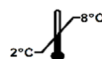


**Instructions for use**  
**DHEA Saliva ELISA**

**REF**

**SA E-7000R**



**RUO**

For research  
use only –  
Not for use  
in diagnostic  
procedures

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## DHEA Saliva ELISA

### 1. INTRODUCTION

#### 1.1 Intended use

The DHEA Saliva ELISA is an enzyme immunoassay for the quantitative determination of Dehydroepiandrosterone (DHEA) in human saliva.

The assay is intended for *research* use by professional users only. Manual processing is recommended. The usage of laboratory automats is the user's sole responsibility. The kit is intended for single use only.

#### 1.2 Description of the analyte

Dehydroepiandrosterone (DHEA; androstenedione; 3 $\beta$ -hydroxy-androst-5-en-17-one) is a C<sub>19</sub> 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 (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.

Since very little DHEA is produced by the gonads, measurement of DHEA levels can help to locate the source of androgen under virilizing conditions [1].

Salivary DHEA concentrations show good correlation with serum, and decreasing values with increasing age in adults have been well-documented [2].

### 2. PRINCIPLE

The DHEA Saliva ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the competition principle. An unknown amount of antigen present in the sample and enzyme-labeled antigen compete for the binding sites of antibodies coated onto the wells. After incubation, any unbound sample antigen and 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 color developed is inversely proportional to the concentration of DHEA in the sample. The enzymatic reaction is stopped by addition of stop solution and the optical density (OD) is measured. A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

### 3. WARNINGS AND PRECAUTIONS

1. This kit is for *research* use only. For professional use only.
2. 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.
3. The microtiter plate contains break-apart strips. Unused wells must be stored at 2 – 8 °C in the sealed foil pouch and used in the frame provided.
4. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
5. Use reservoirs or multipipette tips only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing substrate solution that had previously been used for conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
6. Mix the contents of the microtiter plate wells thoroughly to ensure good test results. Do not reuse wells.
7. Do not let wells dry during assay, add reagents immediately after completing the washing steps.
8. Allow the reagents to reach room temperature (18 – 25 °C) before starting the test. Temperature will affect the optical density of the assay.
9. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
10. Do not smoke, eat, drink, or apply cosmetics in areas where specimens or kit reagents are handled.
11. Wear disposable protective gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
12. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
13. Do not use reagents beyond expiry date as shown on the kit labels.
14. 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.
15. 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 be slightly different.
16. Avoid contact with Stop Solution. It may cause skin irritation and burns.
17. Some reagents contain Proclin 300, CMIT and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.

18. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
19. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from the manufacturer.
20. If product information, including labeling, is incorrect or inaccurate, please contact the kit manufacturer or supplier.

#### 4. REAGENTS

##### 4.1 Reagents provided

**SA E-7031** 96 **Microtiter plate**  
 Content: Wells coated with an anti-DHEA antiserum (polyclonal).  
 Volume: 12x8 (break-apart) strips, 96 wells.

##### Standards and Controls – Ready to use

Cat.-no.	Component	Concentration	Volume / Vial
<b>SA E-7001</b> *1	<span style="border: 1px solid black; padding: 0 2px;">STANDARD</span> <span style="border: 1px solid black; padding: 0 2px;">A</span>	0 pg/ml	3.0 ml
<b>SA E-7002</b> *1	<span style="border: 1px solid black; padding: 0 2px;">STANDARD</span> <span style="border: 1px solid black; padding: 0 2px;">B</span>	10 pg/ml	1.0 ml
<b>SA E-7003</b> *1	<span style="border: 1px solid black; padding: 0 2px;">STANDARD</span> <span style="border: 1px solid black; padding: 0 2px;">C</span>	40 pg/ml	1.0 ml
<b>SA E-7004</b> *1	<span style="border: 1px solid black; padding: 0 2px;">STANDARD</span> <span style="border: 1px solid black; padding: 0 2px;">D</span>	160 pg/ml	1.0 ml
<b>SA E-7005</b> *1	<span style="border: 1px solid black; padding: 0 2px;">STANDARD</span> <span style="border: 1px solid black; padding: 0 2px;">E</span>	640 pg/ml	1.0 ml
<b>SA E-7006</b> *1	<span style="border: 1px solid black; padding: 0 2px;">STANDARD</span> <span style="border: 1px solid black; padding: 0 2px;">F</span>	2560 pg/ml	1.0 ml
<b>SA E-7051</b> *2	<span style="border: 1px solid black; padding: 0 2px;">CONTROL</span> <span style="border: 1px solid black; padding: 0 2px;">1</span>	For control values and ranges please refer to QC Datasheet.	1.0 ml
<b>SA E-7052</b> *2	<span style="border: 1px solid black; padding: 0 2px;">CONTROL</span> <span style="border: 1px solid black; padding: 0 2px;">2</span>		1.0 ml

