Diagnostics Biochem Canada Inc.
Manufacturing Innovative IVD for the World
Since 1973

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

EU: IVD CAN: IVD USA: For Research Use Only. Not For Use in Diagnostic Procedures.

REF : CAN-VD-510

Version: 6.0
Effective: July 05, 2017

INTENDED USE

For the quantitative determination of 25-hydroxyvitamin D [25(OH)D] in human serum and plasma by an enzyme immunoassay.

PRINCIPLE OF THE TEST

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.

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.

CLINICAL APPLICATIONS

Vitamin D concentration in blood should be measured regularly to ensure that satisfactory physiological levels are maintained year round (see references). 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 [25(OH)D].

PROCEDURAL CAUTIONS AND WARNINGS

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Each laboratory is suggested to establish its own internal QC materials and procedure for assessing the reliability of results.
3. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
4. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
5. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
6. All kit reagents and specimens should be at room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and serum and plasma specimens.
7. Avoid exposing kit reagents, serum and plasma specimens to light.
8. A calibrator curve must be established for every run. The kit controls should be included in every run and fall within established confidence limits.
9. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
10. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
11. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the presence of a blue colour, in which case it should not be used.
12. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
13. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
14. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges. The performance of this assay is markedly influenced by the correct execution of the washing procedure.
15. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

1. This kit is specifically designed and validated for the determination of 25(OH)D in human serum and plasma. The kit is not validated for the determination of 25(OH)D in other specimens of human or animal origin.
2. The 25(OH)D level depends on multiple factors. Therefore, only carefully prepared serum or plasma samples are suitable for this test. Do not use grossly haemolysed, lipaemic, icteric serum or plasma, and any sample that was not handled properly according to the instructions.
3. Bacterial contamination, prolonged exposure to light or high temperatures, damage during transportation, repeated freeze and thaw cycles may affect the assay results.
4. The interpretation of the results should recognise the conditions that can affect vitamin D levels, such as medications, food supplements or extreme exposure to sun light or UV rays.
5. Modification of the test procedure, exchange of reagents from different lots, use of reagents after their expiry date, exposure of reagents to intense light or improper transportation of the product can negatively affect the results and the validity of the test.
6. The results obtained with this kit should not be used as the sole basis for clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animal products. If false results are suspected, such as unusually elevated values, samples should be tested using an alternative method.
7. Any sample result greater than 160 ng/mL should not be diluted further. Rather, the sample should be reported as >160 ng/mL.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

All reagents in this kit should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen. Human material used in the preparation of this product has been tested and found negative for the presence of HIV I / II, Hepatitis B surface antigen, HCV (NAT), HIV-1 (NAT) and RPR by FDA approved methods. Notwithstanding, the reagents should be handled as if capable of transmitting infections and in accordance with good laboratory practices.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

A minimum of 0.05 mL of serum or plasma is required per duplicate determination. Appropriate sample collection is essential for the accurate determination of 25(OH)D.

Serum: Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date.

Plasma: Collect 4–5 mL of blood into EDTA plasma tubes. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Single and multi-channel pipettes and disposable tips
2. Distilled or deionized water
3. Disposable glass test tubes or glass bottles
4. Microplate absorbance reader equipped with a 450 nm filter

REAGENTS PROVIDED

1. Anti-25(OH)D Antibody Coated Break-Apart Well Microplate — Ready To Use

Contents: One 96-well antibody coated microplate in a resealable pouch with desiccant.
Storage: Refrigerate at 2–8°C.
Stability: 12 months or as indicated on label.

2. 25(OH)D-Biotin Conjugate Concentrate — Requires Preparation X100

Contents: One glass bottle containing 25-hydroxyvitamin D-Biotin conjugate in a stabilizer with a non-mercury preservative.
Volume: 1.0 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.
Preparation: See preparation of working conjugate solution section.

3. Streptavidin-HRP Conjugate Concentrate — Requires Preparation X100

Contents: One plastic bottle containing Streptavidin-Horse Radish Peroxidase (HRP) conjugate concentrate in a stabilizer with a non-mercury preservative.
Volume: 0.3 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months in unopened vials or as indicated on label.
Preparation: See preparation of working conjugate solution section.

4. 25(OH)D Calibrators — Ready To Use

Contents: Six glass bottles containing 25-hydroxyvitamin D in human plasma with a non-mercury preservative. Calibrators are traceable to NIST SRM 972 and to concentrations determined by UV spectrophotometric analysis using a molar extinction coefficient of 18,300 M-1cm-1 at 264 nm.
Calibrator concentrations*: 0, 10, 20, 40, 80 and 160 ng/mL.
* Approximate value. Please refer to bottle labels for exact concentrations.
Volume: Calibrators A–F: 1 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months in unopened vials or as indicated on label.

5. Controls — Ready To Use

Contents: Two glass bottles containing 25(OH)D in human plasma with a non-mercury preservative. Refer to bottle labels for acceptable ranges. Controls are traceable to NIST SRM 972 and to concentrations determined by UV spectrophotometric analysis using a molar extinction coefficient of 18,300 M-1cm-1 at 264 nm.
Volume: Controls Low and High: 1 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months in unopened vials or as indicated on label.

6. Incubation Buffer — Ready To Use

Contents: One bottle containing a buffer with a blue dye and non-mercury preservative.
Volume: 20 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

7. Assay Buffer — Ready To Use

Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
Volume: 20 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

8. Wash Buffer Concentrate — Requires Preparation X10

Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
Volume: 50 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

9. TMB Substrate — Ready To Use

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in buffer.
Volume: 16 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

10. Stopping Solution — Ready To Use

Contents: One bottle containing 1M sulfuric acid.
Volume: 6 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

PREPARATION OF WORKING CONJUGATE SOLUTION

1. To a disposable glass test tube or glass bottle (**do not use plastic containers**), first add the required amount of assay buffer and then to this add a 1:100 volume of 25(OH)D-Biotin Conjugate Concentrate and a 1:100 volume of Streptavidin-HRP Conjugate Concentrate.

   For example, if the whole plate is to be used, to 16 mL of assay buffer in a glass test tube or bottle, add 0.16 mL of the 25(OH)D-Biotin Conjugate Concentrate and 0.16 mL of the Streptavidin-HRP Conjugate Concentrate.

Note: It is very important to add the assay buffer to the glass tube or bottle ***first*** *and then add the conjugate concentrates to the assay buffer. Failure to prepare the working conjugate solution in this order can lead to decreased OD values.*

2. Mix the working conjugate thoroughly and store in a dark place until it is used in step 7 of the assay procedure.

Note: It is essential to prepare the working conjugate solution ***before*** *the assay procedure is started. The working conjugate solution is stable for up to four hours, therefore it can be prepared between 0 and 120 minutes before starting the assay.*

ASSAY PROCEDURE

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. After all kit components have reached room temperature mix gently by inversion. Prepare working solutions of the conjugate (see preparation of working conjugate solution section) and wash buffer (see wash buffer concentrate under reagents provided section).
2. Remove the required number of microplate strips and assemble into a plate frame. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 25 µL of each calibrator, control and serum or plasma sample into correspondingly labelled wells in duplicate.
4. Pipette 150 µL of the incubation buffer into each well (the use of a multichannel pipette is recommended). Tap the microplate gently by hand for 10 seconds to mix the contents in the wells.
5. Incubate the microplate for 60 minutes at room temperature in a dark place (no shaking).
6. Wash the wells 3 times each time with 300 µL/well of diluted wash buffer. After washing tap the plate firmly against absorbent paper to remove any residual liquid (the use of an automatic strip washer is strongly recommended). The performance of this assay is markedly influenced by the correct execution of the washing procedure.
7. Pipette 150 µL of the working conjugate solution into each well (the use of a multichannel pipette is recommended). Tap the microplate gently by hand for 10 seconds to mix the contents in the wells.
8. Incubate the microplate for 30 minutes at room temperature in a dark place (no shaking).
9. Wash the wells 3 times using the same procedure as in step 6.
10. Pipette 150 µl of the TMB substrate into each well (the use of a multichannel pipette is recommended). Tap the microplate gently by hand for 10 seconds to mix the contents in the wells.
11. Incubate the microplate for 10–15 minutes at room temperature in a dark place (no shaking).
12. Add 50 µL of stopping solution to each well and mix thoroughly by gently tapping the plate by hand for 10 seconds to mix the contents in the wells.
13. Measure the absorbance at 450 nm in all wells with a microplate reader within 0–20 minutes after addition of the stopping solution.

CALCULATIONS

Using immunoassay software, choose either a 4-parameter or 5-parameter curve fitting method for calculating results.

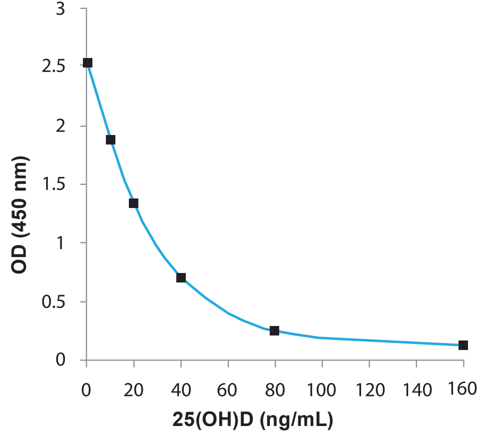
TYPICAL TABULATED DATA

Sample data only. **Do not** use to calculate results.

Calibrator	25(OH)D (ng/mL)	Mean OD (450 nm)	Binding (%)
A	0	2.556	100
B	10	2.207	86
C	20	1.906	75
D	40	1.475	58
E	80	0.711	28
F	160	0.253	10

TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



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 = $\mu_B + 1.645\sigma_B + 1.645\sigma S$,

where σB and σS are the standard deviation of the blank and low value sample and μ_B 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 D2	< 1.0
Vitamin D3	< 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.

CLINICAL DECISION VALUES

The Institute of Medicine at Washington DC (2) concluded that the levels of vitamin D can be associated with health conditions as in the following table:

25(OH)D, ng/mL	Health Status
< 12	Vitamin D deficiency leading to rickets in infants and children and osteomalacia in adults.
12–20	Generally considered inadequate for bone and overall health in healthy individuals.
≥ 20	Generally considered adequate for bone and overall health in healthy individuals.
> 60	Emerging evidence links potential adverse effects to such high levels.

Another source reports the following threshold levels:

25(OH)D, ng/mL	Health Status
< 10	Severe deficiency. Could be associated with osteomalacia or rickets.
10–19	Mild to moderate deficiency. May be associated with increased risk of osteoporosis or secondary hyperparathyroidism.
20–50	Optimum levels in the healthy population; patients with bone disease may benefit from higher levels within this range.
51–80	Increased risk of hypercalciuria. Sustained levels > 50 ng/mL 25OH-VitD along with prolonged calcium supplementation may lead to hypercalciuria and decreased renal function.
> 80	Toxicity possible. 80 ng/mL is the lowest reported level associated with toxicity in patients without primary hyperparathyroidism who have normal renal function. Most patients with toxicity have levels > 150 ng/mL. Patients with renal failure can have very high 25(OH)D levels without any signs of toxicity, as renal conversion to the active hormone 1,25(OH)D is impaired or absent.

These reference ranges represent clinical decision values that apply to males and females of all ages, rather than population-based reference values.

REFERENCES

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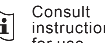
SYMBOLS

European Comformity

In vitro diagnostic device

 Consult instructions for use

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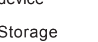
 Storage Temperature

Legal Manufacturer

Use by

Catalogue Number

Authorized representative

Lot number

Dilute 1: # Before use

25 HYDROXY VITAMIN D ELISA

fast & accurate

no sample preparation
short assay time
LC-MS/MS matching results
automatable

OVERVIEW

The worldwide aging and chronically ill population is increasing rapidly. It is forecasted that the Global Vitamin D Testing Market will grow at a Compound Annual Growth Rate (CAGR) of 32% over the period 2013–2018.

The market has been witnessing also an increase in the number of suppliers of Vitamin D tests. Strict regulatory requirements however, pose a challenge for both suppliers and distributors.

Diagnostics Biochem Canada Inc. (DBC) has more than 40 years of experience in the immunoassay market. With dozens of products that are registered with the FDA, Health Canada and that bear the CE mark, DBC exports to more than 70 countries and has solid reputation in new product development, approval and commercialization.

Answering the increasing demand for Vitamin D Tests, DBC has launched a new ELISA for Vitamin D analysis.

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 D_2 and vitamin D_3) or produced in the skin by sun exposure (vitamin D_3).

The body stores both vitamin D_2 and vitamin D_3 mainly in the form of 25-hydroxyvitamin D_2 or 25-hydroxyvitamin D_3 respectively, therefore the best approach to assess the physiological levels of vitamin D is to analyze the total concentration of both hydroxylated forms.

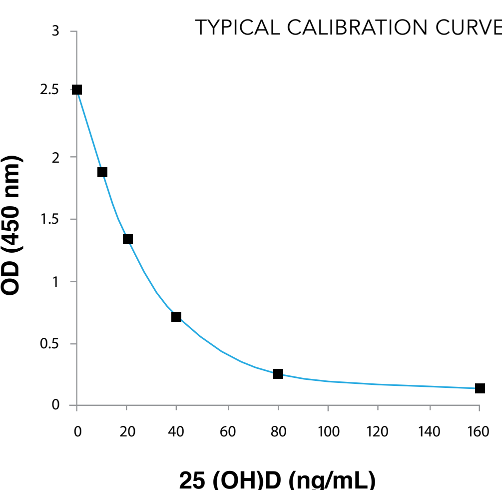
PRINCIPLE OF THE TEST

This kit measures the total concentration of both 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 (25(OH)D). The results are expressed in ng/mL.

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, unlabeled 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.

DBC 25(OH)D ELISA Method in 4 easy steps:

1. Add sample and incubation buffer into microplate wells. Incubate for 1 hour.
2. Wash microplate and add conjugates. Incubate for 30 minutes.
3. Wash microplate and add enzyme substrate. Incubate for 10–15 minutes.
4. Add stopping solution and read at 450 nm.



DBC 25-Hydroxyvitamin D ELISA

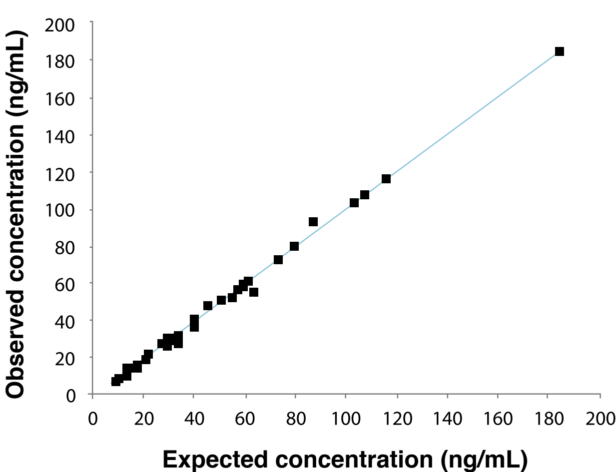
PERFORMANCE

Parameter	DBC	Competitor 1	Competitor 2	Competitor 3	Competitor 4
Total assay time, h	< 2	3	3	3	2
Sample pre-treatment	NONE	YES	YES	YES	YES
Reporter	HRP, colorimetric	^{125}I, radioactive	^{125}I, radioactive	HRP, colorimetric	HRP, colorimetric
Sample size, µL	25	50	50	20	25
Sample type	serum, plasma	serum, plasma	serum, plasma	serum, plasma	serum, plasma
Precision CV%					
Repeatability	2.4–5	—	—	—	—
Within Lab	8.1–10.3	—	—	—	—
Inter-assay	—	8.6–12.5	5.3–6.1	5	8.84
Intra-assay	—	8.2–11	7.3–8.2	7.8	12.7
Range, ng/mL	0–160	0–100	0–160	0–120	0–130

PERFORMANCE

LINEARITY

Combined results from ten samples diluted to three levels output the following correlation between observed and expected concentration values.



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 on the table below:

Sample	Mean (ng/mL)	Repeatability SD	Repeatability CV%	Within Lab SD	Within Lab CV%
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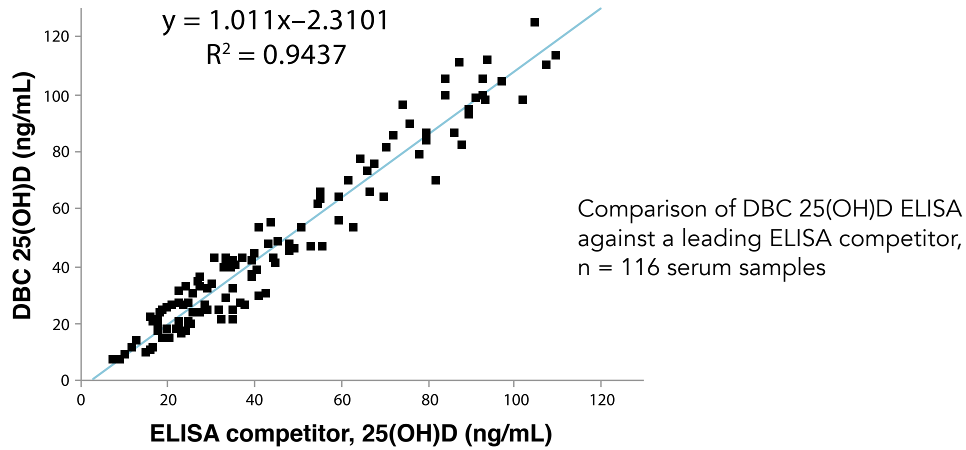
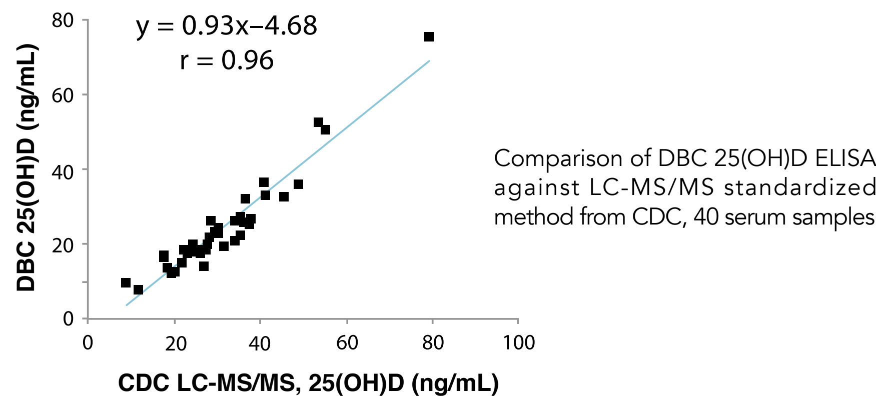
4	60.25	2.82	4.7	6.21	10.3

PERFORMANCE

COMPARATIVE STUDIES



Comparison of DBC 25(OH)D ELISA against a leading ELISA competitor, n = 116 serum samples



Comparison of DBC 25(OH)D ELISA against LC-MS/MS standardized method from CDC, 40 serum samples

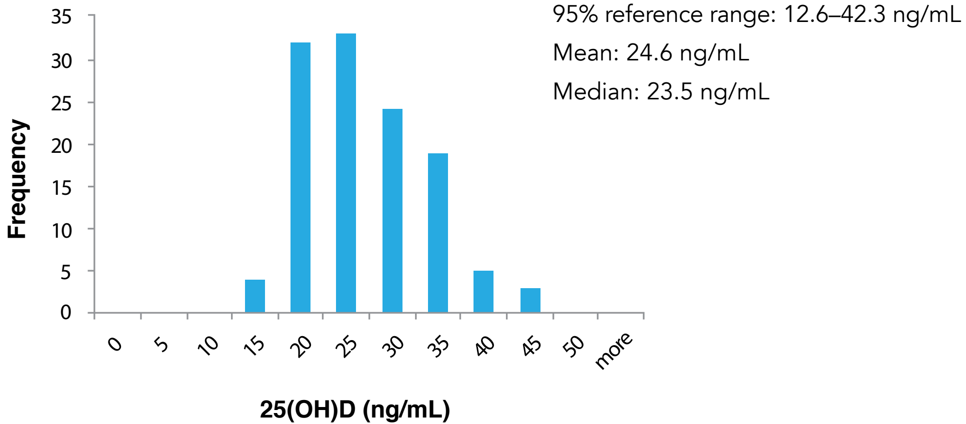
PERFORMANCE

Comparison of DBC 25(OH)D ELISA against LC-MS/MS results: International controls and pooled samples.

		LC-MS/MS ng/mL		DBC 25(OH)D, ng/mL
		AVE	Range	
Assigned value controls (Bio-Rad)	Level 1	7.42	4.54–10.3	8.30 ± 2.6
	Level 2	13.1	8.99–17.2	13.2 ± 4.0
	Level 3	33.6	24.1–43.1	26.9 ± 5.0
	Level 4	91.9	67.1–117	84.6 ± 15
Assigned value controls (Fujerebio)	Level 1	12.3	8.6–16	10.7 ± 2.3
	Level 2	34.1	23.9–44.4	33.4 ± 5.6
	Level 3	76.2	53.3–99	76.4 ± 9.9
Pooled serum samples	Men	35.3		39.4 ± 6.2
	Women Post-menopausal	30.0		31.6 ± 49
	Women Post-menopausal	30.9		31.4 ± 5.3

PERFORMANCE

Distribution of 25(OH)D values in 120 putatively normal individuals from Florida, mixed races, between 18 and 65 years old.



IMPORTANT

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) during the month of August from putatively healthy Black, White and Hispanic individuals of both genders, between 20 and 60 years old. Population reference ranges for 25(OH)D vary widely depending on age, ethnic background, geographic place 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.

RECOMMENDED LEVELS

The Institute of Medicine at Washington DC concluded that the levels of vitamin D can be associated with health conditions as in the following table:

25(OH)D, ng/mL	Health Status
< 12	Vitamin D deficiency leading to rickets in infants and children and osteomalacia in adults.
12–20	Generally considered inadequate for bone and overall health in healthy individuals.
≥ 20	Generally considered adequate for bone and overall health in healthy individuals.
> 60	Emerging evidence links potential adverse effects to such high levels.

Another source reports the following threshold levels:

25(OH)D, ng/mL	Health Status
< 10	Severe deficiency. Could be associated with osteomalacia or rickets.
10–19	Mild to moderate deficiency. May be associated with increased risk of osteoporosis or secondary hyperparathyroidism.
20–50	Optimum levels in the healthy population; patients with bone disease may benefit from higher levels within this range.
51–80	Increased risk of hypercalciuria. Sustained levels > 50 ng/mL 25OH-VitD along with prolonged calcium supplementation may lead to hypercalciuria and decreased renal function.
> 80	Toxicity possible. 80 ng/mL is the lowest reported level associated with toxicity in patients without primary hyperparathyroidism who have normal renal function. Most patients with toxicity have levels > 150 ng/mL. Patients with renal failure can have very high 25(OH)D levels without any signs of toxicity, as renal conversion to the active hormone 1,25(OH)D is impaired or absent.

LITERATURE

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2. Dong Y, et al. Low 25-hydroxyvitamin D levels in adolescents: Race, Season, Adiposity, Physical activity, and Fitness. *Pediatrics.* 2010; 125:1104–1111.
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12. Zadshir A, et al. The prevalence of hypovitaminosis D among US adults: Data from the NHANES III. Ethnicity and disease, 2005, vol 3. S5-97–S5-101.
13. Zerwekh JE. Blood Biomarkers of Vitamin D status. *Am J Clin Nutr.* 2008; 87:1087S–1091S.

25
HYDROXY
VITAMIN
D

ELISA

at a glance

DBC 25(OH)D ELISA REF CAN-VD-510

Sample: 25 µL of human serum or plasma

No sample preparation

Total assay time: less than 2 hours

Number of calibrators: 6

Number of supplied internal controls: 2

Sensitivity: 5.5 ng/mL

Cross-reactivity: 100% 25(OH)D_2
100% 25(OH)D_3
< 1.0% Vitamin D_2
< 1.0% Vitamin D_3

Results match LC-MS/MS

Automatable

FOR MORE INFORMATION, PLEASE CONTACT DBC AT:
DIAGNOSTICS BIOCHEM CANADA
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TEL: 519-681-8731 • FAX: 519-681-8734 • dbc@dbc-labs.com • www.dbc-labs.com

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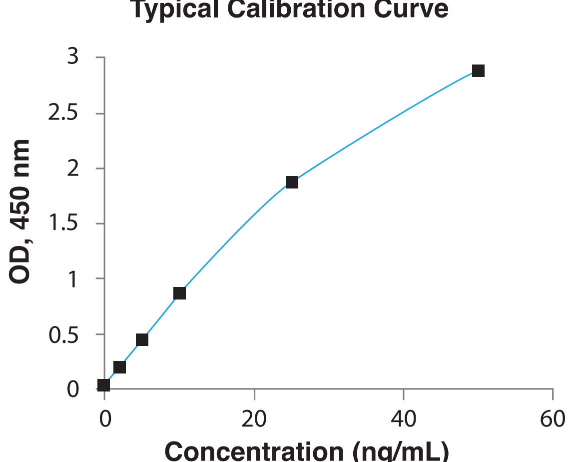
*Manufacturing Innovative IVD
for the World Since 1973*

DBC is proud to introduce the *most sensitive* Adiponectin ELISA kit in the market.

There is a growing diagnostic market for metabolism, inflammatory and coronary artery diseases. Adiponectin is a marker for : Type 2 diabetes, Metabolic syndrome, Energy metabolism , body weight regulation, Coronary Artery Disease and Atherosclerosis.



ASSAY PRINCIPLE

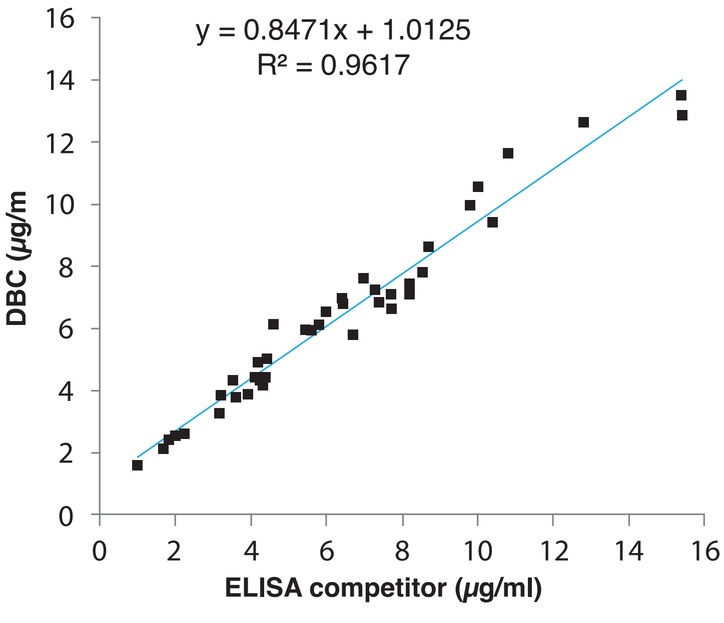
The DBC Adiponectin ELISA is a Sandwich immunoassay that uses a monoclonal anti-Adiponectin antibody for capture and a biotinylated monoclonal anti-Adiponectin antibody for detection.

ASSAY PROCEDURE

- Dilute samples.
- 50 µl calibrators/samples.
- 100 µl of Detection antibody.
- 1 h room temperature /shaking.
- Wash 3X.
- 100 µl Streptavidin-HRP conjugate.
- 30 min room temperature /shaking.
- Wash 3X.
- 150 µl TMB
- 15 min room temperature /shaking.
- 50 µl of stop solution.
- Read in a plate reader at 450 nm.

COMPARISON OF DBC ADIPONECTIN ELISA AGAINST ELISA COMPETITORS

Parameter	DBC	Competitor 1	Competitor 2
Total assay time, h	1h:45 min	2h:30 min	1h:45 min
Ready to use reagents	Yes	No	No
Range	0 - 50	0 - 100	0 - 100
Sample size, µl	50	100	100
Sample type	Serum, plasma	Serum, plasma	Serum, plasma
Precision, %			
Intra-assay	5.5–7.5	3.3–4.4	2.35–4.66
Inter-assay	6.6–8.4	5.8–6.2	5.8–6.72
Inter-lot	2.1–3.1	No data available	No data available
Sensitivity			
LoD, ng/mL	0.055	0.47	0.6
LoQ, ng/mL	0.15	No data available	No data available



ADVANTAGES OF THE DBC ADIPONECTIN ELISA KIT:

Ultrasensitive

Short Incubation Time

Ready Reagents

current contact information:

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41 Byron Avenue
Dorchester, Ontario, Canada N0L 1G0
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after October 31, 2016:

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DBC Aldosterone ELISA

new ALDOSTERONE ELISA kit

DBC's NEW ALDOSTERONE ELISA KIT INCLUDES A READY-TO-USE CONJUGATE AND BLOCKING AGENTS

REF CAN-ALD-500

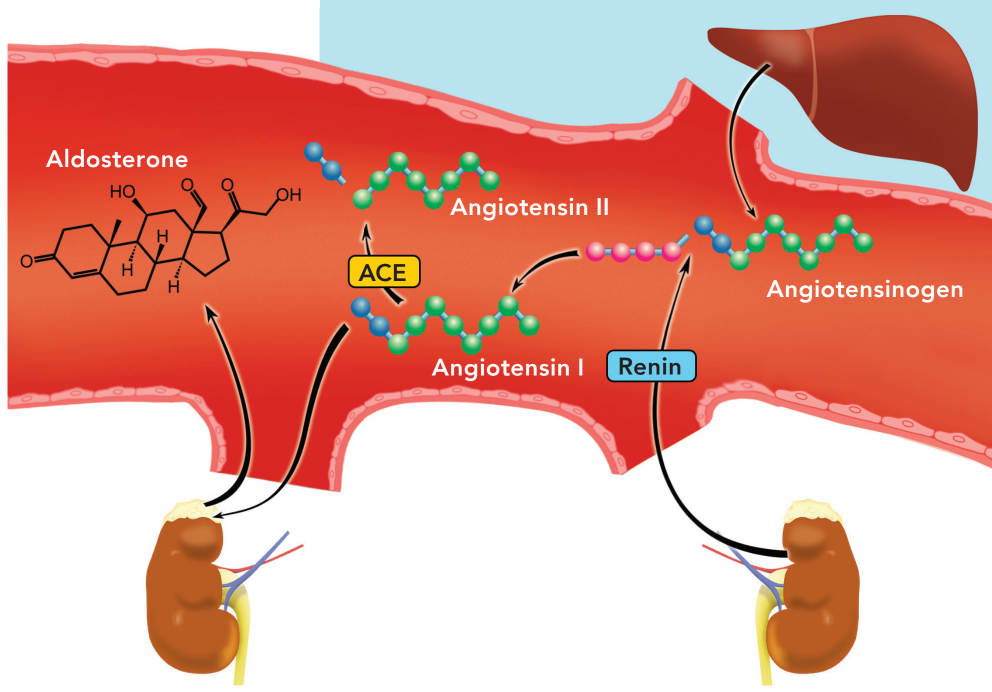
DBC Aldosterone ELISA

A significant proportion of hypertensive individuals suffer from resistant hyper-tension due to factors such as non-compliance, poor nutritional practice (high salt, alcohol, licorice) or secondary hypertension (20% of all resistant hypertension cases). Diagnosing the cause of hypertension is of paramount importance to select the correct therapy.

Heart failure, stroke, renal conditions and dementia are some of the common consequences of uncontrolled hyper-tension; the occurrence of these conditions however, can be reduced when the underlying source of resistant hypertension is identified and a follow up therapy is applied.

THE RENIN-ANGIOTENSIN-ALDOSTERONE AXIS plays a key role in resistant hypertension.

During normal homeostasis, renin is released under conditions of dehydration or low blood pressure (see figure below). **Renin enzymatic activity** then promotes the cleavage of Angiotensinogen and generation of Angiotensin I, which in turn is transformed into Angiotensin II and activates **aldosterone** release (which causes salt and water retention, and excretion of potassium, magnesium, and other ions).



When this metabolism is altered, three patterns of aldosterone and renin activity levels can be produced:

1. Primary hyperaldosteronism causes salt and water retention, feeding back to suppress renin activity.
2. Renal or renovascular causes of hypertension lead to elevated renin activity with secondary hyperaldosteronism.
3. Impairment of the renal tubular epithelial sodium channel (such as Liddle's syndrome) causes salt and water retention and suppresses both renin activity and aldosterone.

ALDOSTERONE MEASUREMENT is therefore an outstanding tool to determine the physiological causes of resistant hypertension, permitting the physician to choose the most appropriate therapy.[1-3]

The following algorithm was used in a study of resistant hypertension in three hypertension clinics in Africa, in a study funded by Grand Challenges Canada. This approach increased systolic blood pressure control from 25% in usual care to 75% in individualized care based on aldosterone/renin profiling.[4]

	Primary Aldosteronism	Liddle's Variants, Adducin Polymorphisms	Renal or Renovascular
Aldosterone	High	Low	High
Renin	Low	Low	High

Primary treatment	Aldosterone antagonists: Spironolactone Eplerenone (Amiloride for men where eplerenone is not available) (rarely surgery)	Amiloride Angiotensin receptor blockers Aliskiren (rarely revascularization)

The precise and accurate measurement of aldosterone by enzyme immunoassay can be an important tool for the diagnosis of the underlying cause of hypertension, leading to appropriate therapy.

This approach not only improves blood pressure control, thus reducing the risk of stroke, heart failure and renal failure, but also reduces adverse effects of medication and may reduce the cost of medication by identifying specific therapy.

DBC Aldosterone ELISA

DBC has launched a new Aldosterone kit (CAN-ALD-500) that includes a ready-to-use conjugate and blocking agents that prevent interferences by sample endogenous substances.

ASSAY PRINCIPLE

The new DBC Aldosterone ELISA kit (CAN-ALD-500) is a competitive immunoassay that uses innovative chemistry and a specific anti-aldosterone antibody that binds quantitatively to all isomers of aldosterone.

PROCEDURE

50 µL calibrators/samples

100 µL of Ready-to-Use Conjugate

1 h room temperature/shaking

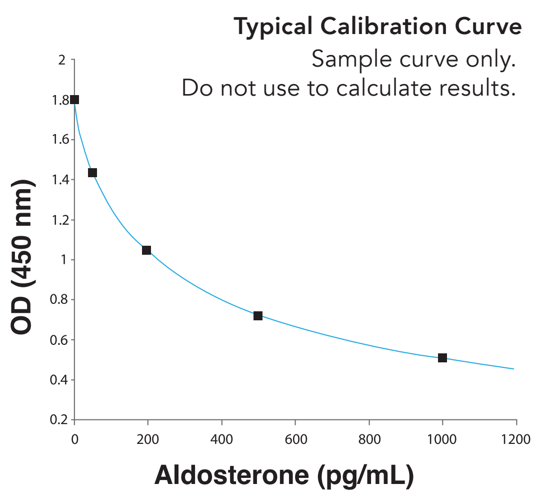
Wash 3x

150 µL TMB

20 min room temperature/shaking

50 µL of stop solution

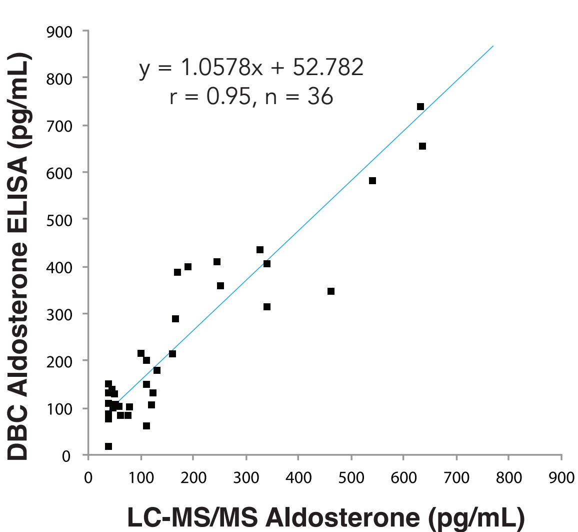
Read in a plate reader at 450 nm



PERFORMANCE

Parameter	DBC	Competitor 1	Competitor 2	LC-MS/MS
Total assay time	1h 20min	1h 30min	Overnight + 1h	N/A
Ready to use reagents	Yes	Yes	No	N/A
Dynamic Range, pg/mL	10–1000	5.7–1000	4.7–250	40–1000
Sample size, µL	50	50	100	600
Sample pre-treatment				
Serum, Plasma	No	No	Yes	Yes
Urine	No	Yes	Yes	Yes
Sensitivity, pg/mL	9.1	5.7	4.7	40
Precision, CV%				
Intra-assay	5.5–9.4	3.8–9.7	4.5–6.6	
Inter-assay	7.6–12.8	8.6–11.5	10.8–16.3	

Comparative analysis of serum samples results between the new DBC Aldosterone kit (CAN-ALD-500) and LC-MS/MS performed at Mayo Clinic.



PERFORMANCE

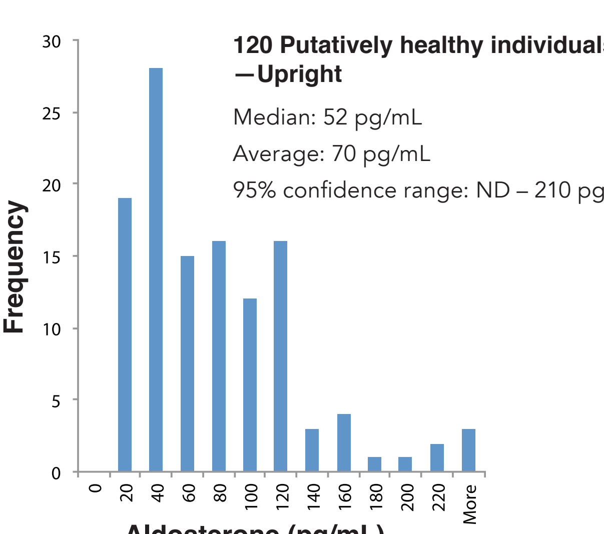
Evaluation of International Controls

The RfB international controls were assayed with the DBC ELISA kit (CAN-ALD-500). The results for the kit are the mean ± SD of 4 independent experiments (pg/mL).

The results match both LC-MS/MS results from Mayo Clinic and fall within the range of results established from all methods.

RfB lot	HM 2/15 A	HM 2/15 B
LC-MS/MS (Mayo Clinic)	570	110
LC-MS/MS (RfB)	***Target***	
	634	122
	Range (16P–84P)	
	419–764	111–172
All Methods (RfB)	***Target***	
	529	110
	Range (16P–84P)	
	468–658	90–144
CAN-ALD-500 (DBC)	636 ± 70	105 ± 16

REFERENCE RANGE



120 Putatively healthy individuals —Upright

Median: 52 pg/mL

Average: 70 pg/mL

95% confidence range: ND – 210 pg/mL

Aldosterone concentration range in the population depends on the ethnic and social composition and nutritional factors.

Each laboratory must determine its own reference ranges!

LITERATURE

1. Spence JD. Lessons from Africa: the importance of measuring plasma renin and aldosterone in resistant hypertension. *Can J Cardiol*. 2012; 28(3):254–7.

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Catalogue number:	CAN-ALD-500
Number of test wells:	96
Sensitivity:	9.1 pg/mL
Sample Volume:	50 µL
Total assay time:	80 mins.
Validated against:	LC-MS/MS

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for the World Since 1973

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PLASMA RENIN ACTIVITY (PRA)

Advantages of DBC's PRA Kits Over Existing Radioimmunoassays:

- Safer to use, transport and dispose of
- Much longer shelf life
- Generic microplate-based assays
- Smaller sample volume
- Shorter incubation time
- Competitive price

BACKGROUND

Measurement of PRA is important for the clinical evaluation of hypertensive patients. PRA, in contrast to the determination of Renin concentration, is a more accurate indicator of primary hyperaldosteronism (PHA):

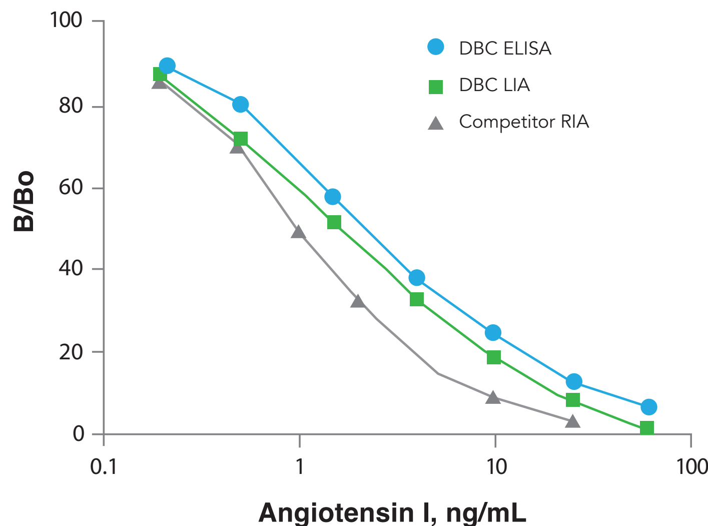
1. PRA is the expression of the rate of Ang-I formation through the enzymatic action of Renin on its substrate, angiotensinogen.
2. The sensitivity of the PRA assay can be enhanced by increasing the incubation time during the generation step.
3. When an inhibitor is bound to the Renin active site PRA is inhibited which is reflected using the DBC PRA kits. The presence of the inhibitor is not reflected in current direct Renin immunoassays which only measure the concentration of Renin and provide no information on the actual activity of Renin.

METHOD

1. Equally divide each sample into two aliquots. Incubate one aliquot at 0°C and the other at 37°C with DBC's buffer to allow a degradation-free generation of Ang-I by Renin (1.5-3 h).
2. Run the ELISA or LIA immunoassay (2 h).
3. Collect results and calculate PRA.

CALIBRATION CURVES

Fig. 1. Typical calibration curves of the PRA enzyme immunoassays as they compare to a radioimmunoassay.



COMPARATIVE STUDIES

Fig. 2. Comparison between DBC's PRA ELISA and LIA.

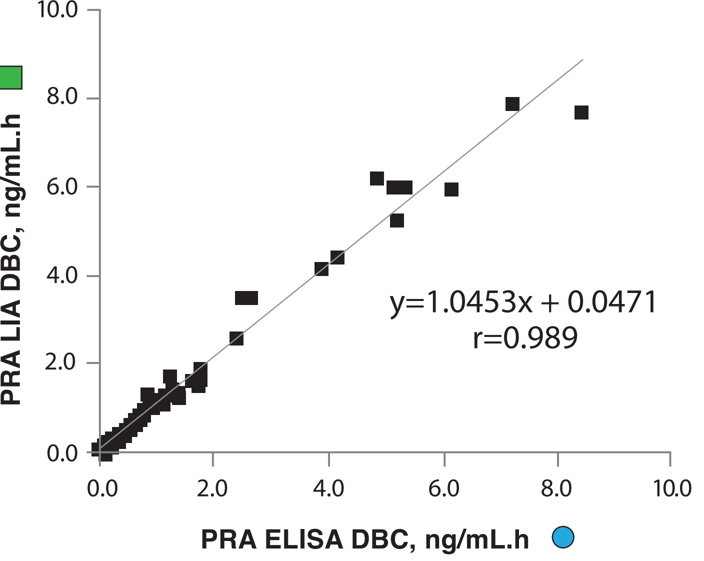


Table 1. Validation of ELISA and LIA PRA assays against Lyphochek® Hypertension Markers control (Bio-Rad), ng/mL.h.

Level	ELISA	LIA	DiaSorin ^{125}I GammaCoat	DiaSorin RENCTK
1	2.00	1.85	2.40	1.35
2	4.92	4.13	5.30	2.70
3	13.4	15.1	13.5	6.70

PRA vs. DIRECT RENIN

Fig. 3. PRA plotted against Renin concentration determined by an ELISA direct assay. Out of 37 samples, 11 samples were under the detection limit for the direct Renin assay. In those 11 samples PRA was between 0.03 and 0.3 ng/mL.h. In the rest of the samples the correlation was as shown in this figure.

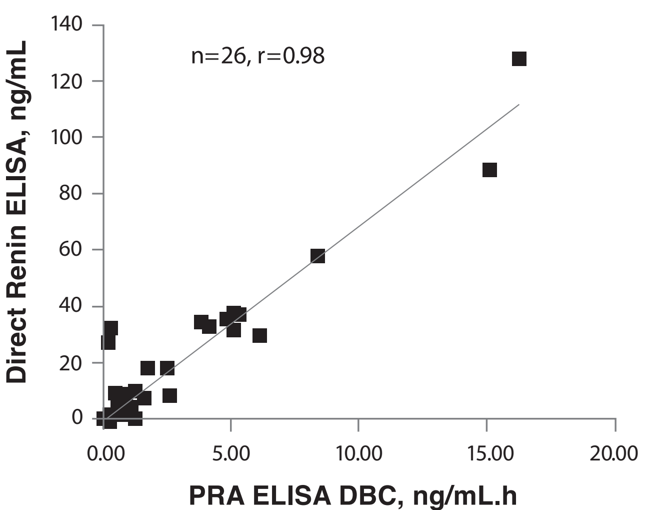
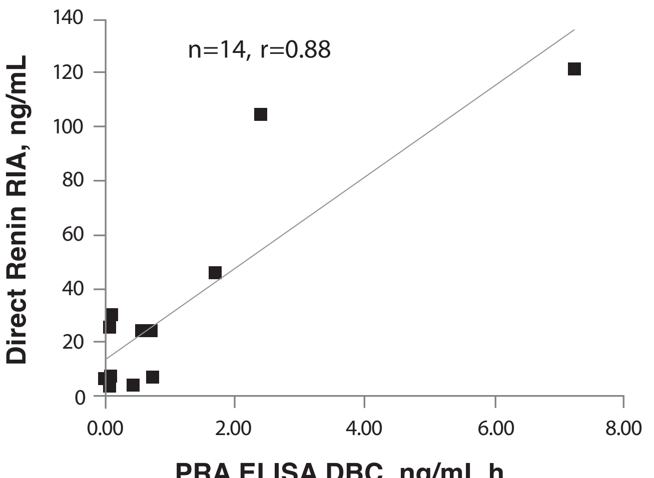


Fig. 4. PRA plotted against Renin concentration determined by a RIA direct assay. Results for PRA LIA were similar as in figs. 3 & 4 (not shown).



We are an **ISO 9001:2008** and **ISO 13485:2003** Registered Company.

PERFORMANCE

Table 2. Output characteristics of DBC's ELISA and LIA assays and how they compare to competitors' RIA kits.

Parameter	DBC ELISA	DBC LIA	RIA Competitors		
			1	2	3
Platform	2x96-well Microplates	2x96-well Microplates	Plastic Test Tubes	Plastic Test Tubes	Plastic Test Tubes
Sensitivity, ng/mL Ang-I	0.038	0.08	0.033	0.2	0.18
Accuracy (recovery % ± SD)	98 ± 8	97 ± 4	100 ± 7	97 ± 3	Not reported
Dilution Linearity (recovery % ± SD)	101 ± 9	98 ± 7	98 ± 6	101 ± 4	Not reported
Intra-assay precision, CV%	6–8	6–9	3–6	5–10	5–10
Inter-assay precision, CV%	4–7	7–10	4–6	7–12	6–8
Range, ng/mL, Ang-I	0–60	0–60	0–25	0–50	0–50
Incubation time after generation step, h	2	2	18–20	3–24	3

PLASMA RENIN ACTIVITY (PRA)

ORDERING INFORMATION

Kit	Format	REF
PRA ELISA	2x96-well Microplates	CAN-RA-4600
PRA LIA	2x96-well Microplates	CAN-RA-7070

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Endocrinology ELISA Kits	Catalogue Number	Total Assay Time	Sensitivity	Format
5α-Androstane 3α, 17-diol Glucuronide (3α-Diol G)	CAN-DG-460	45 minutes	0.1 ng/mL	96-Well Breakapart Strips
17-Hydroxyprogesterone (17-OHP)	CAN-P-320	70 minutes	0.11 ng/mL	96-Well Breakapart Strips
Aldosterone	CAN-ALD-450	75 minutes	10 pg/mL	96-Well Breakapart Strips
Aldosterone NEW	CAN-ALD-500	80 minutes	9.1 pg/mL	96-Well Breakapart Strips
Androstenedione	CAN-AD-208	75 minutes	0.04 ng/mL	96-Well Breakapart Strips
Cortisol	CAN-C-270	60 minutes	0.4 µg/dL	96-Well Breakapart Strips
Cortisol Saliva	CAN-C-290	60 minutes	1 ng/mL	96-Well Breakapart Strips
Dehydroepiandrosterone (DHEA)	CAN-DH-490	70 minutes	0.1 ng/mL	96-Well Breakapart Strips
Dehydroepiandrosterone Sulfate (DHEAS)	CAN-DHS-480	60 minutes	0.005 µg/mL	96-Well Breakapart Strips
Dihydrotestosterone (DHT)	CAN-DHT-280	70 minutes	6 pg/mL	96-Well Breakapart Strips
Estradiol	CAN-E-430	70 minutes	10 pg/mL	96-Well Breakapart Strips
Estrone	CAN-E-420	70 minutes	10 pg/mL	96-Well Breakapart Strips
Growth Hormone (hGH)	CAN-GH-4070	70 minutes	0.2 ng/mL	96-Well Breakapart Strips
Pregnenolone	CAN-PRE-4500	70 minutes	0.05 ng/mL	96-Well Breakapart Strips
Progesterone	CAN-P-305	70 minutes	0.1 ng/mL	96-Well Breakapart Strips
Progesterone Saliva	CAN-P-310	70 minutes	20 pg/mL	96-Well Breakapart Strips
Testosterone Saliva	CAN-TE-300	100 minutes	1 pg/mL	96-Well Breakapart Strips
Testosterone, Free	CAN-FTE-260	70 minutes	0.17 pg/mL	96-Well Breakapart Strips
Testosterone, Total	CAN-TE-250	70 minutes	0.022 ng/mL	96-Well Breakapart Strips

Cardiovascular ELISA Kits	Catalogue Number	Total Assay Time	Sensitivity	Format
Adiponectin NEW	CAN-APN-5000	105 minutes	0.06 ng/mL	96-Well Breakapart Strips
Aldosterone	CAN-ALD-450	75 minutes	10 pg/mL	96-Well Breakapart Strips
Aldosterone	CAN-ALD-500	80 minutes	9.1 pg/mL	96-Well Breakapart Strips
C-Reactive Protein, High Sensitivity (hs-CRP)	CAN-CRP-4360	55 minutes	10 ng/mL	96-Well Breakapart Strips
Cortisol	CAN-C-270	60 minutes	0.4 µg/dL	96-Well Breakapart Strips
Cortisol Saliva	CAN-C-290	60 minutes	1 ng/mL	96-Well Breakapart Strips
Ferritin	CAN-F-4280	40 minutes	7.5 ng/mL	96-Well Breakapart Strips
Plasma Renin Activity (PRA)	CAN-RA-4600	3.5 hours	0.038 ng/mL of Angiotensin-I	96-Well Breakapart Strips

Bone and Mineral Metabolism ELISA Kits	Catalogue Number	Total Assay Time	Sensitivity	Format
25-Hydroxyvitamin D	CAN-VD-510	100 minutes	3.4 ng/mL	96-Well Breakapart Strips
Cortisol	CAN-C-270	60 minutes	0.4 µg/dL	96-Well Breakapart Strips
Cortisol Saliva	CAN-C-290	60 minutes	1 ng/mL	96-Well Breakapart Strips
Ferritin	CAN-F-4280	40 minutes	7.5 ng/mL	96-Well Breakapart Strips

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Diabetes/Metabolism ELISA Kits	Catalogue Number	Total Assay Time	Sensitivity	Format
5α-Androstane 3α, 17-diol Glucuronide (3α-Diol G)	CAN-DG-460	45 minutes	0.1 ng/mL	96-Well Breakapart Strips
Adiponectin NEW	CAN-APN-5000	105 minutes	0.06 ng/mL	96-Well Breakapart Strips
C-Peptide	CAN-CP-4380	105 minutes	0.2 ng/mL	96-Well Breakapart Strips
Growth Hormone (hGH)	CAN-GH-4070	70 minutes	0.2 ng/mL	96-Well Breakapart Strips
Insulin-Like Growth Factor Binding Protein-1 (IGFBP-1)	CAN-IGF-4140	70 minutes	0.5 µg/L	96-Well Breakapart Strips
Leptin	CAN-L-4260	100 minutes	0.42 ng/mL	96-Well Breakapart Strips

Fertility ELISA Kits	Catalogue Number	Total Assay Time	Sensitivity	Format
Chorionic Gonadotropin (hCG)	CAN-HCG-4120	70 minutes	0.7 IU/L	96-Well Breakapart Strips
Follicle Stimulating Hormone (FSH)	CAN-FSH-4060	75 minutes	1 IU/L	96-Well Breakapart Strips
Luteinizing Hormone (LH)	CAN-LH-4040	75 minutes	0.2 IU/L	96-Well Breakapart Strips
Prolactin	CAN-PRL-4100	70 minutes	10 µIU/mL	96-Well Breakapart Strips
Sex Hormone Binding Globulin (SHBG)	CAN-SHBG-4010	55 minutes	0.1 nmol/L	96-Well Breakapart Strips

Oncology ELISA Kits	Catalogue Number	Total Assay Time	Sensitivity	Format
Beta-2-Microglobulin (β2-M)	CAN-B-4300	75 minutes	0.1 mg/L	96-Well Breakapart Strips
Prostate-Specific Antigen, Free (fPSA)	CAN-FPSA-4400	70 minutes	0.05 ng/mL	96-Well Breakapart Strips
Prostate-Specific Antigen, Total (PSA)	CAN-TPSA-4300	100 minutes	0.1 ng/mL	96-Well Breakapart Strips

