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.

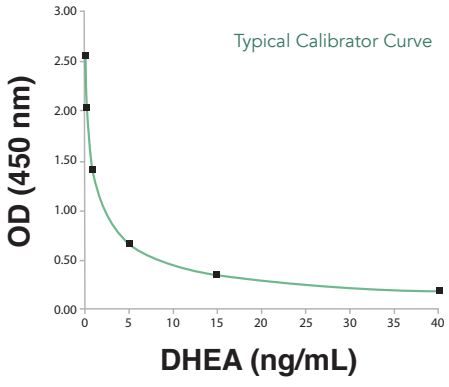
The physiological functions of DHEA are still the subject of investigation. DHEA reportedly plays a role in immune function, lipid metabolism, cholesterol, the nervous system, ageing and protection against viral infection. Serum DHEA levels are relatively high in the fetus and neonates, low during childhood, 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 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.



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%. ND = Not detectable.

Steroid	% Cross-Reactivity
DHEA	100
11-Deoxycortisol	0.17
17-Hydroxypregnenolone	2.09
17α-Hydroxyprogesterone	0.19
Aldosterone	0.11
Androstenedione	0.40
Androsterone	0.14
Cholesterol	ND
Cortisol	0.07
Corticosterone	0.12
DHEAS	< 0.02
DHT	0.37
Epiandrosterone	2.49
Estradiol	0.49
Estrone	0.22
Pregnenolone	9.48
Progesterone	0.23
Testosterone	0.31

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**

DIAGNOSTICS BIOCHEM CANADA
384 NEPTUNE CRESCENT • LONDON, ONTARIO, CANADA • N6M 1A1
TEL: 519-681-8731 • FAX: 519-681-8734 • dbc@dbc-labs.com • www.dbc-labs.com