\*1 Buffered matrix spiked with defined quantity of DHEA.

\*2 Buffered matrix with defined quantity of DHEA.

**SA E-7040** CONJUGATE **Enzyme Conjugate** – Ready to use  
 Content: DHEA conjugated to horseradish peroxidase.  
 Volume: 1 x 11 ml/vial

**AR E-0055** SUBSTRATE **Substrate Solution** – Ready to use  
 Content: Contains tetramethylbenzidine (TMB).  
 Volume: 1 x 22 ml/vial

**AR E-0080** STOP-SOLN **Stop Solution** – Ready to use  
 Content: Contains 2 N hydrochloric acid solution.  
 Avoid contact with the stop solution. It may cause skin irritations and burns.  
 Volume: 1 x 7 ml/vial

Hazards identification:



H290 May be corrosive to metals.  
 H314 Causes severe skin burns and eye damage.  
 H335 May cause respiratory irritation.

**AR E-0030** WASH-CONC 10x **Wash Solution** – 10x concentrated  
 Volume: 1 x 50 ml/vial  
 see "Reagents preparation" (4.4)

## 4.2 Material required but not provided

- Microtiter plate reader capable for endpoint measurement at 450 nm
- Calibrated variable precision micropipettes and multichannel pipettes with disposable pipette tips
- Microtiter plate mixer operating at 900 rpm
- Manual or automatic equipment for microtiter plate washing
- Absorbent paper
- Deionized water
- Timer
- Semilogarithmic graph paper or software for data reduction
- Vortex mixer
- Microcentrifuge

## 4.3 Storage conditions

When stored at 2 – 8 °C unopened reagents will be stable until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2 – 8 °C. After first opening the reagents are stable for 30 days if used and stored properly. Keep away from heat and direct sunlight. Microtiter wells must be stored at 2 – 8 °C. Take care that the foil bag is sealed tightly.

## 4.4 Reagents preparation

Allow the reagents and the required number of wells to reach room temperature (18 – 25 °C) before starting the test.

### Wash Solution

Dilute 50 ml of 10x concentrated Wash Solution with 450 ml deionized water to a final volume of 500 ml. The diluted Wash Solution is stable for at least 12 weeks at room temperature (18 – 25 °C). Precipitates may form when stored at 2 – 8 °C, which should dissolve again by swirling at room temperature (18 – 25 °C). The Wash Solution should only be used when the precipitates have completely dissolved.

## 4.5 Disposal of the kits

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

## 4.6 Damaged test kits

In case of any severe damage of the test kit or components, the manufacturer has to be informed written, 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.

## 5. SPECIMEN COLLECTION AND PREPARATION

Samples containing sodium azide should not be used in the assay. The saliva samples should be completely colorless. Even the slightest red color shows blood contamination. Such blood contamination will give false concentration values. In case of visible blood contamination the donor should discard the sample, rinse the collection device with water, also rinse the mouth with (preferably) cold water, wait for 10 minutes and take a new sample.

### 5.1 Specimen Collection

For the correct collection of saliva we recommend to use only appropriate devices made from ultra-pure polypropylene. Do not use any PE devices or Salivettes for sampling. In most cases this will result in significant interferences. Glass tubes can be used as well, but in this case special attention is necessary for excluding any interference caused by the stopper. