5.9
2	8.24	0.48	5.8
3	12.01	0.82	6.8
4	24.98	1.45	5.8

COMPARATIVE STUDIES

The DBC Leptin ELISA (DBC) was compared against a leading competitor's Leptin EIA kit (Kit X).

Thirty-eight serum samples ranging from 1.05–75.62 ng/mL were assayed with both kits, yielding the following results: Regression:
Kit X = 0.9644 (DBC) + 1.5489
r = 0.98
Kit X Mean: 21.13
DBC Mean: 20.30

RECOVERY

Spiked samples were prepared by adding defined amounts of leptin to three patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	3.89	-	-
+ 3.06	6.28	6.95	90.4
+ 8.06	10.98	11.95	91.9
+ 23.06	25.43	26.95	94.4
2 Unspiked	7.89	-	-
+ 1.06	8.82	8.95	98.5
+ 6.06	15.03	13.95	107.7
+ 21.06	30.32	28.95	104.7
3 Unspiked	11.61	-	-
+ 4.2	15.71	15.81	99.4
+ 12.8	25.42	24.41	104.1
+ 29.46	41.18	41.07	100.3

LINEARITY

Three patient serum samples were serially diluted with leptin assay buffer. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	3.03	-	-
1:2	1.42	1.52	93.4
1:4	0.71	0.76	93.4
1:8	0.35	0.38	92.1
2	11.27	-	-
1:2	5.93	5.64	105.1
1:4	3.05	2.82	108.2
1:8	1.35	1.41	95.7
3	27.91	-	-
1:2	14.91	13.96	106.8
1:4	6.74	6.98	96.6
1:8	3.29	3.49	94.3

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Mean (ng/mL)	Range (ng/mL)
Lean Women	7.4	3.7–11.1
Lean Men	3.8	2.0–5.6

Leptin values are approximately 2.5 times higher in women than men per unit BMI.

Ordering Information: **REF CAN-L-4260**

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Diagnostics Biochem Canada

Luteinizing Hormone (hLH) ELISA

REF CAN-LH-4040

ASSAY PROCEDURE

- Bring kit components to room temperature. Prepare working solutions.
- Pipette 25 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of assay buffer into each well.
- Incubate on a plate shaker for 30 minutes at room temperature.
- Wash 3 times.
- Pipette 100 µL of conjugate working solution.
- Incubate on a plate shaker for 30 minutes at room temperature.
- Wash 3 times. Pipette 100 µL of TMB substrate.
- Incubate on a plate shaker for 15–20 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

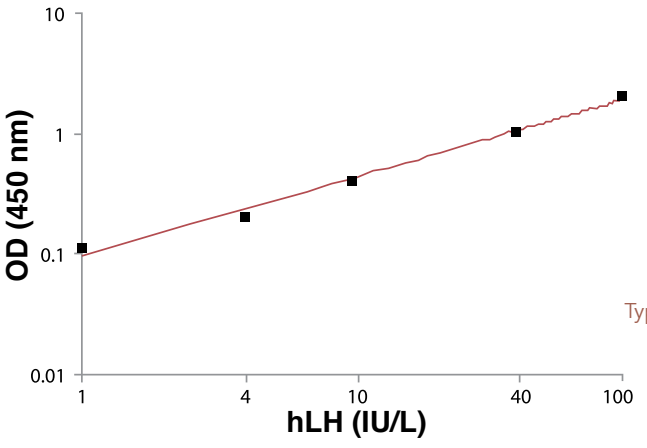
Human luteinizing hormone (hLH) is a glycoprotein synthesized by the anterior lobe of the pituitary gland. This hormone consists of two subunits: α and β. The α subunit of LH is similar to the α subunit found in both the FSH and TSH glycoprotein hormones (which are also synthesized by the pituitary gland) as well as the α subunit of hCG (produced by the placenta). However, the β subunit of each of these hormones are unique. Therefore, the specificity of these four hormones are due to the β peptide chains. It is to be noted that the α chain by itself has no biological activity.

The hypothalamic decapeptide, namely the gonadotropin releasing hormone (GnRH), stimulates the release of LH. Both the LH and FSH hormones in men act on the testis, which have two functions: Leydig cells secrete androgens while sperm are formed by the seminiferous tubules. The secretion of testosterone and dihydrotestosterone by the Leydig cells is under the direct control of LH.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for LH is immobilized onto the microplate and another monoclonal antibody specific for a different region of LH is conjugated to horse radish peroxidase (HRP). LH from the sample and standards are allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second 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 by the enzymatic reaction is directly proportional to the concentration of LH in the sample.

A set of standards is used to plot a standard curve from which the amount of LH in patient samples and controls can be directly read.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

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) plus 2 SD. Therefore, the sensitivity of the DBC Direct hLH ELISA kit is **0.2 IU/L**.

SPECIFICITY (CROSS-REACTIVITY)

The specificity of the Direct hLH ELISA kit was determined by measuring the apparent hLH value of the following compounds:

Substance	Concentration (IU/L)	Apparent hLH Value (IU/L)
hCG Calibrated against WHO 1st IS 75/537	50,000 25,000 10,000 5,000 1,000	55 22 7.8 3.4 < 1.0
hFSH Calibrated against WHO 1st IS 83/575	1000 500 100 50 20	13 6.2 1.7 1.5 1.2
hTSH Calibrated against WHO 2nd IS 80/558	500 250 100 50 5	< 1.0 < 1.0 < 1.0 < 1.0 < 1.0

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in IU/L) are tabulated below:

Sample	Mean	SD	CV %
1	4.84	0.22	4.5
2	16.58	0.44	2.7
3	53.28	1.53	2.9

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in IU/L) are tabulated below:

Sample	Mean	SD	CV %
1	5.15	0.32	5.1
2	17.37	1.40	8.1
3	51.50	4.70	9.2

RECOVERY

Spiked samples were prepared by adding defined amounts of hLH to three patient serum samples. The results (in IU/L) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked + 4.9 + 48.79	0.00 5.06 53.79	- 4.90 48.79	- 103.3 110.2
2 Unspiked + 3.9 + 39.0	2.12 5.76 40.22	- 6.02 41.12	- 95.7 97.8
3 Unspiked + 3.9 + 19.5	5.81 9.10 22.05	- 9.71 25.31	- 93.7 87.1

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in IU/L) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 1:2 1:4 1:8	9.28 5.02 2.48 1.16	- 4.64 2.32 1.16	- 108.2 106.9 100.0
2 1:2 1:4 1:8	37.52 20.49 10.73 5.44	- 18.76 9.38 4.69	- 109.2 114.4 116.0
3 1:2 1:4 1:8	42.33 20.56 11.20 5.74	- 21.17 10.58 5.29	- 97.1 105.9 108.5

REFERENCE VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (IU/L)
Males	1.5–9.3
Females: Follicular Phase Midcycle Peak Luteal Phase Postmenopausal	1.9–12.5 8.7–76.3 0.5–16.9 5.0–52.3

HIGH DOSE HOOK EFFECT

The Direct hLH ELISA kit did not experience a high dose hook effect when it was tested up to a hLH concentration of 20,000 IU/L.

Ordering Information: **REF CAN-LH-4040**

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Diagnostics Biochem Canada

Plasma Renin Activity (PRA) ELISA

REF CAN-RA-4600

ASSAY PROCEDURE

- Bring kit components to room temperature. Prepare working solutions.
- Pipette 50 µL of each calibrator, control and pre-treated specimen sample.
- Pipette 100 µL of Biotin Conjugate into each well.
- Incubate on a microplate shaker for 60 minutes at room temperature.
- Wash 5 times.
- Pipette 150 µL of Streptavidin-HRP Conjugate Working Solution into each well.
- Incubate on a microplate shaker for 30 minutes at room temperature.
- Wash 5 times. Pipette 150 µL of TMB Substrate.
- Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of Stopping Solution.
- Read the plate on a microplate reader at 450 nm.

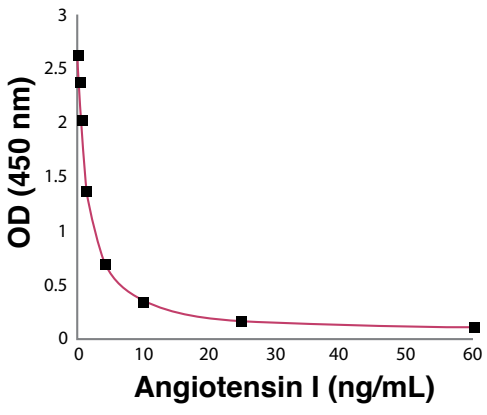
Measurement of PRA is important for the clinical evaluation of hypertensive patients. In particular, determination of plasma renin activity can help in the diagnosis of primary hyperaldosteronism (5–13% of hypertensive cases) and assist in the therapy and management of other forms of hypertension.

PRINCIPLE OF THE TEST

Prior to testing plasma samples with the PRA ELISA, a specimen pre-treatment step is required. First, a protease inhibitor (PMSF) is added to the sample to prevent the degradation of angiotensin-I. Next, the generation buffer is added to bring the pH of the sample to approximately 6.0. The plasma sample is then pipetted into two aliquots. One aliquot is incubated at 0°C (ice bath) and the other is incubated at 37°C. Angiotensin-I will be generated by plasma renin in the fraction incubated at 37°C.

The PRA ELISA is a competitive immunoassay. In the first incubation step, competition occurs between angiotensin-I present in calibrators, controls, specimen samples and an angiotensin-I-biotin conjugate (biotin conjugate) for a limited number of anti-angiotensin-I antibody binding sites on the microplate wells. During this incubation, protease inhibitors are present to prevent the degradation of angiotensin-I into smaller peptides.

In the second incubation step, streptavidin-HRP conjugate is added, which binds specifically to any bound biotin conjugate. Unbound streptavidin HRP conjugate is removed by a washing step. Next, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue coloured product that is inversely proportional to the amount of angiotensin-I present. The enzymatic reaction is terminated by the addition of the stopping solution, converting the blue colour to a yellow colour. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the concentration of angiotensin-I in specimen samples and controls can be directly read.



The plasma renin activity concentration in the plasma sample is calculated from the angiotensin-I concentration in the 0°C and 37°C aliquots and the generation time used. The plasma renin activity results are expressed in terms of the mass of angiotensin-I generated per volume of human plasma per unit of time (ng/mL/h).

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The analytical sensitivity study was performed according to the CLSI EP17-A2 guideline. The Limit of Background (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) for both Angiotensin-I and PRA are summarized in the table below:

Parameter	Angiotensin-I (ng/mL)	PRA (ng/mL/h)
LoB	0.093	0.024
LoD	0.166	0.059
LoQ	0.166	0.090

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with angiotensin-I cross-reacting at 100%:

Compound	% Cross-Reactivity
Angiotensin I	100
Angiotensin II	< 0.001
Angiotensin III	< 0.001
Angiotensin 1-5	< 0.001
Angiotensin 1-7	< 0.001
Angiotensin 1-9	0.0122
Renin Substrate	0.015

INTERFERENCES

An interference study was performed according to the CLSI EP07-Ed3 guideline. Three human plasma samples were spiked with potentially interfering substances. No significant interference was detected up to the concentrations shown in the table below.

Interferent	Test Concentration
Acetaminophen	30 µg/mL
Acetylcysteine	15 mg/dL
Acetylsalicylic Acid	3 mg/dL
Ampicillin Na	7.5 mg/dL
Bilirubin Conjugated	20 mg/dL
Bilirubin Unconjugated	40 mg/dL
Biotin	2.4 µg/mL
Captopril	1000 ng/mL
Captopril disulfide	10 µg/mL
Cathepsin B	100 ng/mL
Cathepsin D	10 ng/mL
Cefoxitin Na	300 mg/dL
Cyclosporine	0.18 mg/dL
Doxycycline HCl	1.8 mg/dL
Enalaprilat dihydrate	200 ng/mL

Interferent	Test Concentration
Furosemide (Lasix)	50 µg/mL
Haemoglobin	1.25 g/L
HAMA	1000 ng/mL
Heparin	3300 U/L
Human Serum Albumin	52 g/L
Ibuprofen	21.9 mg/dL
Insulin	150 µIU/mL
Levodopa	0.75 mg/dL
Methyldopa	2.25 mg/dL
Metronidazole	12.3 mg/dL
Nicardipine (Loxen)	200 ng/mL
Phenylbutazone	32.1 mg/dL
Rheumatoid Factor (RF)	200 IU/mL
Rifampicin	4.8 mg/dL
Theophylline	25 µg/mL

RECOVERY

Spiked samples were prepared by adding defined amounts of angiotensin-I to three EDTA plasma samples. The angiotensin-I results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	1.09	-	-
+ 1	2.16	2.09	103.3
+ 15	15.3	16.1	95.0
+ 50	41.8	51.1	81.8
2 Unspiked	1.72	-	-
+ 1	2.70	2.72	99.3
+ 15	16.8	16.7	100.6
+ 50	54.1	51.7	104.6
3 Unspiked	1.01	-	-
+ 1	1.76	2.01	87.6
+ 15	12.7	16.0	79.4
+ 50	41.7	51.0	81.8

LINEARITY

The linearity study was according to the CLSI EP06-Ed2 guideline using three human EDTA plasma samples.