Thyroid ELISA Kits	Catalogue Number	Total Assay Time	Sensitivity	Format
Thyroid Stimulating Hormone (TSH)	CAN-TSH-4080	70 minutes	0.1 µIU/mL	96-Well Breakapart Strips
Thyroxine, Free (fT4)	CAN-FT4-4340	70 minutes	1 pg/mL	96-Well Breakapart Strips
Thyroxine, Total (T4)	CAN-T4-4240	45 minutes	0.6 µg/dL	96-Well Breakapart Strips
Triiodothyronine, Free (fT3)	CAN-FT3-4230	70 minutes	0.3 pg/mL	96-Well Breakapart Strips
Triiodothyronine, Reverse (rT3) NEW	CAN-RT3-100	105 minutes	0.009 ng/mL	96-Well Breakapart Strips
Triiodothyronine, Total (T3)	CAN-T3-4220	70 minutes	0.16 ng/mL	96-Well Breakapart Strips

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Endocrinology LIA Kits	Catalogue Number	Total Assay Time	Sensitivity	Format
5α-Androstane 3α 17β-diol Glucuronide (3α Diol G)	CAN-DG-6010	45 minutes	0.1 ng/mL	96-Well Breakapart Strips
Aldosterone	CAN-ALD-6000	30 minutes	7.2 pg/mL	96-Well Breakapart Strips
Cortisol	CAN-C-7010	75 minutes	0.15 µg/dL	96-Well Breakapart Strips
Dehydroepiandrosterone Sulfate (DHEAS)	CAN-DHS-7020	40 minutes	0.02 µg/mL	96-Well Breakapart Strips
Estriol Saliva	CAN-E-7030	80 minutes	0.03 ng/mL	96-Well Breakapart Strips
Estrone	CAN-E-6060	70 minutes	8.8 pg/mL	96-Well Breakapart Strips
Estrone Saliva	CAN-E-7000	95 minutes	1 pg/mL	96-Well Breakapart Strips
Progesterone	CAN-P-6050	70 minutes	0.1 ng/mL	96-Well Breakapart Strips
Testosterone, Free	CAN-FTE-6080	70 minutes	0.17 pg/mL	96-Well Breakapart Strips
Testosterone Saliva	CAN-TE-6090	95 minutes	1 pg/mL	96-Well Breakapart Strips

Thyroid LIA Kits	Catalogue Number	Total Assay Time	Sensitivity	Format
Thyroid Stimulating Hormone (TSH)	CAN-TSH-6030	65 minutes	0.002 µIU/mL	96-Well Breakapart Strips
Thyroxine, Free (fT4)	CAN-FT4-6040	75 minutes	1 pg/mL	96-Well Breakapart Strips

Cardiovascular LIA Kits	Catalogue Number	Total Assay Time	Sensitivity	Format
Aldosterone	CAN-ALD-6000	30 minutes	7.2 pg/mL	96-Well Breakapart Strips
Cortisol	CAN-C-7010	75 minutes	0.15 µg/dL	96-Well Breakapart Strips
Cortisol Saliva	CAN-C-290	60 minutes	1 ng/mL	96-Well Breakapart Strips
Ferritin	CAN-F-4280	40 minutes	7.5 ng/mL	96-Well Breakapart Strips
Plasma Renin Activity (PRA)	CAN-RA-7070	3.5 hours	0.08 ng/mL of Angiotensin-I	96-Well Breakapart Strips

Oncology LIA Kits	Catalogue Number	Total Assay Time	Sensitivity	Format
Prostate-Specific Antigen, Total (PSA)	CAN-TPSA-6020	90 minutes	0.002 ng/mL	96-Well Breakapart Strips

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DBC'S panel of thyroid hormone immunoassays is now more comprehensive with the new Reverse T3 ELISA kit.

Reverse T3 (rT3), or reverse triiodothyronine (3,3′,5′-Triiodo-L-thyronine), differs from triiodothyronine (T3; 3,3′,5-Triiodo-L-thyronine) in the positions of the iodine atoms in the molecule.

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 (see diagram) is inhibited in a number of non-thyroidal conditions such as fasting, malnutrition, diabetes mellitus, stress, severe trauma or infection, and others. This scenario is known as "Sick euthyroid" syndrome or "Low T3" syndrome.

T_3 T_4 $3,3'T_2$ rT_3

In non-thyroidal illness the inhibition of 5′-monodeiodinase produces decreased conversion of T4 into T3 and rT3 into 3,3′-T2.

5′-monodeiodinase inhibition reduces production of T3 and metabolism of rT3

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.

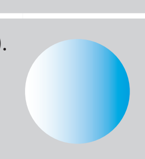
Currently clinical diagnostic rT3 testing is commercially available only through expensive technologies—LC-MS/MS or RIA—which are out of the reach for many laboratories in the World.

Recognizing this, DBC developed a simple and accurate ELISA test.

PRINCIPLE OF THE TEST

The DBC rT3 ELISA is a competitive enzyme immunoassay.

Four Easy Steps

Step		Solution Colour in Well
1	Load calibrators, controls and samples; add biotin conjugate. Incubate 1 hour at 37°C.	
2	Wash and load Streptavidin-HRP conjugate. Incubate 30 minutes at 37°C.	
3	Wash and load substrate (TMB). Incubate 15 minutes at 37°C.	
4	Add stopping solution. Read in a microplate reader at 450 nm.	

PERFORMANCE

SPECIFICITY (CROSS-REACTIVITY)

The quantitative evaluation of the cross-reactivity was performed using the method of Abraham.

Compound	% Cross Reactivity
rT3	100
T3	< 0.001
T4	0.005
3,5-T2	0.004

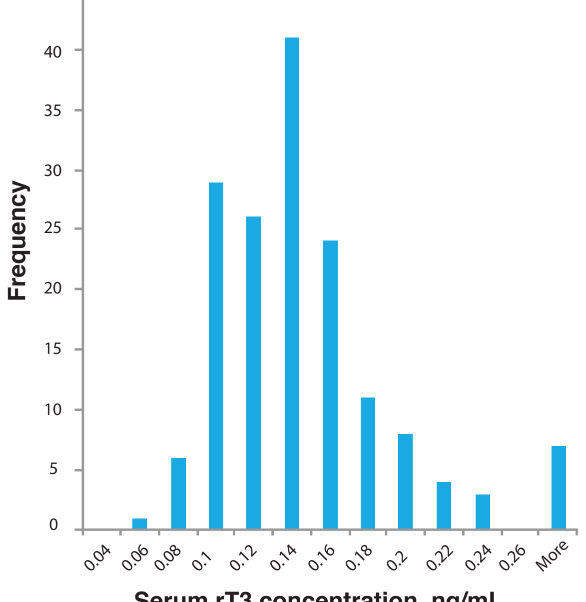
INTERFERENCES

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 5.5 mg/mL and Biotin up to 40 µg/mL.

REFERENCE RANGES

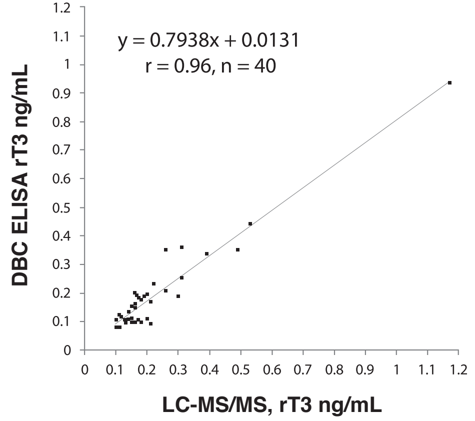
Reference ranges calculated using a non-parametric method, using commercial samples from putatively normal individuals 20 years or older. Data in ng/mL.

Group	n	Median	95% Confidence Range	Total Range	Published Range at Mayo Clinic
Serum adults	160	0.13	0.08–0.31	0.06–0.76	0.1–0.24
Plasma adults	120	0.14	0.08–0.29	0.049–0.65	



COMPARATIVE STUDIES

The new device was compared to a leading competing technology: LC-MS/MS. The comparison was performed with 40 commercial human serum samples and shows that the devices produce commutable results:



DBC REVERSE T3 ELISA AT A GLANCE:

- Catalogue Number: **CAN-RT3-100**
- Sample: 25 µL of human serum or plasma
- No sample preparation
- Total assay time: less than 2 hours
- Number of calibrators: 6
- Number of supplied internal controls: 2
- Sensitivity: 0.005 ng/mL
- Cross-reactivity: T3 < 0.001%; T4 0.005%; 3,5-T2 0.004%
- Correlates with LC-MS/MS results
- Automatable

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DBC Reverse T3 ELISA

DON'T LET YOUR THYROID TAKE OVER

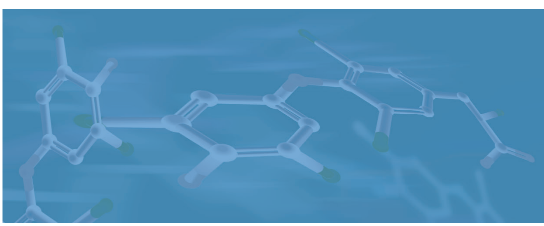
Test Your Hormones

DBC's panel of thyroid hormone immunoassays is now more comprehensive with the **new Reverse T3 ELISA kit**

Reverse T3 is the Best Marker of Tissue Thyroid Hormone Levels

INTRODUCTION

For years thyroid hormone testing has been concentrated on TSH and T4. Serum TSH and T4 levels however, do not correlate well with intracellular thyroid hormone levels.[1, 2] This recent finding counters the long-held misconception that the rate and extent of uptake of thyroid hormones into the cells occurs by simple diffusion (propelled by the concentration of the free hormones in serum).



Instead, the transport of T3 and T4 into the cells across the cellular membranes is active and requires cellular energy, which affects the T4 transporter more than the T3 transporter. Therefore, TSH and T4 serum concentrations are poor indicators of tissue thyroid levels and should not be used to diagnose if the individual is euthyroid (normal thyroid hormone concentration) at the tissue level. Moreover, high T4 levels have been negatively correlated with the conversion of T4 to T3 (the active thyroid hormone). In spite of overwhelming support for this mechanism, the misconceived "diffusion hypothesis" continues to be held by endocrinologists and primary physicians, sometimes leading to inadequate prescriptions of T4 preparations such as Synthroid and Levoxyl for restoring tissue euthyroidism.

Furthermore, the thyroid hormone status is disturbed in non-thyroid illnesses such as sepsis, surgery, myocardial infarction, starvation, and others where the prevalence of abnormalities in thyroid function tests is between 40 and 70% with consequent difficulties in interpretation of the results that leads to patient mismanagement.

What then, is the best marker of tissue thyroid hormone levels?

The answer lies in a structural isomer of T3:
Reverse T3

Reverse T3 (rT3), or reverse triiodothyronine (3,3′,5′-Triiodo-L-thyronine), differs from triiodothyronine (T3; 3,3′,5-Triiodo-L-thyronine) in the positions of the iodine atoms in the molecule. Additionally, the majority of circulatory rT3 is synthesized by peripheral deiodination of thyroxine (T4).

