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Labor Diagnostika Nord GmbH & Co.KG

Instructions for use

DHEA Saliva ELISA

REF SA E-7000

96

+2 +8 °C

IVD

CE

Introduction

Intended Use

An enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of Dehydroepiandrosterone (DHEA) in saliva.

Summary and Explanation

Dehydroepiandrosterone (DHEA; androstenolone; 3β-hydroxy-5-androsten-17-one) is a C_{19} steroid produced in the adrenal cortex and, to a lesser extent, gonads. DHEA serves as a precursor in testosterone and estrogen synthesis. Due to the presence of a 17-oxo (rather than hydroxyl) 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 relatively low affinity for sex-hormone binding globulin. These factors may enhance the physiologic biopotency of DHEA.

The physiologic role of DHEA has not been conclusively defined. A variety of in vitro effects, including antiproliferative effects in different cell lines and effects on enzyme-mediated cell metabolism, have been reported. In vivo studies suggest that DHEA may affect cholesterol and lipid metabolism, insulin sensitivity and secretion and immune function. Abnormal DHEA levels have been reported in schizophrenia and obesity. Therapeutic administration of DHEA has been proposed for several conditions, including obesity and cardiovascular disease.

PRINCIPLE of the test

The **Salivary DHEA ELISA** Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.
The microtiter wells are coated with a polyclonal antibody directed towards an antigenic site of the DHEA molecule.
Endogenous DHEA of a patient sample competes with a DHEA-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.
The amount of bound peroxidase conjugate is inversely proportional to the concentration of DHEA in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of DHEA in the patient sample.

WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.

15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from the manufacture.

REAGENTS

Reagents provided

96 **SA E-7031 Microtiterwells**

12x8 (break apart) strips, 96 wells; Wells coated with a anti-DHEA antibody (polyclonal).

Standards

	Cat. no.	Standard	Concentration	Volume/Vial
STANDARD A	**SA E-7001**	Standard 0	0 pg/mL	1 ml
STANDARD B	**SA E-7002**	Standard 1	10 pg/mL	1 ml
STANDARD C	**SA E-7003**	Standard 2	60 pg/mL	1 ml
STANDARD D	**SA E-7004**	Standard 3	120 pg/mL	1 ml
STANDARD E	**SA E-7005**	Standard 4	480 pg/mL	1 ml
STANDARD F	**SA E-7006**	Standard 5	1440 pg/mL	1 ml

Contain a non-mercury preservative.

CONTROL 1 **+** CONTROL 2 **SA E-7051 + SA E-7052 Control Low and High**

2 vials, 1.0 mL each, ready to use; For control values and ranges please refer to vial label or QC-Datasheet.
Contain a non-mercury preservative.

CONJUGATE **SA E-7040 Enzyme Conjugate**

1 vial, 14 mL, ready to use; DHEA conjugated to horseradish peroxidase;
Contains a non-mercury preservative.

SUBSTRATE **FR E-0055 Substrate Solution**

1 vial, 14 mL, ready to use; Tetramethylbenzidine (TMB).

STOP-SOLN **FR E-0080 Stop Solution**

1 vial, 14 mL, ready to use; contains 0.5M H_2SO_4.

Avoid contact with the stop solution. It may cause skin irritations and burns.

WASH- CONC 40x **FR E-0030 Wash Solution**

1 vial, 30 mL (40X concentrated); see „Preparation of Reagents".

Note: Additional *Standard 0* for sample dilution is available upon request.

Materials required but not provided

- A microtiter plate calibrated reader (450±10 nm), (e.g. the Instruments Microtiter Plate Reader).
- Calibrated variable precision micropipettes (50 µL, 100 µL, 200 µL).
- Absorbent paper.
- Distilled or deionized water
- Timer (60 min. range).
- Semi logarithmic graph paper or software for data reduction

Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for 8 weeks if stored as described above.

Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Wash Solution

Add deionized water to the 40X concentrated *Wash Solution*.
Dilute 30 mL of concentrated *Wash Solution* with 1170 mL deionized water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 2 weeks at room temperature.

Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheet.

Damaged Test Kits

In case of any severe damage to the test kit or components, the manufacture has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

SPECIMEN Collection and Preparation

Eating, drinking, chewing gums or brushing teeth should be avoided for 30 minutes before sampling. Otherwise, it is recommended to rinse mouth thoroughly with cold water 5 minutes prior to sampling.

Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).

If there is visible blood contamination the patient specimen, it should be discarded, rinse the sampling device with water, wait for 10 minutes and take a new sample.

Note: Samples containing sodium azide should not be used in the assay.

Specimen Collection

Saliva samples should be collected only using special saliva sampling devices (vial and straw)
SALI-TUBES 100 (SLV-4158).

Do not use any cotton swab for sampling, such as Salivettes; in most cases this will result in artificially high values.

Due to the cyclic secretion pattern of steroid hormones it is important to care for a proper timing of the sampling. In order to avoid arbitrary results we recommend that 5 samples always be taken within a period of 2 – 3 hours (*multiple sampling*) preferably before a meal.
As food might contain significant amounts of steroid hormones samples preferably should be taken while fasting. If fasting should be a problem the collection period should be timed just before lunch or before dinner.

Specimen Storage and Preparation

Specimens should be capped and may be stored for up to one week at 2 – 8 °C prior to assaying.
Specimens held for a longer time should be frozen –20 °C prior to assay. Even repeated thawing and freezing is no problem.

Each sample has to be frozen, thawed, and centrifuged at least once in order to separate the mucins by centrifugation.
Upon arrival of the samples in the lab the samples have to stay in the deep freeze at least overnight. Next morning the frozen samples are warmed up to room temperature and mixed carefully.
Then the samples have to be centrifuged for 5 to 10 minutes (at 3000 - 2000 x g).
Now the clear colorless supernatant is easy to pipette.

If a set of multiple samples is to be tested, the lab (after at least one freezing, thawing, and centrifugation cycle) has to mix the 5 single samples in a separate sampling device and perform the testing from this mixture.

Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and re-assayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) Dilution 1:10: 10 µl saliva + 90 µl *Standard 0* (mix thoroughly)
b) Dilution 1:100: 10 µl of dilution a) + 90 µl *Standard 0* (mix thoroughly).

Assay procedure

General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

Test Procedure

Each run must include a standard curve.

1.	Secure the desired number of Microtiter wells in the frame holder.
2.	Dispense **50 µL** of each ***Standard,*** **control and samples** with new disposable tips into appropriate wells.
3.	Dispense **100 µL *Enzyme Conjugate*** into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4.	Incubate for **60 minutes** at room temperature.
5.	Briskly shake out the contents of the wells. Rinse the wells 5 times with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets. **Important note:** The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6.	Add **100 µL** of ***Substrate Solution*** to each well.
7.	Incubate for **20 minutes** at room temperature.
8.	Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
9.	Determine the absorbance (OD) of each well at **450 ± 10 nm** with a microtiter plate reader. It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred calculation method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 1440 pg/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard		Optical Units (450 nm)
Standard 0	0 pg/mL	1.85
Standard 1	10 pg/mL	1.56
Standard 2	60 pg/mL	1.28
Standard 3	120 pg/mL	1.14
Standard 4	480 pg/mL	0.77
Standard 5	1440 pg/mL	0.49

Expected normal values

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with 198 apparently normal healthy men aged 21 - 75 years and 200 apparently normal healthy women aged 21 - > 60 years, using the Salivary DHEA ELISA the following values are observed:

	Men			Women		
Age Group [Years]	**5% - 95% Percentile [pg/mL]**	**Median [pg/mL]**	**n**	**5% - 95% Percentile [pg/mL]**	**Median [pg/mL]**	**n**
21 - 30	103.9 – 578.3	296.3	40	82.5 – 496.1	206.3	40
31 - 40	116.2 – 471.8	202.7	38	75.4 – 328.5	175.4	40
41 - 50	109.1 – 475.3	187.8	40	54.4 – 412.0	121.5	40
51 - 60	86.1 – 488.0	134.0	40	43.8 – 236.1	95.4	40
60 - 75	41.8 – 184.3	98.1	40	33.8 – 229.7	89.4	40

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

Quality Control

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit.
The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or the manufacture directly.

Performance Characteristics

Assay Dynamic Range

The range of the assay is between 2.2 – 1440 pg/mL.

Specificity of Antibodies (Cross Reactivity)

The following materials have been evaluated for cross reactivity.

Steroid	% Cross reactivity
DHEA	100
17-OH Pregnenolone	0.072
Androsterone	0.056
Desoxycorticosterone	0.052
Progesterone	0.23
Pregnenolone	0.013
11-Desoxycortisol	0.012
Corticosterone	0.004
DHEA-S	0.0037
Testosterone	0.002
5-α Dihydrotestosterone	0.0007
Cortisol	0.0007
17α-Hydroxyprogesterone	0.0004
Aldosterone	0.0003
Estradiol 17ß	n.d.
Estradiol 17α	n.d.
Estrone	n.d.
Estriol	n.d.

* n.d. = non detectable

Sensitivity

The analytical sensitivity of the Salivary DHEA ELISA was calculated by subtracting 2 standard deviations from the mean of twenty (20) replicate analyses of *Standard 0 (S_0)*.
The analytical sensitivity of the assay is 2.2 pg/mL.

The functional sensitivity of the Salivary DHEA ELISA was determined by repeated measurements of two saliva samples.
The functional sensitivity of the assay is 5.6 pg/mL.

Reproducibility

Intra-Assay

The intra-assay (within-run) variation of the Salivary DHEA ELISA was determined by repeated measurements of four saliva samples.

Sample	1	2	3	4
Mean (pg/mL)	66.4	318.6	150.4	31.4
SD (pg/mL)	4.4	18.1	3.5	2.5
CV (%)	6.6	5.7	2.3	8.0
n	20	20	20	20

Inter-Assay (Between-Run)

The inter-assay (between-run) variation was determined by repeated measurements of five saliva samples in duplicates in 10 different runs.

Sample	**1**	**2**	**3**	**4**	**5**
Mean (pg/mL)	239.9	71.7	48.9	65.9	98.8
SD (pg/mL)	8.2	3.0	2.3	3.0	4.1
CV (%)	3.4	4.2	4.7	4.5	4.2
n	20	20	20	20	20

Inter-Assay (Between-Lot)

The inter-assay (between-lot) variation was determined by duplicate measurements of five saliva samples in three different kit lots.

Sample	**1**	**2**	**3**	**4**	**5**
Mean (pg/mL)	56.9	104.9	241.6	80.4	112.8
SD (pg/mL)	3.2	3.3	12.2	3.3	4.0
CV (%)	5.6	3.1	5.0	4.2	3.6
n	18	18	18	18	18

Recovery

Recovery of the ELISA was determined by adding increasing amounts of the analyte to three different saliva samples containing different amounts of endogenous analyte. Each sample (native and spiked) was assayed and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and measured values of the samples

Sample		**1**	**2**	**3**
Concentration [pg/mL]		136.3	370.9	477.6
Average Recovery		103.3	103.5	104.2
Range of Recovery [%]	from	98.8	96.2	101.5
	to	108.2	108.3	107.1

Linearity

Three saliva samples containing different amounts of analyte were serially diluted with zero standard and assayed with the ELISA. The percentage recovery was calculated by comparing the expected and measured values.

Sample		**1**	**2**	**3**
Concentration [pg/mL]		590.4	276.3	90.8
Average Recovery		95.4	101.1	91.6
Range of Recovery [%]	from	92.0	95.5	85.2
	to	99.2	106.6	95.1

Limitations of Use

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

Interfering Substances

Blood contamination of more than 0.04% in saliva samples will affect results,
Concentrations of sodium azide ≥ 0.02% interferes in this assay and may lead to false results.

Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of DHEA in a sample.

High-Dose-Hook Effect

No hook effect was observed in this test

Legal Aspects

Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact the manufacture.

Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!