Each plasma sample was pre-treated according to the Angiotensin-I Generation Procedure to produce a 0°C and 37°C aliquot. Each aliquot was diluted using calibrator A at several equidistant concentration levels and up to a 1:10 dilution. Samples were tested in quadruplicate, and the results compared to the predicted concentrations. The statistical analysis shows that the assay is sufficiently linear up to a 1:10 dilution when using calibrator A as the diluent. The results (in ng/mL/h) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	33.7	-	-
1:2	17.9	16.9	105.9
1:4	8.33	8.43	98.8
1:10	3.24	3.37	96.1
2	7.11	-	-
1:2	3.33	3.56	93.5
1:4	1.59	1.78	89.3
1:10	0.60	0.71	84.5
3	1.66	-	-
1:2	0.68	0.83	81.9
1:4	0.33	0.42	78.6
1:10	0.13	0.17	76.5

REFERENCE RANGES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. Data from Literature reference.[1]

N	PRA Mean (ng/mL.h)	PRA Range (10^{th}-90^{th} percentile) (ng/mL.h)
533	0.75	0.06–4.69

1. Brossaud J, Corcuff JB. Pre-Analytical and Analytical Considerations for the Determination of Plasma Renin Activity. *Clin Chim Acta*. 2009; 410(1–2):90–2.

Ordering Information: **REF CAN-RA-4600**

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Diagnostics Biochem Canada

Pregnenolone ELISA

REF CAN-PRE-4500

ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 50 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of conjugate working solution into each well.
- Incubate on a plate shaker for 1 hour at room temperature.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

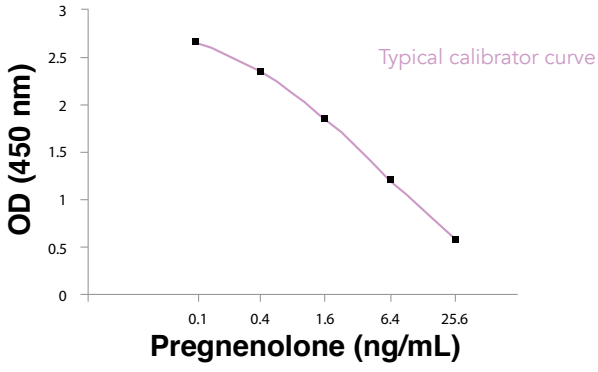
Pregnenolone (3β-hydroxypregn-5-en-20-one) is the first steroid to be derived from cholesterol in the pathway of steroidogenesis, and it is the common precursor for all of the adrenal and gonadal steroids. Its production occurs in the mitochondrion by cleavage of the C-20 side chain of cholesterol by the P-450SCC enzyme. Once produced, pregnenolone may be utilized by two pathways of steroidogenesis. Pregnenolone may either be converted to 17-OH pregnenolone via the enzymatic action of 17α-hydroxylase or to progesterone via the enzymatic action of 3β-hydroxysteroid dehydrogenase.

Elevated pregnenolone levels occur in forms of congenital adrenal hyperplasia (CAH), due to 3β-hydroxysteroid dehydrogenase deficiency or 17α-hydroxylase deficiencies. Higher levels have also been reported in women with idiopathic hirsutism. Studies on pregnenolone levels in regard to sex and age differences indicate that maximum levels occur at approximately 17 and 16 years of age for women and men, while minimum levels occur at approximately 37 and 38 years of age for women and men, respectively. In general, women were found to have slightly higher values when compared to men.

Many areas of pregnenolone physiology remain to be investigated. Current research indicates that the determination of pregnenolone in serum may be useful for studying its metabolite, pregnenolone sulfate, which has been reported to have various effects in the mammalian brain and central nervous system.

PRINCIPLE OF THE TEST

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 patient 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 pregnenolone in the sample. A set of standards is used to plot a standard curve from which the concentration of pregnenolone in patient samples and controls can be directly read.



PERFORMANCE CHARACTERISTICS

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 DBC Direct Pregnenolone ELISA kit is **0.05 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct Pregnenolone ELISA kit with pregnenolone crossreacting at 100%.

Steroid	% Cross-Reactivity
Pregnenolone	100
Progesterone	6.0
Dehydroisoandrosterone	5.2
5α-Androstandiol	4.7
Epiandrosterone	1.0
Pregnenolone Sulfate	0.4
Androstandione	0.3
5α-Androsterone	0.3
DHEAS	0.2
Etiocholanolone	0.1

The following steroids were tested but cross-reacted at less than 0.1%: Adrenosterone, Aldosterone, Androstenedione, Cholesterol, Corticosterone, 5α-DHT, 17β-Estradiol, Estriol and Testosterone.

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.19	0.02	10.6
2	1.04	0.85	8.2
3	4.77	0.37	7.8

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.22	0.03	14.5
2	1.14	0.14	12.3
3	4.56	0.44	9.6

RECOVERY

Spiked samples were prepared by adding defined amounts of pregnenolone to four patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked + 4.14	0.37 5.31	- 4.51	- 117.7
2 Unspiked + 4.01	0.77 5.69	- 4.78	- 119.0
3 Unspiked + 3.98	0.85 5.18	- 4.83	- 107.2
4 Unspiked + 3.78	1.47 6.31	- 5.25	- 120.2

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 1:2 1:4 1:8	5.31 2.89 1.26 0.71	- 2.66 1.33 0.66	- 108.6 94.7 107.6
2 1:2 1:4 1:8	6.51 2.75 1.54 0.80	- 3.26 1.63 0.81	- 84.4 94.5 98.8
3 1:2 1:4 1:8	8.34 3.78 2.15 1.05	- 4.17 2.09 1.04	- 90.6 102.9 101.0

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	N	Mean (ng/mL)	Abs. Range (ng/mL)
Males	30	0.50	0.1–3.4
Females	50	0.55	0.1–3.8

Ordering Information: **REF CAN-PRE-4500**

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Diagnostics Biochem Canada

Progesterone Saliva ELISA

REF CAN-P-310

ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 50 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of conjugate working solution.
- Incubate on a plate shaker for 1 hour at room temperature.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a plate shaker for 10–20 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

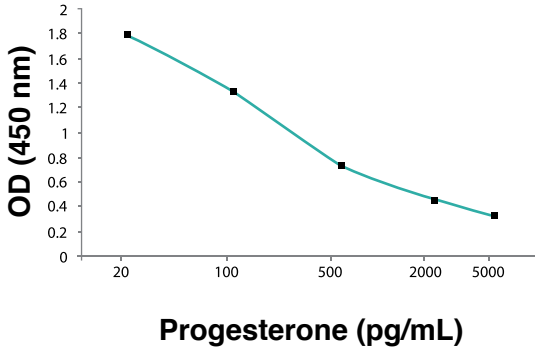
Progesterone is a C-21 female sex steroid hormone with a variety of physiological effects. In the follicular phase of the menstrual cycle, progesterone is produced in low levels. It increases to the LH peak and then sharply rises 3 to 4 days later to higher levels, remaining elevated through the 10th to 12th days after the LH peak. Then there is a sharp decline to the low levels of the follicular phase. It is responsible for the induction of the cyclic changes in the endometrium of the uterus allowing implantation and successful growth of the fertilized ovum and maintenance of pregnancy.

Progesterone measurements are useful in documenting ovulation and in the management of difficulties during the first trimester of pregnancy. Levels of progesterone may be useful in the evaluation of sterility due to luteal phase defects, prediction of impending abortion, and the diagnosis of ectopic pregnancy.

Drugs such as, oral contraceptives, superovulatory drugs, estrogen replacement therapy medication, and GnRH analogues may affect normal values of progesterone. The removal of ovarian function following surgical oophorectomy or chemotherapy may influence salivary progesterone values. The determination of salivary progesterone combines a highly sensitive technique and non-invasive sample collection that is of value in clinical and research studiess.

PRINCIPLE OF THE TEST

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 patient 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 progesterone in the sample. A set of standards is used to plot a standard curve from which the amount of progesterone in patient samples and controls can be directly read.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

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 DBC Progesterone Saliva ELISA kit is **20 pg/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct Progesterone Saliva ELISA kit with progesterone cross-reacting at 100%.

Steroid	% Cross-Reactivity
Progesterone	100
11α-OH-Progesterone	100
Deoxycorticosterone	1.7
17-OH-Progesterone	0.4
5α-Androstane-3β, 17β-diol	0.3
Corticosterone	0.3
Pregnenolone	0.2

The following steroids were tested but cross-reacted at less than 0.1%: Cortisol, Cortisone, Danazol, DHEAS, Estradiol, 5β-Pregnan-3α, 17α, 21α-triol-20-one, 5β-Pregnan-3α, 17-diol-20-one, Pregnan-3α, 20α-diol and Testosterone.

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	32.93	4.39	13.3
2	78.73	4.63	5.9
3	302.67	22.30	7.37

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	30.83	3.90	12.7
2	75.03	7.73	7.7
3	241.06	26.23	10.9

RECOVERY

Spiked samples were prepared by adding defined amounts of progesterone to two patient saliva samples (1:1). The results (in pg/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	4.38	-	-
+ 100	58.93	52.19	112.9
+ 500	240.57	252.19	95.4
+ 2000	851.70	1002.19	85.0
2 Unspiked	7.49	-	-
+ 100	46.27	53.75	86.1
+ 2000	894.58	1003.75	89.1
+ 5000	2694.49	2503.75	107.6

LINEARITY

Three patient saliva samples were diluted with calibrator A. The results (in pg/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	1005.66	-	-
1:2	473.10	502.83	94.1
1:4	218.29	251.41	86.8
1:8	115.63	125.71	92.0
2	1462.5	-	-
1:2	700.48	731.25	95.8
1:4	327.69	365.62	89.6
1:8	172.12	182.81	94.1
3	2279.9	-	-
1:2	1061.0	1139.95	93.1
1:4	497.67	569.98	87.3
1:8	239.59	284.99	84.1

REFERENCE 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	
Follicular Phase	< 100
Luteal Phase	100–500
Postmenopausa	< 50

Ordering Information: REF CAN-P-310

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Diagnostics Biochem Canada

Progesterone Serum ELISA

REF CAN-P-305

ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 25 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of conjugate working solution.
- Incubate on a plate shaker for 1 hour at room temperature.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a plate shaker for 10–20 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

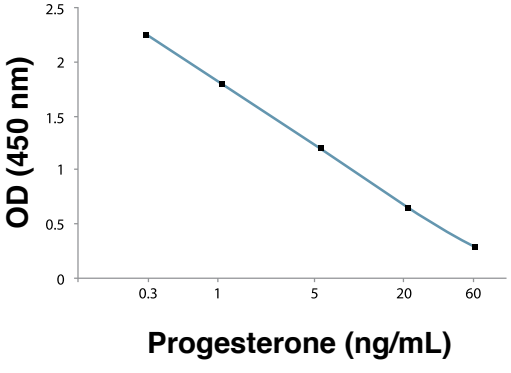
Progesterone is a C-21 female sex steroid hormone with a variety of physiological effects. In the follicular phase of the menstrual cycle, progesterone is produced in low levels. It increases to the LH peak and then sharply rises 3 to 4 days later to higher levels, remaining elevated through the 10th to 12th days after the LH peak. Then there is a sharp decline to the low levels of the follicular phase. It is responsible for the induction of the cyclic changes in the endometrium of the uterus allowing implantation and successful growth of the fertilized ovum and maintenance of pregnancy.

Progesterone measurements are used in documenting ovulation and in the management of difficulties during the first trimester of pregnancy. Levels of progesterone may be useful in the evaluation of sterility due to luteal phase defects, prediction of impending abortion, and the diagnosis of ectopic pregnancy.

Normal values of progesterone may be affected by drugs such as, oral contraceptives, superovulatory drugs, estrogen replacement therapy medication, and GnRH analogues. The removal of ovarian function following surgical oophorectomy or chemotherapy may influence serum progesterone values.

PRINCIPLE OF THE TEST

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 patient 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 progesterone in the sample. A set of standards is used to plot a standard curve from which the amount of progesterone in patient samples and controls can be directly read.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

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 DBC Direct Progesterone ELISA kit is **0.1 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct Progesterone ELISA kit with Progesterone crossreacting at 100%.

Steroid	% Cross-Reactivity
Progesterone	100
11α-OH-Progesterone	100
Deoxycorticosterone	1.7
17-OH-Progesterone	0.4
5α-Androstane-3β, 17β-diol	0.3
Corticosterone	0.3
Pregnenolone	0.2

The following steroids were tested but cross-reacted at less than 0.1%: Cortisol, Cortisone, Danazol, DHEAS, Estradiol, 5β-Pregnan-3α, 17α, 21α-triol-20-one, 5β-Pregnan-3α, 17-diol-20-one, Pregnan-3α, 20α-diol and Testosterone.

INTRA-ASSAY PRECISION

Two samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	1.89	0.20	10.6
2	14.24	1.45	10.2

INTER-ASSAY PRECISION

Two samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	2.63	0.33	12.6
2	10.15	1.04	10.2

RECOVERY

Spiked samples were prepared by adding defined amounts of progesterone to two patient serum samples (1:1). The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	20	-	-
+ 5.0	13	12.5	104
+ 20.0	16	20	80
+ 60.0	31	40	78
2 Unspiked	45	-	-
+ 5.0	31	25	124
+ 20.0	35	32.5	108
+ 60.0	48	52.5	91

LINEARITY

Two patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	10	-	-
1:2	5.3	5	106
1:4	2.16	2.5	86
1:8	1.38	1.25	110
1:16	0.59	0.63	95
2	20	-	-
1:2	10	9.1	91
1:4	5	4.2	84
1:8	2.5	2.0	81
1:16	1.25	1.1	88

COMPARATIVE STUDIES

The DBC Direct Progesterone ELISA kit (Kit A) was compared with a competitor's coated tube RIA kit (Kit B). The results (in ng/mL) are tabulated below:

Group	Kit A Mean	Kit B Mean
33 Samples	3.05	2.76

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Mean (ng/mL)	Abs. Range (ng/mL)
Males	0.53	ND–1.35
Females	8.37	ND–70.0
Postmenopausal Female	0.46	ND–4.0

Ordering Information: REF CAN-P-305

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Diagnostics Biochem Canada

Prolactin (PRL) ELISA

REF CAN-PRL-4100

ASSAY PROCEDURE

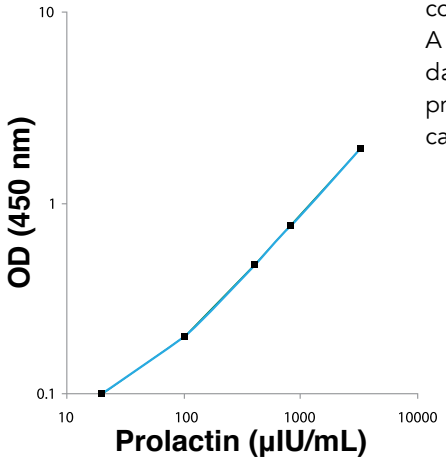
- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 25 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of conjugate working solution into each well.
- Incubate on a plate shaker for 1 hour at room temperature.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

Prolactin (PRL) is a polypeptide hormone synthesized by the lactotropic cells of the anterior pituitary gland. Structurally, it is similar to two other polypeptide hormones namely, growth hormone and placental lactogen. PRL is a polypeptide containing 199 amino acids, while growth hormone and placental lactogen each have 191 amino acids. There is approximately 100 µg of prolactin in the human pituitary gland, which is a very small amount when compared to growth hormone, which is present at 8–10 mg.

The target organ of prolactin is the breast (mammary gland). Its main physiological action is not only to initiate but also to sustain lactation. The hypothalamus secretes dopamine, which has a direct effect of inhibition of the secretion of PRL. If dopamine is not available or absent the secretion of PRL is autonomous.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical one-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for prolactin is immobilized onto the microplate and another monoclonal antibody specific for a different region of prolactin is conjugated to horse radish peroxidase (HRP). Prolactin from the sample and standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. 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 by the enzymatic reaction is directly proportional to the concentration of prolactin in the sample. A set of standards is used to plot a standard curve from which the amount of prolactin in patient samples and controls can be directly read.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

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) plus 2 SD. Therefore, the sensitivity of the DBC Prolactin ELISA kit is **10 µIU/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The specificity of the DBC Prolactin ELISA kit was determined by measuring the apparent Prolactin values of the following compounds:

Substance	Concentration Range	Apparent PRL Value (µIU/mL)
hCG (WHO 75/537)	100–2500 IU/L	Not Detected
FSH (WHO 1st 83/575)	25–4000 IU/L	Not Detected
hGH (WHO 80/505)	10–1000 mg/L	Not Detected
PL	0.1–50 mg/L	Not Detected
TSH (WHO 80/558)	25–1000 mIU/L	Not Detected

The specificity towards other structural forms of prolactin, including macroprolactin has not been determined.

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in µIU/mL) are tabulated below:

Sample	Mean	SD	CV %
1	202	14	6.9
2	586	68	11.6
3	1320	136	10.3

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in µIU/mL) are tabulated below:

Sample	Mean	SD	CV %
1	237	18	7.6
2	589	85	14.4
3	1725	277	13.2

RECOVERY

Spiked samples were prepared by adding defined amounts of prolactin to three patient serum samples. The results (in µIU/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	55	-	-
+ 62	95	117	81.2
+ 130	172	185	93.0
+ 542	508	597	85.1
2 Unspiked	59	-	-
+ 49	127	108	117.6
+ 145	258	204	126.5
+ 775	792	834	95.0
3 Unspiked	707	-	-
+ 145	807	852	94.7
+ 385	1356	1092	124.2
+ 775	1868	1482	126.0

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in µIU/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	292	-	-
1:2	124	146	84.9
1:4	87	73	119.2
1:8	41	37	110.8
2	444	-	-
1:2	202	222	91.0
1:4	116	111	104.5
1:8	69	56	123.2
3	1965	-	-
1:2	1014	983	103.2
1:4	427	491	87.0
1:8	209	246	85.0

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Absolute Range (µIU/mL)
Males	67–360
Females	55–2500
Postmenopausal	< 400

Ordering Information: REF CAN-PRL-4100

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Diagnostics Biochem Canada

Free Prostate Specific Antigen ELISA

REF CAN-FPSA-4400

ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 50 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of conjugate working solution into each well.
- Incubate on a plate shaker for 1 hour at room temperature.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

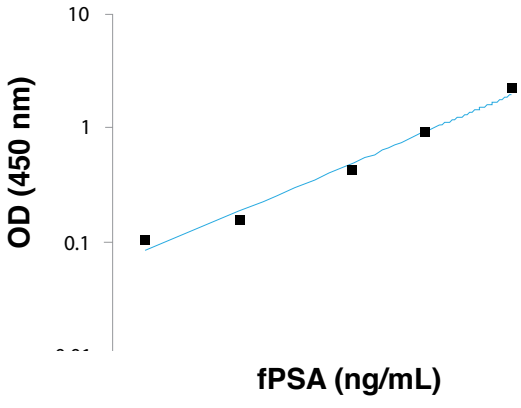
Prostate specific antigen (PSA) is a 3 3-kDa glycoprotein secreted by epithelial cells of the prostate gland. In human serum, PSA is primarily complexed with α1-antichymotrypsin, and to a lesser extent with other serum proteins. Only a small fraction of PSA is present as the free form (free PSA).

The expected normal level of PSA in male serum is lower than 4 ng/mL. A rise in the concentration of PSA indicates prostate pathology, including benign prostatic hyperplasia (BPH) and prostate cancer.

Free PSA (fPSA) has been studied in attempts to help distinguish BPH from untreated prostate cancer. These studies have shown that the ratio of free PSA/total PSA is lower in untreated prostate cancer than in patients with BPH.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical one-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for fPSA is immobilized onto the microplate and another monoclonal antibody specific for a different region of fPSA is conjugated to horse radish peroxidase (HRP). fPSA from the sample and standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. 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 by the enzymatic reaction is directly proportional to the concentration of fPSA in the sample. A set of standards is used to plot a standard curve from which the amount of fPSA in patient samples and controls can be directly read.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

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) plus 2 SD. Therefore, the sensitivity of the DBC Direct fPSA ELISA kit is **0.05 ng/mL**.

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.24	0.02	9.6
2	0.79	0.06	7.2
3	1.75	0.10	5.8

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.24	0.03	10.5
2	1.15	0.13	11.1
3	3.13	0.21	6.6

HIGH DOSE HOOK EFFECT

The Direct fPSA ELISA kit did not experience a high dose hook effect when it was tested up to a fPSA concentration of 200 ng/mL.

RECOVERY

Spiked samples were prepared by adding defined amounts of fPSA to three patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked + 0.63	1.74 2.37	- 2.03	- 116.7
2 Unspiked + 1.19	1.74 3.30	- 2.93	- 112.6
3 Unspiked + 1.63	3.60 5.23	- 4.48	- 116.7

LINEARITY

Two patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 1:2 1:4 1:8	1.22 0.72 0.32 0.19	- 0.61 0.31 0.15	- 118.0 103.2 126.7
2 1:2 1:4 1:8	12.30 7.04 3.58 1.56	- 6.15 3.08 1.54	- 114.5 116.2 101.3

COMPARATIVE STUDIES

The DBC Direct fPSA E LISA kit (y) was compared with a competitor's fPSA ELISA kit (x). The comparison of 16 serum samples yielded the following linear regression results:

$y = 0.8406x + 0.7096$, $r = 0.96$

Ordering Information: **REF CAN-FPSA-4400**

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Diagnostics Biochem Canada

Total Prostate Specific Antigen ELISA

REF CAN-TPSA-4300

ASSAY PROCEDURE

- Bring kit components to room temperature. Prepare working solutions.
- Pipette 25 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of the assay buffer into each well.
- Incubate on a plate shaker for 1 hour at room temperature.
- Wash 3 times. Pipette 100 µL of the conjugate working solution into each well.
- Incubate on a plate shaker for 30 minutes at room temperature.
- Wash 3 times. Pipette 100 µL of TMB substrate into each well.
- Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of stopping solution into each well.
- Read the plate on a microplate reader at 450 nm.

Prostate specific antigen (PSA) is a 3 3-kDa glycoprotein secreted by epithelial cells of the prostate gland. In human serum, PSA is primarily complexed with α1-antichymotrypsin, and to a lesser extent with other serum proteins. Only a small fraction of PSA is present as the free form (free PSA).

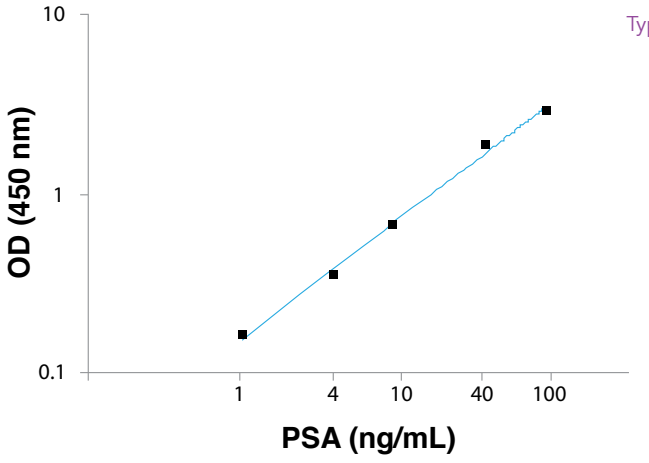
The expected normal level of PSA in male serum is lower than 4 ng/mL. A rise in the concentration of PSA indicates prostate pathology, including benign prostatic hyperplasia (BPH) and prostate cancer.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for total PSA is immobilized onto the microplate and another monoclonal antibody specific for a different region of PSA is conjugated to horse radish peroxidase (HRP). Total PSA from the sample and standards are allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second 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 by the enzymatic reaction is directly proportional to the concentration of total PSA in the sample.

A set of standards is used to plot a standard curve from which the amount of total PSA in patient samples and controls can be directly read.

Typical calibrator curve



PERFORMANCE CHARACTERISTICS

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) plus 2 SD. Therefore, the sensitivity of the DBC Direct Total PSA ELISA kit is **0.1 ng/mL**.

INTRA-ASSAY PRECISION

Two samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	5.59	0.02	6.1
2	22.89	0.06	4.6

INTER-ASSAY PRECISION

Two samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.92	0.09	9.5
2	3.64	0.14	3.8

RECOVERY

Spiked samples were prepared by adding defined amounts of PSA to three patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	7.00	-	-
+ 0.86	7.79	7.86	99.1
+ 9.43	16.42	16.43	99.9
+ 26.57	39.66	33.57	118.1
2 Unspiked	2.72	-	-
+ 2.08	4.65	4.80	96.8
+ 10.65	14.97	13.37	111.9
+ 27.8	34.92	30.52	114.4
3 Unspiked	14.26	-	-
+ 7.35	20.32	21.61	94.0
+ 24.49	39.85	38.75	102.8
+ 32.15	47.49	46.41	102.3

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	39.66	-	-
1:2	18.33	19.83	92.4
1:4	8.93	9.92	90.0
1:8	4.56	4.96	91.9
2	34.92	-	-
1:2	17.80	17.46	101.9
1:4	8.16	8.73	93.5
1:8	4.08	4.36	93.6
3	47.49	-	-
1:2	23.24	23.75	97.9
1:4	12.90	11.87	108.7
1:8	6.67	5.94	112.3

COMPARATIVE STUDIES

The DBC Direct Total PSA ELISA kit (y) was compared with a competitor's Total PSA ELISA kit (x). The comparison of 168 serum samples yielded the following linear regression results:

$y = 0.7972x + 0.8551$, $r = 0.9837$

HIGH DOSE HOOK EFFECT

The Direct Total PSA ELISA kit did not experience a high dose hook effect when it was tested up to a PSA concentration of 1000 ng/mL.

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Serum samples from a group (n = 96) of apparently healthy males and females aged from 3–70 years old were assayed with the DBC Direct Total PSA ELISA Kit. Results are as follows:

Group	Range (ng/mL)
Males and Females (3–70 years old)	All < 4.0

Ordering Information: REF CAN-TPSA-4300

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Diagnostics Biochem Canada

Resistin ELISA

REF CAN-RSN-4000

ASSAY PROCEDURE


Bring kit components to room temperature. Mix gently by inversion. Prepare working solutions.

Pipette 50 µL of each calibrator, control and specimen sample.

Pipette 100 µL of monoclonal anti-resistin-biotin conjugate.

Incubate on a plate shaker for 1 hour at room temperature.

Wash 3 times.

Pipette 100 µL of streptavidin-HRP conjugate.

Incubate on a plate shaker for 30 minutes at room temperature.


Wash 3 times. Pipette 150 µL of TMB substrate into each well.

Incubate on a plate shaker for 10–15 minutes at room temperature.


Pipette 50 µL of stopping solution into each well. Mix by gently tapping on plate.

Read the plate on a microplate reader at 450 nm.

Resistin is a 12.5 kDa protein containing 108 amino acids. It is synthesised as a pre-peptide and its hydrophobic signal peptide is cleaved before secretion. Resistin circulates in human blood as a dimeric protein consisting of two 92 amino acid polypeptides that are linked by a disulfide bridge.

Resistin belongs to the resistin-like molecule (RELM) hormone family. The RELM family comprises RELM-α, RELM-β, RELM-γ and resistin. RELM-β is related to resistin and is expressed in the colon. In rodents, resistin is produced by adipose tissue and is a significant regulator of glucose metabolism and insulin sensitivity. Hyperresistinemia in rodents causes insulin resistance and predisposition to type 2 diabetes. In humans, resistin is produced by the macrophages, which stimulates the macrophage secretion of pro-inflammatrory cytokines. Some studies have shown a correlation between increased serum resistin levels and atherosclerosis. Another study shows an increase of resistin levels in mice with atherosclerotic lesions. Many studies have tried to translate the mouse data to humans by answering the question whether levels of resistin are increased in human obesity, insulin resistance, and/or type 2 diabetes. Some groups failed to identify changes in resistin levels with obesity, insulin resistance, or type 2 diabetes while other studies that used diverse populations and different assays, have found significant relationships with one or more of these conditions.

Based on the above studies resistin may be a biomarker and a mediator of metabolic and inflammatory diseases. Many areas of resistin physiology remain to be investigated to determine if it can be used as a marker for energy metabolism and body weight regulation, metabolic syndrome, inflammation and atherosclerosis.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for resistin is immobilized onto the microplate and another monoclonal antibody specific for a different epitope of resistin is conjugated to biotin. During the first step, resistin present in the samples and standards is bound to the immobilized antibody and to the biotinylated antibody, thus forming a sandwich complex. Excess and unbound biotinylated antibody is removed by a washing step. In the second step, streptavidin-HRP is added, which binds specifically to any bound biotinylated antibody. Again, unbound streptavidin-HRP is removed by a washing step. Next, the enzyme substrate is added (TMB), forming a blue coloured product that is directly proportional to the amount of resistin present. The enzymatic reaction is terminated by the addition of the stopping solution, converting the blue colour to a yellow colour. The absorbance is measured on a microtiter plate reader at 450 nm. A set of standards is used to plot a standard curve from which the amount of resistin in patient samples and controls can be directly read.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection (LoD) was determined from the analysis of 60 samples of the blank and calculated as follows:
LoD = 2xSD where SD is the standard deviation of the blank.

LoD = **0.004 ng/mL** of Resistin.

SPECIFICITY (CROSS-REACTIVITY)

The blank was spiked separately with 100 ng/mL of human leptin, human TNF-α, human IL-6, human FABP4, human FABP5, human RELM-β or with 10 ng/mL of human C-peptide or 10 µg/mL of human adiponectin. The signal obtained for each was compared to the signal of resistin at 15 ng/mL. % Cross-Reactivity = (Signal of substance tested / Signal of Resistin at 15 ng/mL) x100.

Analyte	Concentration (ng/mL)	% Cross-Reactivity
Leptin	100	0
TNF-α	100	0.21
IL-6	100	0
C-peptide	10	0
Adiponectin	10000	0
FABP4	100	0
FABP5	100	0
RELM-β	100	0

INTRA-ASSAY PRECISION

Three samples were assayed 20 times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV%
1	3.61	0.068	1.9
2	8.32	0.17	2.0
3	15.79	0.44	2.8

INTER-ASSAY PRECISION

Three samples were assayed in 20 different tests in the span of 20 days. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV%
1	5.79	0.27	4.7
2	10.92	0.43	3.9
3	15.37	0.69	4.5

RECOVERY

Three patient serum samples were spiked by adding defined amounts of resistin. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	6.36	-	-
+ 2 ng/mL	7.25	8.36	87
+ 5 ng/mL	9.70	11.36	85
+ 10 ng/mL	14.42	16.36	88
2 Unspiked	7.98	-	-
+ 2 ng/mL	8.96	9.98	90
+ 5 ng/mL	11.63	12.98	90
+ 10 ng/mL	16.71	17.98	93
3 Unspiked	10.36	-	-
+ 2 ng/mL	10.71	12.36	87
+ 5 ng/mL	13.69	15.36	89
+ 10 ng/mL	19.02	20.36	93

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
Sample 1	14.53	-	-
1:2	7.72	7.26	106
1:4	4.10	3.63	113
1:8	2.26	1.82	124
Sample 2	8.55	-	-
1:2	4.71	4.27	110
1:4	2.55	2.14	119
Sample 3	6.57	-	-
1:2	3.6	3.28	110
1:4	1.96	1.64	120

COMPARATIVE STUDIES

The DBC Resistin ELISA kit (y) was compared with a leading competitor ELISA kit (x). The comparison of 40 serum samples yielded the following linear regression results: y =0.9444x - 0.2202 with r = 0.93.

REFERENCE VALUES

As for all clinical assays each laboratory should collect data and establish their own range of reference values.

Group	n	Mean (ng/mL)	95% Confidence Range (ng/mL)
Male	120	4.28	1.53–8.95
Female	120	4.87	1.59–10.83

Ordering Information: **REF CAN-RSN-4000**

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Diagnostics Biochem Canada

Sex Hormone Binding Globulin (SHBG) ELISA

REF CAN-SHBG-4010

ASSAY PROCEDURE

- Bring kit components to room temperature. Prepare working solutions.
- Pipette 20 µL of each calibrator, control and specimen sample.
- Pipette 200 µL of assay buffer into each well.
- Incubate on a plate shaker for 30 minutes at room temperature.
- Wash 3 times.
- Pipette 150 µL of conjugate working solution into each well.
- Incubate on a plate shaker for 15 minutes at room temperature.
- Wash 3 times. Pipette 150 µL of TMB substrate into each well.
- Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of stopping solution into each well.
- Read the plate on a microplate reader at 450 nm.

Sex hormone binding globulin (SHBG) is a glycoprotein composed of 373 amino acid residues and three carbohydrate side chains. SHBG has been known by many other names including Testosteroneestradiol Binding Globulin (TeBG), Sex steroid Binding Protein (sBP) and Sex Steroid Binding Globulin (SSBG).

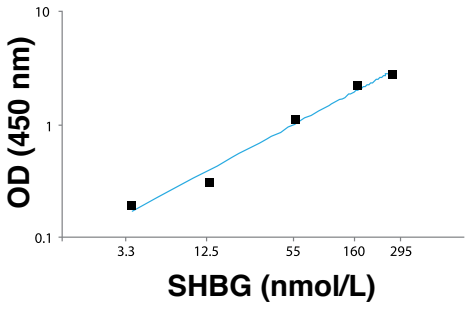
One of the main properties of SHBG is its high affinity for steroids, especially the C18, C19 and 17α-hydroxyl groups. The binding of steroids to SHBG is temperature and pH dependent.

The three steroids that have a high avidity for SHBG are Dihydrotestosterone, Testosterone and Estradiol. Very small amounts of these steroids are free in biological fluid; the majority are bound to SHBG and albumin. These two fractions, that is, free and bound exist in a state of dynamic equilibrium. When the level of SHBG concentration changes, a remarkable change occurs in both albumin-bound hormone and also in the free fraction.

Throughout life SHBG increases until the eighties in both sexes. During the menstrual cycle SHBG does not seem to vary appreciably, however, according to some authors the concentration of SHBG is elevated in the luteal phase. During pregnancy the level of SHBG rises rapidly until about the 30th week of gestation.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for SHBG is immobilized onto the microplate and another monoclonal antibody specific for a different region of SHBG is conjugated to horse radish peroxidase (HRP). SHBG from the sample and standards are allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second 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 by the enzymatic reaction is directly proportional to the concentration of SHBG in the sample.



A set of standards is used to plot a standard curve from which the amount of SHBG in patient samples and controls can be directly read.

PERFORMANCE CHARACTERISTICS

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) plus 2 SD. Therefore, the sensitivity of the DBC SHBG ELISA kit is **0.1 nmol/L**.

SPECIFICITY (CROSS-REACTIVITY)

The specificity of the SHBG ELISA kit was determined by measuring the apparent SHBG value of samples spiked with high levels of Thyroxine Binding Globulin (TBG):

Substance	Concentration Range (mg/L)	Apparent SHBG Value (nmol/L)
Thyroxine Binding Globulin (TBG)	10–500	Not Detected

INTRA-ASSAY PRECISION

Four samples were assayed ten times each on the same calibrator curve. The results (in nmol/L) are tabulated below:

Sample	Mean	SD	CV%
1	4.5	0.39	8.6
2	16	0.68	4.3
3	57	1.70	3.0
4	158	8.4	5.3

INTER-ASSAY PRECISION

Four samples were assayed ten times over a period of four weeks. The results (in nmol/L) are tabulated below:

Sample	Mean	SD	CV%
1	3.8	0.44	11.6
2	19	1.60	8.4
3	63	5.50	8.7
4	194	14.0	7.2

HIGH DOSE HOOK EFFECT

The SHBG ELISA kit did not experience a high dose hook effect when it was tested up to a SHBG concentration of 10,000 nmol/L.

RECOVERY

Spiked samples were prepared by adding defined amounts of SHBG to three patient serum samples. The results (in nmol/L) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	39	-	-
+ 6.5	42	45.5	92.3
+ 28.5	67	67.5	99.3
+ 165	208	204	102.0
2 Unspiked	61	-	-
+ 6.5	63	67.5	93.3
+ 28.5	91	89.5	101.7
+ 165	224	226	99.1
3 Unspiked	157	-	-
+ 6.5	170	163.5	104.0
+ 28.5	210	185.5	113.2
+ 165	307	322.0	95.3

LINEARITY

Three patient serum samples were diluted with assay buffer. The results (in nmol/L) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	58	-	-
1:2	29	29	100.3
1:5	12.4	11.6	106.9
1:10	5.6	5.8	96.6
2	85	-	-
1:2	42.5	42.5	100.0
1:5	19.9	17	117.1
1:10	9.2	8.5	108.2
3	120	-	-
1:2	61.2	60	102.0
1:5	24	24	100.0
1:10	12.2	12	101.7

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	N	Mean (nmol/L)	Range (nmol/L)
Male	104	31	7–70
Female	44	50	15–120

Ordering Information: **REF CAN-SHBG-4010**

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Diagnostics Biochem Canada

Testosterone Saliva ELISA

REF CAN-TE-300

ASSAY PROCEDURE

- Bring kit components to room temperature. Prepare working solutions.
- Pipette 100 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of the testosterone-biotin conjugate working solution into each well.
- Incubate on a plate shaker for 1 hour at room temperature.
- Wash the wells 5 times.
- Pipette 150 µL of the streptavidin-HRP conjugate working solution into each well.
- Incubate on a plate shaker for 30 minutes at room temperature.
- Wash the wells 5 times.
- Pipette 150 µL of TMB substrate. Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of stopping solution into each well.
- Read the plate on a microplate reader at 450 nm.

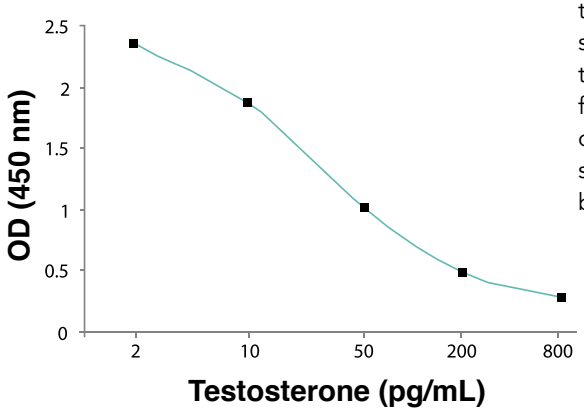
Testosterone is a C-19 steroid secreted from the testis and the adrenal cortex in men and from the adrenal cortex and ovary in women. Testosterone is also produced by peripheral tissues from androstenedione, which is of little physiological significance in men, however in women about half of circulating testosterone is derived from this origin. The action of testosterone is both androgenic or anabolic. Testosterone measurements are used mainly for clinical evaluation of hypogonadism in males and hyperandrogenic states in females.

Most of the circulating testosterone is bound to three proteins: sex hormone binding globulin (44–78%), albumin (20–54%) and cortisol binding globulin (small amount). Only about 2–3% of the total circulating testosterone remains unbound or in the free form. Only the free portion (or the non-SHBG bound fraction) of the circulating testosterone is thought to be available to tissues where it exerts its biological actions.

The salivary hormone assays are advocated for their noninvasive, easy sample collection method. Salivary testosterone is of great clinical value for it represents a filtered fraction of plasma testosterone and is independent of flow rate. Many studies have suggested that salivary testosterone correlates well with either free or non-SHBG bound testosterone.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a two-step competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, controls and patient samples) and a biotin-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate. In the second step, the streptavidin-horseradish peroxidase conjugate binds to any bound biotinylated testosterone. The washing and decanting procedures remove unbound materials. After the last 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 testosterone in the sample. A set of standards is used to plot a standard curve from which the amount of testosterone in patient samples and controls can be directly read.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

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 DBC Testosterone Saliva ELISA kit is **1.0 pg/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Testosterone ELISA kit with testosterone cross-reacting at 100%.

Steroid	% Cross-Reactivity
Testosterone	100
5α-DHT	5.2
Androstenedione	1.4
Androstanediol	0.8
Progesterone	0.5
Androsterone	0.1

The following steroids were tested but cross-reacted at less than 0.1%: Aldosterone, Andrenosterone, Cholesterol, Corticosterone, Dehydroepiandrosterone, Dehydroepiandrosterone Sulfate, Epi-androsterone, 17β-Estradiol, Estriol 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	14.05	1.00	7.1
2	38.19	1.30	3.4
3	122.81	8.19	6.7

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	11.03	1.08	9.8
2	47.24	3.32	7.0
3	122.79	8.19	9.1

RECOVERY

Spiked samples were prepared by adding defined amounts of testosterone to 3 patient saliva samples. The results (in pg/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	5.21	-	-
+ 50 (5:1)	15.89	14.2	112
+ 200 (5:1)	50.82	44.2	115
+ 800 (5:1)	154.48	164.2	94
2 Unspiked	32.50	-	-
+ 50 (5:1)	34.86	36.0	97
+ 200 (5:1)	57.39	66.0	87
+ 800 (5:1)	165.73	186.0	89
3 Unspiked	52.57	-	-
+ 50 (5:1)	53.67	52.06	103
+ 200 (5:1)	85.77	82.06	105
+ 800 (5:1)	179.00	202.06	89

LINEARITY

Three patient saliva samples were diluted with calibrator A. The results (in pg/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	154.48	-	-
1:2	82.44	77.24	107
1:4	39.38	38.62	102
1:8	23.14	19.31	120
2	165.73	-	-
1:2	88.74	82.87	107
1:4	50.47	41.43	122
1:8	16.95	20.72	82
3	188.75	-	-
1:2	104.45	94.38	111
1:4	49.24	47.19	104
1:8	20.31	23.59	86

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	N	Range (pg/mL)
Males	40	38–120
Females	41	5–32

Ordering Information: **REF CAN-TE-300**

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Diagnostics Biochem Canada

Testosterone ELISA

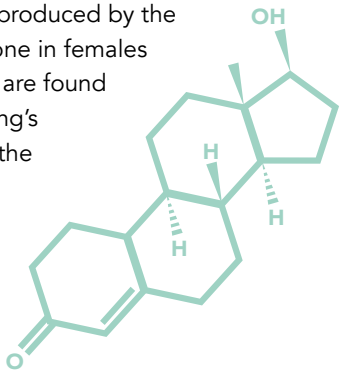
REF CAN-TE-250

ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 50 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of conjugate working solution into each well.
- Incubate on a plate shaker for 1 hour at room temperature.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

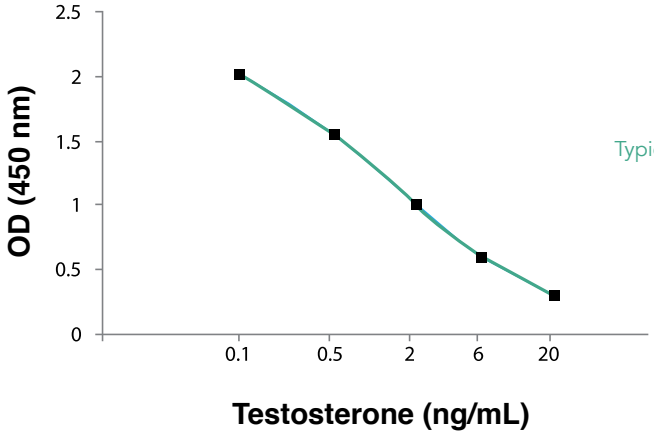
Testosterone is the most important male sex hormone, it is responsible for genital development, beard growth, muscle development and general male characteristics. The measurement of serum or plasma levels is an index of leydig cell function and high or low values correlate well with hypo- or hypergonadism.

In females small amounts of testosterone are produced by the adrenals and ovaries. High levels of testosterone in females indicates excessive androgen production and are found in progressive hirsutism and virilization, Cushing's syndrome and a deficiency in one or more of the specific enzymes required for normal steroid biosynthesis.



PRINCIPLE OF THE TEST

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 patient 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 testosterone in the sample. A set of standards is used to plot a standard curve from which the amount of testosterone in patient samples and controls can be directly read.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

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 DBC Direct Testosterone ELISA kit is **0.022 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct Testosterone ELISA kit with testosterone cross-reacting at 100%.

Steroid	% Cross-Reactivity
Testosterone	100
5α-DHT	5.2
Androstenedione	1.4
Androstanediol	0.8
Progesterone	0.5
Androsterone	0.1

The following steroids were tested but cross-reacted at less than 0.1%: Aldosterone, Andrenosterone, Cholesterol, Corticosterone, Dehydroepiandrosterone, Dehydroepiandrosterone Sulfate, Epiandrosterone, 17β-Estradiol, Estriol and Pregnenolone.

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.75	0.07	9.6
2	0.77	0.06	7.7
3	1.37	0.08	6.6

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.76	0.05	6.1
2	3.29	0.28	8.5
3	4.11	0.30	7.3

COMPARATIVE STUDIES

The DBC Direct Testosterone ELISA kit (x) was compared with a competitors Testosterone ELISA kit (y). The comparison of 40 serum samples yielded the following linear regression results: y = 1.4171x - 0.0941, r = 0.96

RECOVERY

Spiked samples were prepared by adding defined amounts of testosterone to four patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	0.45	-	-
+ 6.67	5.73	7.12	80.5
2 Unspiked	0.67	-	-
+ 6.67	8.08	7.34	110.1
3 Unspiked	1.40	-	-
+ 6.67	7.13	8.07	88.4
4 Unspiked	2.01	-	-
+ 6.67	8.42	8.68	97.0

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	5.73	-	-
1:2	3.23	2.86	112.9
1:4	1.66	1.43	116.1
1:8	0.85	0.72	118.1
2	8.08	-	-
1:2	4.01	4.04	99.3
1:4	2.02	2.02	100.0
1:8	0.96	1.01	95.0
3	8.42	-	-
1:2	3.75	4.21	89.1
1:4	2.01	2.11	95.3
1:8	1.03	1.05	98.1

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. The results of an expected range study with apparently normal healthy subjects yielded the following results (all values are reported in ng/mL):

Group	N	Mean (ng/mL)	Central 95% (ng/mL)
Prepubertal infants	10	0.12	0.05–0.25
Puberty and Males adults	40	4.7	3.0–12.0
Females	40	0.5	0.2–1.0

Ordering Information: REF CAN-TE-250

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Diagnostics Biochem Canada

Free Testosterone ELISA

REF CAN-FTE-260

ASSAY PROCEDURE

- Bring kit components to room temperature. Prepare working solutions.
- Pipette 25 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of conjugate working solution into each well.
- Gently shake the plate for 10 seconds.
- Incubate at 37°C for 1 hour.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate at 37°C for 10–15 minutes.
- Pipette 50 µL of stopping solution into each well.
- Read the plate on a microplate reader at 450 nm.

Testosterone is a C-19 steroid secreted from the testis and the adrenal cortex in men and from the adrenal cortex and ovaries in women. Testosterone is also produced by peripheral tissues from androstenedione, which is of little physiological significance in men; in women however, about half of the circulating testosterone is derived from this origin. Testosterone measurements are used mainly for clinical evaluation of hypogonadism in males and hyperandrogenic states in females.

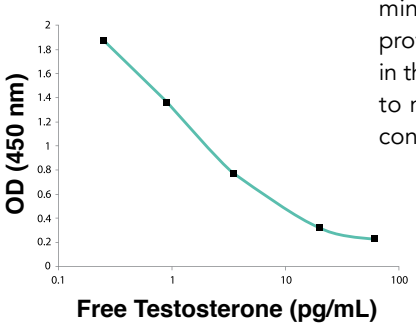
Testosterone circulates in the blood bound to three proteins: **sex hormone binding globulin** (60–80%), **albumin** and **cortisol binding globulin**. Only about 1–2% of the total circulating testosterone remains unbound or free. Even though it is still under investigation, most researchers accept the free testosterone determination as a measure of the biologically active fraction.

Free testosterone determinations are recommended to overcome the influences caused by variations in transport proteins on the total testosterone concentration.

PRINCIPLE OF THE TEST

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 patient 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 free testosterone in the sample. A set of standards is used to plot a standard curve from which the amount of free testosterone in patient samples and controls can be directly read.

The DBC free testosterone kit utilizes a highly specific rabbit anti-testosterone polyclonal antibody at a low binding capacity (Keq x concentration) to keep minimum disturbances of the testosterone-protein equilibrium. The other components in the test system are also optimized in order to not alter the original free testosterone concentration.



Typical calibration curve

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection (LoD) was determined from the analysis of 64 replicates of a low value sample and from the LoB.

LoD = LoB + 1.645σS, where σS is the standard deviation of the low value sample. σS was determined to be 0.0093 based on 64 measurements of a low value sample.

LoD = 0.0025 + (1.645*0.0093) = 0.018 pg/mL.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct Free Testosterone ELISA kit with testosterone cross-reacting at 100%.

Steroid	% Cross-Reactivity
Testosterone	100
5α-DHT	3.5
Androstenedione	0.17
Progesterone	0.007
Androsterone	0.075
Aldosterone	< 0.008
Cholesterol	< 0.0001
Cortisone	0.0025
DHEA	0.071
DHEAS	0.0014
17β-Estradiol	0.15
Estriol	< 0.008
Pregnenolone	0.028

COMPARATIVE STUDIES

The DBC Direct Free Testosterone ELISA Kit (y) was compared with a competitors Free Testosterone Coated Tube RIA Kit (x). The comparison of 60 serum samples yielded the following linear regression results:

y (DBC) = 0.9362x (competitor) + 3.8794, r = 0.97

INTRA-ASSAY PRECISION

Five samples were assayed 24 times each on the same calibrator curve. The results (in pg/mL) are tabulated below:

Sample	Mean	CV%
1	2.24	6.7
2	3.81	6.4
3	13.6	6.0
4	13.7	5.9
5	23.7	4.8

INTER-ASSAY PRECISION

Three samples were assayed twenty times in duplicate over a period of greater than ten days. The results (in pg/mL) are tabulated below:

Sample	Mean	CV%
1	3.53	8.1
2	13.8	11.5
3	23.3	6.9

EFFECT OF SEX HORMONE BINDING GLOBULIN

The purpose of this study was to investigate a possible interference caused by the binding of SHBG to the free testosterone-HRP conjugate. A charcoal-stripped human serum pool was spiked precisely with SHBG at concentrations ranging from 6.25–200 µg/mL and was assayed with the DBC Free Testosterone ELISA Kit. Results tabulated below (in pg/mL):

SHBG Added	OD 450 nm	Percent B/B_0 (%)
0	2.37	100.0
6.25	2.37	99.9
12.5	2.34	98.7
50	2.36	99.5
200	2.27	95.6

The results showed % binding values between 95–100% (B_0 = unspiked serum) even at higher than normal SHBG levels. In conclusion, the results showed that there was no significant binding by SHBG on the free testosterone-HRP conjugate.

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. The results of an expected range study with apparently normal healthy subjects yielded the following results (all values are reported in pg/mL):

Cohort Group; Gender/Age	N	95% Confidence Range	Absolute Range
Males / < 13	44	–	ND–1.6
Males / 13–19	37	–	ND–22.3
Males / 20–39	120	9.1–32.2	–
Males / 40–59	120	5.7–30.7	–
Males / ≥ 60	120	5.9–27.0	–
Females / < 13	63	–	ND–1.3
Females / 13–19	17	–	0.2–2.0
Females / 20–39	120	0.1–6.3	–
Females 40–59	120	0.2–4.1	–
Females / ≥ 60	60	0.5–3.9	–

Ordering Information: REF CAN-FTE-260

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Diagnostics Biochem Canada

Thyroid Stimulating Hormone (TSH) ELISA

REF CAN-TSH-4080

ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 50 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of conjugate working solution into each well.
- Incubate on a plate shaker for 90 minutes at room temperature.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

Thyroid stimulating hormone (TSH) is a glycoprotein hormone secreted by the anterior pituitary gland. TSH has two subunits, namely α and β. The α subunit of TSH is similar to the α subunit found in the LH, FSH and hCG glycoprotein hormones. The β subunit however, is specific and differs from hormone to hormone.

The thyroid hormones are secreted and produced by the thyroid gland. The production of thyroid hormones is under the regulation of TSH. Also, TSH acts as a stimulator of iodide transport and the gland itself is under the positive control of TSH. The concentrations of thyroid hormones control the secretion of TSH, therefore, a negative feedback exists. It is to be noted that the secretion of thyroid hormones are under the direct, positive effect of the sympathetic nervous system.

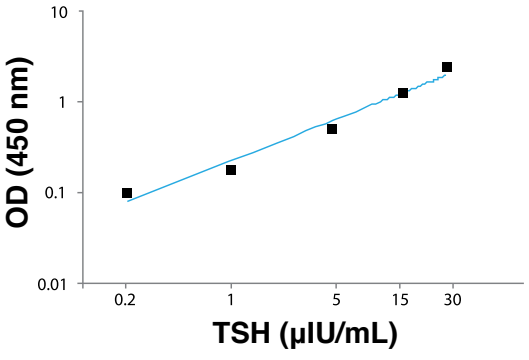
The major protein component of the thyroid gland is thyroglobulin, a glycoprotein of which the secretion in the blood stream is stimulated by TSH. Therefore, TSH plays an important role in the proper function and development of the thyroid gland.

It is recommended to assay both the glycoprotein hormone and the target organ hormones. For example, in primary hypothyroidism the serum level of thyroxine is low while the TSH level is high. In secondary hypothyroidism, both thyroxine and TSH are low. The TSH level is decreased in hyperthyroidism. Today, with all the sensitive assays available, if there were to be only one test to be prescribed for thyroid function, TSH would be the test. TSH determinations are also helpful to monitor patients who receive thyroxine replacement therapy.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical one-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for TSH is immobilized onto the microplate and another monoclonal antibody specific for a different region of TSH is conjugated to horse radish peroxidase (HRP). TSH from the sample and standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. 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 by the enzymatic reaction is directly proportional to the concentration of TSH in the sample.

A set of standards is used to plot a standard curve from which the amount of TSH in patient samples and controls can be directly read.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

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) plus 2 SD. Therefore, the sensitivity of the DBC Direct TSH ELISA kit is **0.1 µIU/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The specificity of the Direct TSH ELISA kit was determined by measuring the apparent TSH values of the following compounds:

Substance	Concentration Range	Apparent TSH Value (µIU/mL)
hCG Calibrated against WHO 1st IS 75/537	10,000–50,000 IU/L	< 0.15
hFSH Calibrated against WHO 1st 83/575	1000–4000 IU/L	< 0.15
hLH Calibrated against WHO 2nd IS 80/552	100–500 IU/L	< 0.15

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in µIU/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.52	0.07	13.3
2	1.54	0.10	6.4
3	9.27	0.72	7.7

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in µIU/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.78	0.07	8.3
2	8.03	0.99	12.3
3	25.42	3.26	12.8

COMPARATIVE STUDY

The DBC Direct TSH ELISA kit (Kit A) was compared with two other competitors ELISA kits (Kit B and Kit C). The results (in µIU/mL) are tabulated below:

Group	N	Kit A Mean	Kit B Mean	Kit C Mean
Random Males and Females	27	2.97	3.36	2.89

RECOVERY

Spiked samples were prepared by adding defined amounts of TSH to three patient serum samples. The results (in µIU/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	1.92	-	-
+ 0.25	2.31	2.17	106.5
+ 3.0	5.12	4.92	104.1
+ 7.5	10.26	9.42	108.9
2 Unspiked	2.01	-	-
+ 0.25	2.27	2.26	100.4
+ 3.0	5.10	5.01	101.8
+ 7.5	9.36	9.51	98.4
3 Unspiked	2.02	-	-
+ 0.25	2.35	2.27	103.5
+ 3.0	4.87	5.02	97.0
+ 7.5	8.57	9.52	90.0

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in µIU/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	9.36	-	-
1:2	4.53	4.68	96.8
1:4	2.31	2.34	98.7
1:8	1.08	1.17	92.3
2	10.89	-	-
1:2	5.65	5.45	103.7
1:4	2.96	2.72	108.8
1:8	1.32	1.36	97.1
3	11.85	-	-
1:2	6.03	5.93	101.7
1:4	2.43	2.96	82.1
1:8	1.18	1.48	79.7

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (µIU/mL)
Normal	0.3–5
Hyperthyroid	< 0.15
Hypothyroid	> 5.7

Ordering Information: REF CAN-TSH-4080

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Diagnostics Biochem Canada

Free Thyroxine (fT4) ELISA

REF CAN-FT4-4340

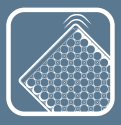
ASSAY PROCEDURE


Bring kit components to room temperature.


Prepare working solutions.


Pipette 25 µL of each calibrator, control and specimen sample.


Pipette 100 µL of conjugate working solution into each well.



Gently shake plate for 10 seconds. Incubate for 1 hour at 37°C.


Wash 3 times.


Pipette 150 µL of TMB substrate into each well.


Incubate for 10–15 minutes at 37°C.


Pipette 50 µL of stopping solution.


Read the plate on a microplate reader at 450 nm.

Thyroxine (T4), the principal thyroid hormone, circulates in blood almost completely bound to carrier proteins. However, only the free (unbound) fraction of thyroxine is considered to be biologically active. The main carriers of thyroxine are thyroxine-binding globulin (TBG), pre-albumin and albumin. The measurement of free thyroxine (fT4) levels correlate better with the clinical status than total thyroxine levels.

The DBC free T4 assay is a one step competitive ELISA system that is rapid and easy to perform compared to equilibrium dialysis and ultrafiltration methods, which are cumbersome and time-consuming. This system employs a highly specific monoclonal antibody and a non-analog tracer that was proved experimentally to have no significant binding to TBG and albumin.

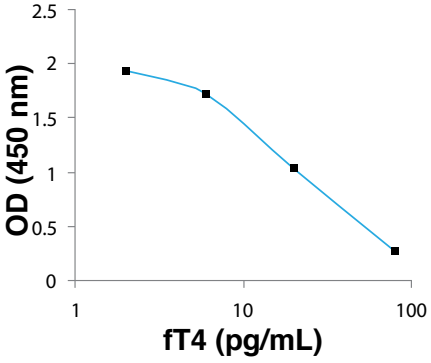
In the euthyroid, normal population the free T4 concentration is 7–22 pg/mL. The level of free T4 is decreased in hypothyroidism while in thyrotoxic patients the level of free T4 is increased.

This assay is used at times with other thyroid tests for in vitro diagnostic purposes and for assessing patients who are receiving thyroid treatments (follow-up).

PRINCIPLE OF THE TEST

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 patient 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 fT4 in the sample. A set of standards is used to plot a standard curve from which the amount of fT4 in patient samples and controls can be directly read.

The labelled T4 (conjugate) employed in this assay system has shown no binding properties towards thyroxine-binding globulin (TBG) and human serum albumin (HSA). The binding sites on the microplates are designed to be of a low binding-capacity in order not to disturb the equilibrium between T4 and its carrying proteins. The assay is carried out under normal physiological conditions of pH, temperature and ionic strength.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

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 DBC Direct fT4 ELISA kit is **1.0 pg/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct fT4 ELISA kit with T4 cross-reacting at 100%.

Compound	% Cross-Reactivity
L-Thyroxine	100
D-Thyroxine	94
3,3′,5′-Triiodo-L-Thyronine (Reverse T3)	86
3,3′,5-Triiodo-L-Thyronine (T3)	3.3
3,3′,5′-Triiodo-D-Thyronine	1.8
3,3′,5′-Triiodothyropropionic acid	0.6

The following compounds were tested but cross-reacted at less than 0.04%: Acetylsalicylic acid, 3,5-Diiodo-L-Thyronine, 3,5-Diiodo-L-Tyrosine and 3-Iodo-L-Tyrosine.

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	3.79	0.16	4.8
2	23.26	1.14	4.9
3	70.60	3.04	4.3

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	4.27	0.53	12.3
2	20.54	2.36	11.5
3	67.34	6.67	9.9

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. The following reference range (pg/mL) was established with 80 apparently healthy adults:

Group	N	Range (pg/mL)
Normal Euthyroid Samples	80	7–22

EFFECT OF BILIRUBIN

Bilirubin was added to a patient sample at concentrations of 50 and 100 µg/mL and assayed with the DBC Direct fT4 ELISA kit. Results are tabulated below:

Sample	fT4 (pg/mL)
Unspiked	8.78
+ 50 µg/mL bilirubin	10.68
+ 100 µg/mL bilirubin	9.72

EFFECT OF HUMAN SERUM ALBUMIN (HSA)

Purified human serum albumin (HSA) was added to a patient sample at concentrations of 10, 20 and 40 mg/mL. Samples were assayed with the DBC Direct fT4 ELISA kit. Results are tabulated below:

Sample	fT4 (pg/mL)
Unspiked	8.78
+ 10 mg/mL	8.81
+ 20 mg/mL	9.46
+ 40 mg/mL	9.90

No binding of labelled fT4 to HSA was found at these concentrations.

EFFECT OF THYROXINE-BINDING GLOBULIN (TBG)

The zero calibrator was spiked precisely with purified TBG at concentrations ranging from 25–200 µg/mL and assayed with the DBC Direct fT4 ELISA kit. Results are tabulated below:

Sample	TBG Added (µg/mL)	OD 450 nm
1	0	1.883
2	25	2.030
3	50	2.149
4	100	2.175
5	200	2.251

No significant binding of labelled fT4 to TBG was found at these concentrations.

EFFECT OF NON-ESTERIFIED FATTY ACIDS

Oleic acid was added to a patient sample at concentrations of 0.5, 5 and 20 mmol/L and assayed with the DBC Direct fT4 ELISA kit. Results are tabulated below:

Sample	fT4 (pg/mL)
Unspiked	24.83
+ 0.5 mmol/L	20.53
+ 5 mmol/L	26.06
+ 20 mmol/L	83.64

At high concentrations of oleic acid, the free T4 level was significantly increased. This is due to the well-known effect that non-esterified fatty acids can dissociate T4 from its carrier proteins.

Ordering Information: **REF CAN-FT4-4340**

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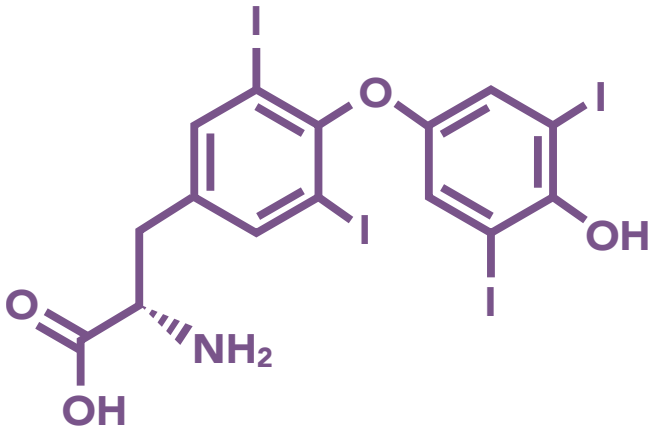
Diagnostics Biochem Canada

Thyroxine (T4) ELISA

REF CAN-T4-4240

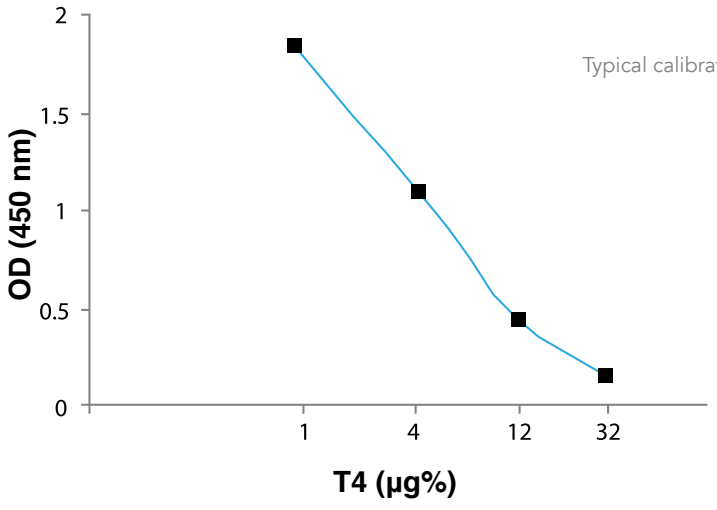
ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 20 µL of each calibrator, control and specimen sample.
- Pipette 150 µL of conjugate working solution into each well.
- Incubate on a plate shaker for 30 minutes at room temperature.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a plate shaker for 15–20 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.



PRINCIPLE OF THE TEST

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 patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate. The washing and decanting procedures remove un-bound 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 T4 in the sample. A set of standards is used to plot a standard curve from which the amount of T4 in patient samples and controls can be directly read.



PERFORMANCE CHARACTERISTICS

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 DBC Direct T4 ELISA kit is **0.6 µg%**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct T4 ELISA kit with T4 cross-reacting at 100%.

Compound	% Cross-Reactivity
L-Thyroxine	100
D-Thyroxine	94
3,3′,5′-Triiodo-L-Thyronine (Reverse T3)	86
3,3′,5-Triiodo-L-Thyronine (T3)	3.3
3,3′,5′-Triiodo-D-Thyronine	1.8
3,3′,5′-Triiodothyropropionic acid	0.6

The following compounds were tested but cross-reacted at less than 0.04%: Acetylsalicylic acid, 3,5-Diiodo-L-Thyronine, 3,5-Diiodo-L-Tyrosine and 3-Iodo-L-Tyrosine.

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in µg%) are tabulated below:

Sample	Mean	SD	CV %
1	2.48	0.23	9.2
2	8.58	0.60	6.9
3	20.46	1.33	6.4

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in µg%) are tabulated below:

Sample	Mean	SD	CV %
1	3.33	0.41	12.3
2	10.30	1.19	11.5
3	14.5	1.44	9.9

RECOVERY

Spiked samples were prepared by adding defined amounts of T4 to three patient serum samples. The results (in µg%) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	2.03	-	-
+ 2.91	4.64	4.94	93.9
+ 7.38	9.28	9.41	98.6
+ 13.70	17.93	15.73	114.0
2 Unspiked	9.43	-	-
+ 2.91	13.17	12.32	106.9
+ 7.38	19.56	16.81	116.4
+ 13.70	25.81	23.13	111.6
3 Unspiked	24.03	-	-
+ 2.91	26.74	26.94	99.3
+ 7.38	30.65	31.41	97.6
+ 13.70	> 32	37.73	-

LINEARITY

Two patient serum samples were diluted with calibrator A. The results (in µg%) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	22.50	-	-
1:2	11.74	11.25	104.4
1:4	5.70	5.63	101.2
1:8	2.71	2.81	96.4
2	25.64	-	-
1:2	14.50	12.82	113.1
1:4	6.90	6.41	107.6
1:8	3.31	3.21	103.1

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (µg%)
Euthyroid	4–12
Hyperthyroid	> 12
Hypothyroid	< 4

Ordering Information: REF CAN-T4-4240

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Diagnostics Biochem Canada

Free Triiodothyronine (fT3) ELISA

REF CAN-FT3-4230

ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 25 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of conjugate working solution into each well.
- Gently shake plate for 10 seconds. Incubate for 1 hour at 37°C.
- Wash the wells 3 times.
- Pipette 150 µL of TMB substrate into each well.
- Incubate for 10–15 minutes at 37°C.
- Pipette 50 µL of stopping solution into each well.
- Read the plate on a microplate reader at 450 nm.

Triiodothyronine (T3) is a thyroid hormone found circulating in the bloodstream. T3 contains three iodine atoms and is produced largely through the extrathyroidal conversion of thyroxine (T4), the principal thyroid hormone with four iodine atoms. Most of the T3 that circulates in the blood is bound to carrier proteins such as TBG, pre-albumin and albumin. The free fraction of T3 (fT3), which represents only 0.25% of the total amount, is considered to be the physiological active fraction.

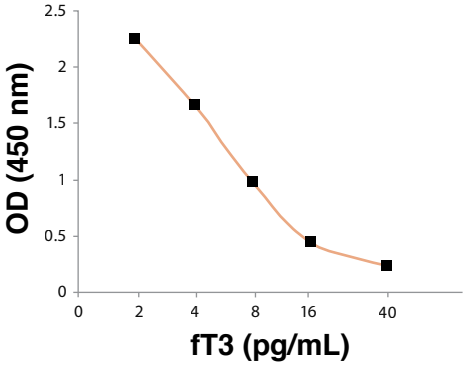
Total T3 levels depend not only on thyroid status and the peripheral conversion of T4 to T3, but also on the concentration of thyroid hormone-binding proteins. Free T3 (fT3) on the other hand, is largely unaffected by variations in these carrier proteins which can occur under conditions such as pregnancy, estrogen therapy and the use of oral contraceptives. Therefore, free T3 typically reflects a patient's actual thyroid status more reliably than total T3.

Measurement of free T3 is generally recommended for patients with symptoms of hyperthyroidism as found in Graves' disease, toxic adenoma and toxic multinodular goiter.

PRINCIPLE OF THE TEST

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 patient 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 fT3 in the sample. A set of standards is used to plot a standard curve from which the amount of fT3 in patient samples and controls can be directly read.

The labelled T3 (conjugate) employed in this assay system has shown no substantial binding properties towards thyroxinebinding globulin (TBG) or human serum albumin (HSA). The binding sites on the microplates are designed to be of a low binding-capacity in order not to disturb the equilibrium between T3 and its carrying proteins. The assay is carried out under normal physiological conditions of pH, temperature and ionic strength.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

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 DBC Direct fT3 ELISA kit is **0.3 pg/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct fT3 ELISA kit with T3 cross-reacting at 100%.

Compound	% Cross-Reactivity
L-Triiodothyronine	100
D-Triiodothyronine	34
Triiodothyropropionic acid	20
Diiodo-D-thyronine	0.5
D-Thyroxine	0.3
L-Thyroxine	0.9

The following compounds were tested but cross-reacted at less than 0.1%: Diiodotyrosine, Iodotyrosine, Phenytoin, Sodium Salicylate and r-Triiodothyronine.

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	5.182	0.501	9.7
2	8.560	0.598	7.0
3	48.200	1.686	3.5

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	3.306	0.284	8.6
2	5.154	0.402	7.8
3	8.698	0.713	8.2

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. The following reference range (pg/mL) was established with 44 apparently healthy adults:

Group	N	Mean	Central 95% Range
Euthyroid Adults	44	3.7	2.2–5.3

EFFECT OF THYROXINE BINDING GLOBULIN (TBG)

The purpose of this study was to investigate a possible interference caused by the binding of TBG to the fT3-HRP conjugate. The zero calibrator was spiked with purified TBG and assayed. The results are tabulated below:

TBG (µg/mL added)	OD	% B/B_0
0	1.255	100
12.5	1.229	98
25	1.170	93
50	1.137	91
100	1.168	93
200	1.174	94
400	1.118	89

The results show no binding of labelled T3 to TBG even at higher than normal levels. In conclusion, results showed that there was no significant influence by TBG in the DBC Direct Free T3 Direct ELISA Kit.

EFFECT OF HUMAN SERUM ALBUMIN (HSA)

The purpose of this study was to investigate a possible interference of HSA on the assay procedure. The zero calibrator was spiked with purified HSA and assayed. The results are tabulated below:

HSA (mg/mL added)	OD	% B/B_0
0	1.255	100
3.125	1.228	98
6.25	1.331	100
12.5	1.245	99
25	1.197	95
50	1.217	97
100	1.063	85

The results show no significant binding of labelled T3 to HSA even at higher than normal levels.

EFFECT OF NON-ESTERIFIED FATTY ACIDS (NEFA)

The purpose of this study was to investigate a possible interference of NEFA on the assay procedure. Two samples were spiked with oleic acid and assayed. The results are tabulated below:

NEFA (mmol/L added)	Sample 1 (pg/mL)	Sample 2 (pg/mL)
0	4.4	8.7
0.5	4.6	7.5
3.5	4.6	8.3
25	4.6	10.9

The results show that NEFA may increase the free T3 values, only at higher than normal concentrations.

Ordering Information: REF CAN-FT3-4230

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Diagnostics Biochem Canada

Reverse Triiodothyronine ELISA

REF CAN-RT3-100

ASSAY PROCEDURE

Bring kit components to room temperature. Prepare working wash buffer.

Pipette 25 µL of each calibrator, control and specimen sample.

Pipette 100 µL of RT3-Biotin conjugate into each well.

Incubate on a microplate shaker for 1 hour at room temperature. Wash 3 times.

Pipette 150 µL of Streptavidin-HRP conjugate.

Incubate on a microplate shaker for 30 minutes at room temperature. Wash 3 times.

Pipette 150 µL of TMB substrate.

Incubate on a microplate shaker for 10–20 minutes at room temperature.

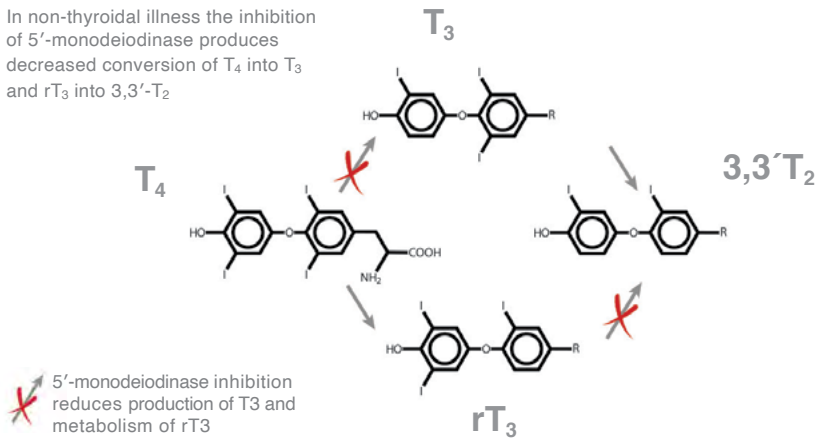
Pipette 50 µL of stopping solution. Gently shake the microplate by hand for 10 seconds.

Read the plate on a microplate reader at 450 nm.

3,3′,5′-Triiodo-L-thyronine also known as **reverse triiodothyronine** or reverse T3 (rT3), differs from 3,3′,5-Triiodo-L-thyronine (T3) in the positions of the iodine atoms in the molecule. The majority of circulatory rT3 is synthesized by peripheral de-iodination of thyroxine (T4).

Both T3 and rT3 bind to thyroid hormone receptors, but in contrast to T3, rT3 has not been found yet to stimulate receptor metabolic activity; it blocks receptor sites from T3 activation. The ratio of rT3 to T3 is a valuable biomarker of the metabolism and function of thyroid hormones because the process of 5′ monodeiodination that converts T4 to T3 and rT3 to 3,3′-T2 is inhibited in a number of non-thyroidal conditions such as fasting, anorexia nervosa, malnutrition, diabetes mellitus, stress, severe trauma or infection, hemorrhagic shock, hepatic dysfunction, pulmonary diseases and others. This scenario is known as “Sick euthyroid” syndrome or “Low T3” syndrome.

An elevated ratio of rT3 over T3 is therefore indicative of “sick euthyroid” syndrome and helps to exclude a diagnosis of hypothyroidism, particularly in critically ill patients. The concentration of rT3 could be high in patients on the following medications: amiodarone, dexamethasone, propylthiouracil, ipodate, propranolol, and the anesthetic halothane. The concentration of rT3 could be low in patients on Dilantin, which decreases rT3 due to its displacement from thyroxine-binding globulin and therefore generates an excessive clearance of rT3.



PRINCIPLE OF THE TEST

The DBC rT3 ELISA is a competitive enzyme immunoassay, where the antigen (rT3 present in calibrators, controls and patient samples) competes with a biotin-labelled antigen (rT3-Biotin conjugate) for a limited quantity of antibody which is coated on the microplate wells. After one hour incubation followed by the first washing, unbound materials are removed and a Streptavidin-HRP conjugate is added and incubated for 30 minutes. Following a second washing, the TMB substrate is added. The enzymatic reaction is terminated by addition of the stopping solution, upon which the color intensity is measured with a microplate reader. The color intensity is inversely proportional to the concentration of rT3 in the sample. The set of kit calibrators that are run simultaneously with the samples is used to plot a calibration curve and determine the concentration of rT3 in samples and controls.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection (LoD) was determined from the analysis of at least 60 samples of the blank and a low value sample in two independent experiments and it was calculated as follows:
LoD = μB + 1.645σB + 1.645σS, where σB and σS are the standard deviation of the blank and low value sample and μB is the mean value of the blank.

The Limit of Detection (LoD) was determined to be **0.014 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with rT3 cross-reacting at 100%:

Steroid	% Cross-Reactivity
rT3	100
T3	0
T4	< 0.1
T2	0

INTERFERENT SUBSTANCES

The following substances did not show significant interference with the assay: hemoglobin up to 2 g/L, free and conjugated bilirubin up to 200 mg/L, triglycerides up to 5.0 mg/mL and Biotin up to 2.4 μg/mL.

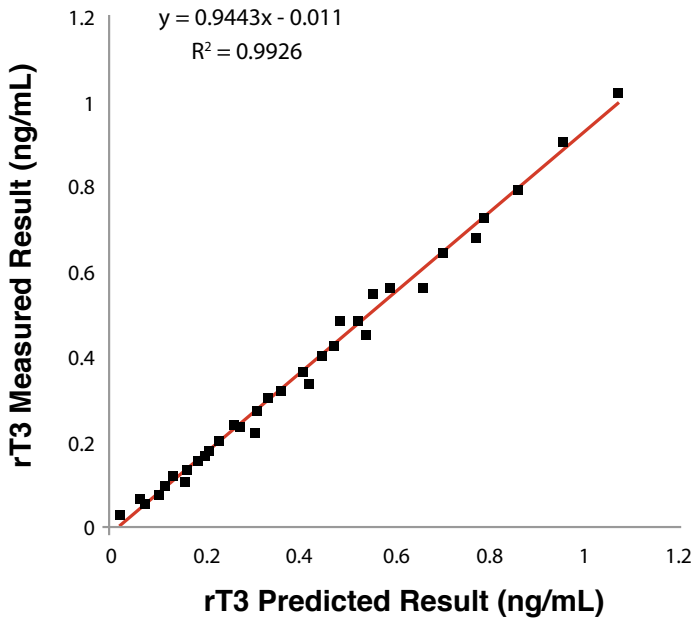
PRECISION

The experimental protocol used a nested components-of-variance design with 2 testing days, two lots and five scientists per day, each scientist ran 2 tests (one test with each lot) per day, and two replicate measurements per run (a 2 x 5 x 2 x 2 design) for each sample. The esults were analyzed with a two-way nested ANOVA and summarized in the table below.

Sample	Mean (ng/mL)	Within Run SD	Within Run CV (%)	Total SD	Total CV (%)
1	0.233	0.01	3.6	0.02	7.0
2	0.535	0.01	2.4	0.05	9.5
3	1.174	0.06	4.9	0.13	10.7
4	0.102	0.01	6.0	0.01	10.4
5	0.082	0.00	5.8	0.01	8.7
6	0.094	0.01	8.1	0.01	11.5
7	0.257	0.01	4.3	0.02	9.7
8	0.480	0.02	4.2	0.04	9.2

LINEARITY

The linearity study was performed with four human serum samples covering the range of the assay and following CLSI guideline EP6-A. The samples were diluted in calibrator A up to a 1:5 dilution, tested in duplicate, and the results compared to the predicted concentration. The statistical analysis shows that the assay is sufficiently linear.



COMPARATIVE STUDIES

The DBC rT3 ELISA kit (y) was tested manually, as well as with automated technology. The comparison of 40 samples yielded the following linear regression results:

y = 0.8452x + 0.0195, r = 0.96

REFERENCE VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	N	Median (ng/mL)	95% Range (ng/mL)	Total Range (ng/mL)
Serum	120	0.15	0.098–0.218	0.069–0.262
Plasma	120	0.15	0.098–0.26	0.072–0.309

Ordering Information: REF CAN-RT3-100

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Diagnostics Biochem Canada

Triiodothyronine (T3) ELISA

REF CAN-T3-4220

ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 50 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of conjugate working solution into each well.
- Incubate on a plate shaker for 1 hour at room temperature.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

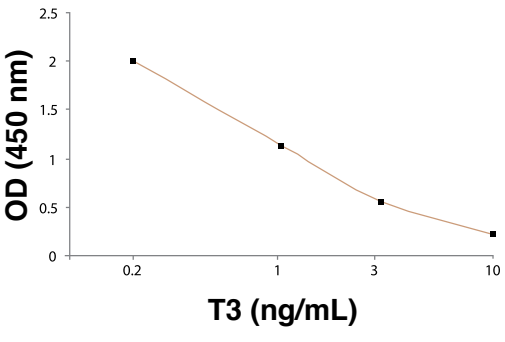
Triiodothyronine (T3) and thyroxine (T4) are the two active thyroid hormones found in the blood stream. About 80% of T3 is produced by the deiodination of T4 in the peripheral tissue and the other 20% is produced directly from the thyroid gland. T3 is transported through the peripheral blood stream bound to serum proteins, namely thyroxine binding globulin, thyroid binding prealbumin and albumin. About 0.3% of the total T3 is unbound and is therefore considered the free fraction.

T3 has an influence on oxygen consumption and heat production in virtually all tissues. The hormone also plays a critical role in growth, development and sexual maturation of growing organisms.

T3 is one parameter used in the clinical diagnosis and differentiation of thyroid disease, particularly hyperthyroidism. In most hyperthyroid patients, both serum T3 and serum T4 levels are elevated. Serum T3 levels are a sensitive indicator of the impending hyperthyroid state often preceeding elevated T4 and free thyroxine index values. Serum T3 levels are clinically significant in both the diagnosis of thyroid disease and in the detection of T3-thyrotoxicosis. However, it has been demonstrated that T3 levels may be affected by a number of medications, acute and chronic stress, and a variety of acute and chronic nonthyrodial illnesses. It is therefore necessary to differentiate those results that are due to thyroid dysfunction from those related to non-thyrodal diseases.

PRINCIPLE OF THE TEST

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 patient 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 T3 in the sample. A set of standards is used to plot a standard curve from which the amount of T3 in patient samples and controls can be directly read.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

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 DBC Direct T3 ELISA kit is **0.16 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct T3 ELISA kit with T3 cross-reacting at 100%.

Compound	% Cross-Reactivity
L-Triiodothyronine	100
D-Triiodothyronine	34
Triiodothyropropionic acid	20
Diiodo-D-thyronine	0.5
D-Thyroxine	0.2
L-Thyroxine	0.1

The following compounds were tested but cross-reacted at less than 0.1%: Diiodotyrosine, Iodotyrosine, Phenytoin, Sodium Salicylate and r-Triiodothyronine.

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.65	0.08	12.3
2	1.19	0.14	11.7
3	5.16	0.21	4.1

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.64	0.07	10.4
2	1.24	0.12	9.7
3	4.86	0.44	9.0

RECOVERY

Spiked samples were prepared by adding defined amounts of T3 to a serum pool. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	1.3	-	-
+ 2.0	3.8	3.3	115.1
+ 3.3	5.0	4.6	108.7
+ 5.0	5.7	6.3	90.5

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	2.90	-	-
1:2	1.50	1.45	103.4
1:4	0.71	0.73	97.3
1:8	0.40	0.36	111.1
2	5.10	-	-
1:2	2.60	2.55	102.0
1:4	1.20	1.28	93.8
1:8	0.80	0.64	125.0
3	8.00	-	-
1:2	4.45	4.00	112.3
1:4	2.30	2.00	115.0
1:8	1.00	1.00	100.0

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (ng/mL)
Healthy Normal Males and Females	0.7–2.1

Ordering Information: **REF CAN-T3-4220**

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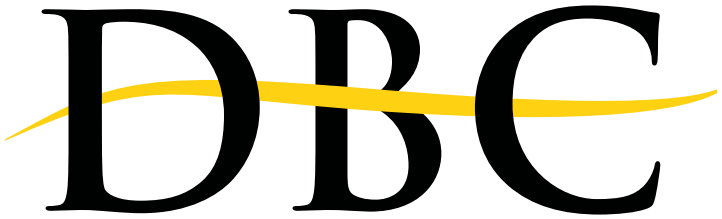

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