Both T3 and rT3 bind to thyroid hormone receptors; but, in difference to T3, rT3 has not been found yet to stimulate receptor metabolic activity. It does however, block 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 (see diagram on p.3) 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 (except renal failure and AIDS). 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.[1-11]

The concentration of rT3 could be high in patients on the following medications: amiodarone, dexamethasone, propylthiouracil, ipodate, propranolol, and the anesthetic halothane; on the other side, 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 excessive clearance of rT3.

In non-thyroidal illness the inhibition of 5′-monodeiodinase produces decreased conversion of T4 into T3 and rT3 into 3,3′-T2.

5′-monodeiodinase inhibition reduces production of T3 and metabolism of rT3

T_3

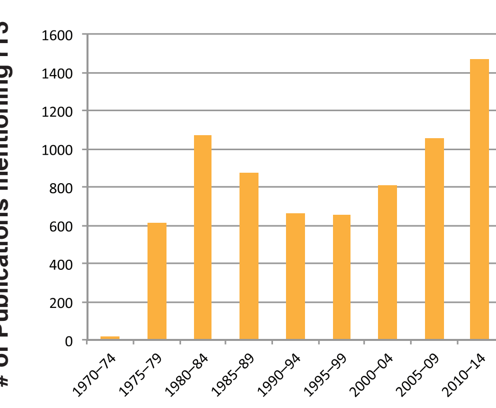
T_4

$3,3'T_2$

rT_3

Most authors agree that measurements of rT3 are considered useful in differentiating nonthyroidal illness (high rT3) from secondary hypothyroidism (low TSH and possible low rT3) with one report in 1995 claiming poor diagnostic specificity.[12] Lately, however, the diagnostic importance of rT3 tests has been recognized and the interest on this hormone has increased dramatically.

The number of publications on rT3 more than doubled in the last decade and rocketed in the last few years.



Currently clinical diagnostic rT3 testing is commercially available only through expensive technologies—LC-MS/MS or RIA—which are out of the reach for many laboratories in the World.

Recognizing this, DBC developed a simple and accurate ELISA test for the determination of rT3 in serum, and plasma.

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 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 followed by a second washing and addition of TMB, the HRP substrate. The enzymatic reaction is terminated by addition of stopping solution upon which the color intensity—measured with a microplate reader—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.

FOUR EASY STEPS

			Solution Colour in Well
1	Load calibrators, controls and samples; add biotin conjugate	Incubate 1 hour at 37°C	
2	Wash and load Streptavidin-HRP conjugate	Incubate 30 minutes at 37°C	
3	Wash and load substrate (TMB)	Incubate 15 minutes at 37°C	
4	Add stopping solution	Read in a microplate reader at 450 nm	

PERFORMANCE

SPECIFICITY (CROSS-REACTIVITY)

The quantitative evaluation of the cross-reactivity was performed using the method of Abraham.[13]

Compound	%Cross Reactivity
rT3	100
T3	< 0.001
T4	0.005
3,5-T2	0.004

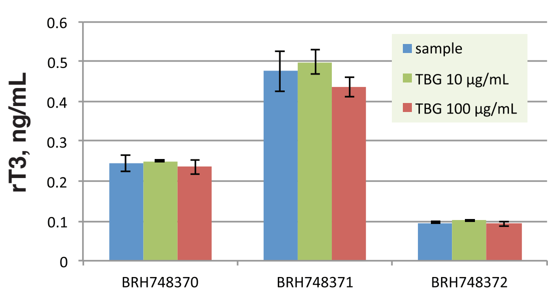
INTERFERENCES

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 5.5 mg/mL and Biotin up to 40 µg/mL.

Effect of TBG concentration in samples on rT3 concentration results measured with DBC's rT3 ELISA kit.

To demonstrate that DBC's rT3 ELISA blocks interference of TBG binding to rT3, TBG was added to human serum samples at concentrations of 10 and 100 µg/mL. The sample results are not affected significantly with the presence of additional TBG in the sample. TBG's reference concentration range in serum is 11–27 µg/mL.

Error bars represent the SD of the mean of two independent experiments each with two replicated measurements.



PERFORMANCE

INTRA-ASSAY PRECISION

Four serum samples were assayed 24 times each on the same calibrator curve.

Sample	Mean (ng/mL)	SD (ng/mL)	%CV
1	0.089	0.0024	2.7
2	0.250	0.020	8.0
3	0.455	0.019	4.1
4	1.018	0.140	13.4

INTER-ASSAY PRECISION

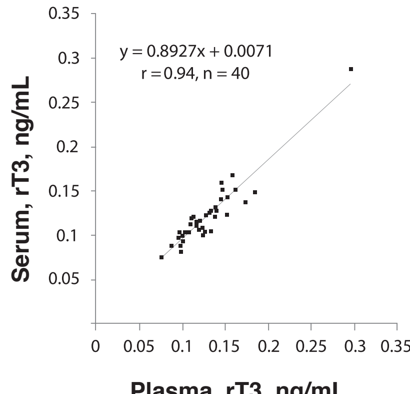
Four serum samples were assayed in 20 different tests in the period of 20 days. The results are tabulated below:

Sample	Mean (ng/mL)	SD (ng/mL)	%CV
1	0.127	0.016	12.7
2	0.304	0.038	12.4
3	0.469	0.057	12.1
4	0.847	0.083	9.8

PERFORMANCE

SAMPLE MATRIX COMPARISON

The option to use plasma samples was investigated using 40 matched sample pairs of each serum and plasma (di-sodium EDTA) from the same individuals. The regression study demonstrates that there is strong equivalence between matrices, therefore the type of matrix, serum or plasma, does not affect the analytical result of the test.



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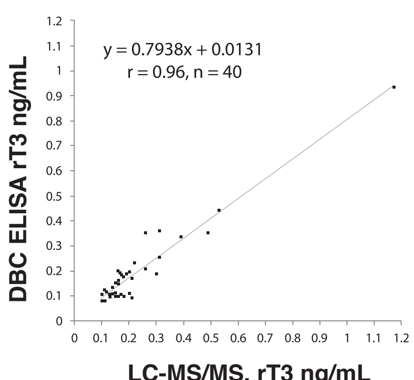

PERFORMANCE

Reference ranges calculated using a non-parametric method, using commercial samples from putatively normal individuals 20 years or older. Data in ng/mL.

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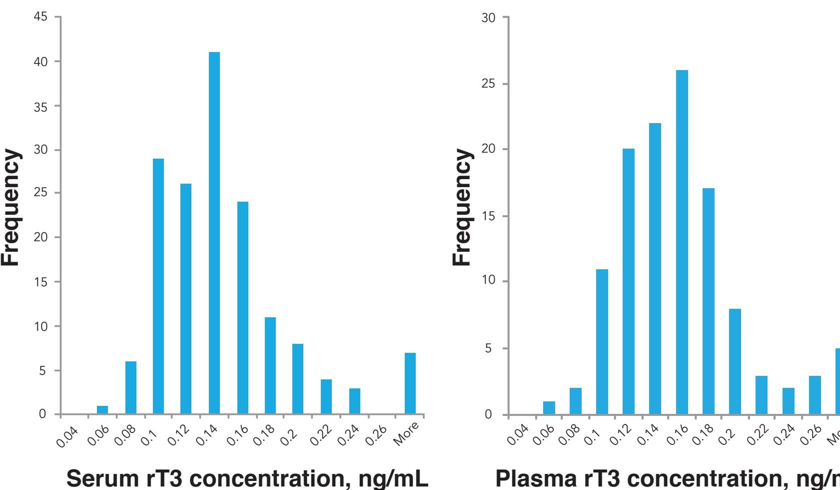

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- DBC rT3 ELISA Catalogue Number: CAN-RT3-100
- Sample: 25 µL of human serum or plasma
- No sample preparation
- Total assay time: less than 2 hours
- Number of calibrators: 6
- Number of supplied internal controls: 2
- Sensitivity: 0.005 ng/mL
- Cross-reactivity:

T3	< 0.001%
T4	0.005%
3,5-T2	0.004%

- Correlates with LC-MS/MS results
- Automatable

For more information, please contact DBC at:

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PLANNING the Test

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TECHNICAL SUPPORT

techsupport@dbc-labs.com

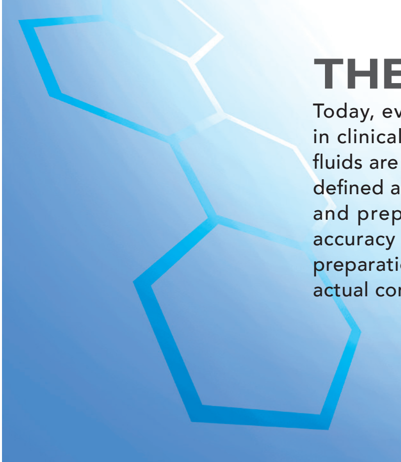
Introduction

Enzyme-linked immunosorbent assay (ELISA), also known as enzyme immunoassay (EIA), is one of the most clever and economical analytical technologies ever implemented in clinical diagnostics. Forty years after its invention, ELISA is used worldwide and produces millions of diagnostic results daily.

Diagnostics Biochem Canada Inc. (DBC) embraced the technology since its inception in medical practice and continues to supply dozens of ELISA product lines to more than one hundred countries. For forty years DBC has mastered the technology to deliver products that—when a correct operation and laboratory practices are followed—rival in accuracy more sophisticated technologies.

The goal of this brochure is to share with ELISA users important tips that will help to obtain outstanding results and avoid costly mistakes.

Specimen Preparation



THE BASICS

Today, every biological fluid is used as a specimen in clinical diagnostics, but the most common bodily fluids are serum, plasma, saliva and urine. Each fluid, defined also as matrix, demands elaborate collection and preparation procedures which determine the accuracy of the analytical method. Improper sample preparation can lead to results very distant from the actual concentration of the analyte.

GENERAL GUIDELINES

1. Read test Instructions for Use (IFU) and ensure the test is intended for the specimen.
2. Collect and store specimens following the IFU. Some tests can be inaccurate if the specimen has been frozen/thawed too many times.
3. Investigate that specimens do not contain preservatives or chemicals that are incompatible with the test.
4. Label the specimens accurately.
5. Inspect the specimens for extraneous materials or visible deterioration.
6. Ensure the specimen is uniform and separate enough sample for the test. Store the rest according to the IFU.
7. Plan enough time for the sample to reach room temperature before starting the test.
8. Follow additional preparation steps according to the IFU.

INSIDER TIPS

SERUM AND PLASMA

Read IFU to ensure that you are following sample preparation instructions specific for each kit.

The tolerance of ELISAs to lipids, hemoglobin and bilirubin changes between kits. Find limits in the IFU and ensure that your sample is not excessively white, red or yellow.

SALIVA

Process the saliva samples according to the IFU.

The concentration of most analytes in saliva is much smaller than in blood. Do not use saliva samples that might be contaminated with blood or food.

URINE

Process only the volume that will be used immediately and store the rest (since excess of diluted or processed samples should be discarded). Do not store diluted or processed urine samples.

Test Planning

THE BASICS

Careful planning and organization of the test is often underestimated, leading to costly mistakes or, even worse, to inadvertent inaccuracies. It is essential to fully study the test instructions for use (IFU), prior to the running the test and confirm that trained personnel, time and material resources are in place to run the test.

GENERAL GUIDELINES

1. Read the IFU carefully and ensure that you have everything needed (including equipment, materials and reagents not provided with the kit and enough sample volume).
2. Inspect the components of the kit and ensure the kit is not expired and that bottles contain enough reagent volume for the test.
3. Defrost the specimens; check that they are properly labelled and separate only enough sample for the test; store the rest of the specimen.
4. Ensure that all reagents, samples and microplate reach room temperature.
5. Do not open the microplate bag until it has reached room temperature; water vapour could condense on cold wells and shorten the shelf life of those strips that are not used immediately.

INSIDER TIPS

PLAN WELL AHEAD

Choose a reliable transportation agency and ensure that they respect the symbols in the package, such as storage temperature and positional arrows.

Even though DBC's kits have an expiry date of approximately one year when stored at 2–8°C, it is recommended to use them within the first six months; shelf life can be reduced by unaccounted offenses, such as too high or too low temperatures, wrong orientation (kit transported upside down) and excessive shaking during transportation and customs storage.

Check that your pipettes are calibrated and clean. One common cause of errors is cross-contamination of samples with dirty pipettes.

Prime your microplate washer with the kit's wash buffer and ensure that the machine is working properly.

Again, read and understand well the IFU before the test. If you have any questions contact DBC.

Assay Execution

THE BASICS

Running the assay is very easy. It is critical nonetheless, to possess good pipetting skills and training in handling biological specimens. Ensure that equipment has received proper maintenance.

GENERAL GUIDELINES

1. Use gloves, lab coat and necessary protective equipment; biological samples should be treated —always—as capable of transmitting disease.
2. Run duplicates of each calibrator, control and sample, as recommended in the IFU, to produce accurate results.
3. To dispense an accurate volume, prime pipette tips with each calibrator, control and sample. Use a new tip for each sample; the same tip can be used for each replicate of the same calibrator, control and sample.
4. Use the same pipetting technique (angle, speed) throughout the plate.
5. Regulate pipetting speed to avoid bubbles or air penetration into the tip.
6. Prepare conjugate in glass or HDPE disposable tubes or bottles since other materials can adsorb the conjugate or contaminate the solution.
7. To dilute the conjugate concentrate, add the buffer first to a tube or bottle and then, add the conjugate concentrate to this solution. Do not place the conjugate concentrate in the bottle before adding the buffer (especially in kits that include more than one conjugate).
8. Do not immerse the tip deep into the sample; specimens are viscous and substantial volume could be carried over to the well from outside of the tip.

INSIDER TIPS

MANUAL

Serum, plasma and saliva are viscous fluids. Therefore, take extra care in regulating the pipetting speed to load the exact volume.

Use multichannel pipettes to dispense buffers, conjugates, substrates and stop solution and regulate pipetting speed to avoid splashing liquid and splashing out tip contents.

Do not allow microplate wells to dry after washes, load next solution immediately.

Clean bottom surface of wells before reading.

AUTOMATED

Regulate dispensing speed to avoid splashing of liquid and check coefficients of variation (CVs) between replicates are within accepted limits.

Ensure that temperature conditions are those specified in the IFU. In those tests that require 37°C incubation, heating should only start once the microplate is loaded with calibrators, controls samples and conjugate solution.

Maintain and clean your equipment according to manufacturer's manual.

Results Analysis

THE BASICS

Accurate diagnoses can only be attained with careful and critical analysis of the results together with other tests and the rest of patient's history. No single test alone should be used as a decision point for therapeutic intervention.

GENERAL GUIDELINES

1. Ensure that the test results meet the specifications of the QC certificate and whenever possible use additional external controls.
2. Use the algorithm recommended in the IFU to build the calibration curve; **do not use point-to-point analysis for immunoassays—ever**.
3. Take in to consideration the patient's history of medications and exposure to animals or animal products, particularly if a result looks suspiciously far from the expected value.
4. If a result is out of the dynamic range of the test, label it as <(Limit of Detection) or >(highest calibrator value); samples could be diluted if the result is too high (but for most tests this is unnecessary).

INSIDER TIPS

Reference intervals, also known as reference ranges should be determined for each cohort group with population where the test is performed. For example, it is known that mean aldosterone levels change not only with age and race, but also with the geographic location; therefore, use the intervals published in the IFU only as a rough approximation. Consult CLSI guideline C28-A3c and the literature listed at the end for more information.

Do not confuse reference intervals with normal ranges since reference intervals might include individuals with sub-optimal levels and therefore "non-normal".

If a sample result is on the borderline of a reference range, do not declare it positive or negative automatically; check the confidence level of the reference range and the histogram of value distribution to determine the probability of the sample being positive or negative.

Literature

ELISA – GENERAL

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Jones G, Barker A. Reference Intervals. *Clin Biochem Rev.* 2008; 29:S93–S97.

Benefits of DBC Kits

THE DBC ADVANTAGE

DBC immunoassay technology is oriented to provide the highest possible accuracy and reliability through the development of robust products. To achieve these targets DBC delivers an ELISA platform that comprises both sandwich assays for peptides and proteins, and competitive assays for small molecule analyses such as steroid and thyroid hormone tests. Both methods use high quality Corning stripwell microplates that are coated with specific antibodies produced in house. Our proprietary coating technology ensures that immobilized antibodies are active much longer than the expiry date shown in the labels, making the microplates robust enough to resist unexpected insults during transportation, customs and storage.

DBC kits cover a wide range of clinical diagnostic applications including areas such as bone metabolism, cancer and hypertension; the main focus however, has been Endocrinology and specifically the quantitative analysis of steroids and thyroid hormones in human fluids. The immunological analysis of those molecules poses significant challenges because they are present at very low concentrations and bind strongly to one or several proteins that compete with the antibody for binding. DBC immunoassays overcome those difficulties by using advanced chemistry solutions, while providing kits that are easy to use and accurate.

DBC's ELISA technology is developed by a team of scientists with more than one hundred years of combined experience; we are always open to listening our customers' feedback and continue to develop assays in new frontiers of medical diagnostics.

Medical Applications of DBC Kits

ELISA

Product	Catalogue No.	Endocrinology	Bone & Mineral Metabolism	Oncology	Thyroid	Diabetes	Cardiovascular & Renal	Autoimmune	Fertility
5α-Androstane-3α, 17β diol Glucuronide (3α-Diol G)	CAN-DG-460	X				X			
17-Hydroxyprogesterone	CAN-P-320	X							X
25-Hydroxyvitamin D	CAN-VD-510	X	X	X	X	X	X	X	X
Adiponectin	CAN-APN-5000	X				X	X		
Aldosterone	CAN-ALD-450	X	X				X		
Aldosterone	CAN-ALD-500	X	X				X		
Androstenedione	CAN-AD-208	X							
B2-Microglobulin	CAN-B-4300	X						X	
C-Peptide	CAN-C-P-4380	X				X	X		
C-Reactive Protein (hs-CRP)	CAN-CRP-4360	X		X				X	
Chorionic Gonadotropin (hCG)	CAN-HCG-4120	X		X					
Cortisol	CAN-C-270	X	X	X	X		X		X
Cortisol Saliva	CAN-C-290	X	X	X	X		X		
Dehydroepiandrosterone (DHEA)	CAN-DH-490	X		X		X	X	X	
Dehydroepiandrosterone Sulfate (DHEAS)	CAN-DHS-480	X							
Dihydrotestosterone (DHT)	CAN-DHT-280	X		X					X
Estradiol	CAN-E-430	X							X
Estrone	CAN-E-420	X							X
Ferritin	CAN-F-4280	X	X	X	X	X	X	X	X
Follicle Stimulating Hormone (hFSH)	CAN-FSH-4060	X							X
Growth Hormone (hGH)	CAN-GH-4070	X		X	X	X			X
Insulin-Like Growth Factor Binding Protein-1 (IGFBP-1)	CAN-IGF-4140	X		X		X			
Leptin	CAN-L-4260	X	X	X		X	X		
Luteinizing Hormone (hLH)	CAN-LH-4040	X							X
Plasma Renin Activity (PRA)	CAN-RA-4600	X					X		
Pregnenolone	CAN-PRE-4500	X		X					
Progesterone	CAN-P-305	X							X
Progesterone Saliva	CAN-P-310	X							X
Prolactin	CAN-PRL-4100	X		X	X				
Prostate Specific Antigen, Free (fPSA)	CAN-FPSA-4400	X		X					
Prostate Specific Antigen, Total (PSA)	CAN-TPSA-4300	X		X					

Medical Applications of DBC Kits

ELISA

Product	Catalogue No.	Endocrinology	Bone & Mineral Metabolism	Oncology	Thyroid	Diabetes	Cardiovascular & Renal	Autoimmune	Fertility
Sex Hormone Binding Globulin (SHBG)	CAN-SHBG-4010	X		X					
Testosterone, Free	CAN-FTE-260	X		X			X		X
Testosterone,Total	CAN-TE-250	X		X			X		X
Testosterone Saliva	CAN-TE-300	X		X			X		X
Thyroid Stimulating Hormone (TSH)	CAN-TSH-4080	X	X		X				
Thyroxine, Free (fT4)	CAN-FT4-4340	X	X		X				
Thyroxine, Total (T4)	CAN-T4-4240	X	X		X				
Triiodothyronine, Free (fT3)	CAN-FT3-4230	X	X		X				X
Triiodothyronine, Reverse	CAN-RT3-100	X	X		X				
Triiodothyronine, Total (T3)	CAN-T3-4220	X	X		X				

LIA

Product	Catalogue No.	Endocrinology	Bone & Mineral Metabolism	Oncology	Thyroid	Diabetes	Cardiovascular & Renal	Autoimmune	Fertility
5α-Androstane-3α, 17β diol Glucuronide (3α-Diol G)	CAN-DG-6010	X			X				
Aldosterone	CAN-ALD-6000	X	X				X		
Cortisol	CAN-C-7010	X	X	X	X		X		X
Dehydroepiandrosterone Sulfate (DHEAS)	CAN-DHS-7020	X							
Estrone	CAN-E-6060	X							X
Estrone Saliva	CAN-E-7000	X							X
Plasma Renin Activity (PRA)	CAN-RA-7070	X					X		
Progesterone	CAN-P-6050	X							X
Prostatic Specific Antigen, Total (PSA)	CAN-TPSA-6020	X		X					
Testosterone, Free	CAN-FTE-6080	X		X					X
Testosterone Saliva	CAN-TE-6090	X		X					X
Thyroid Stimulating Hormone (TSH)	CAN-TSH-6030	X			X				X
Thyroxine, Free (fT4)	CAN-FT4-6040	X			X				

DBC

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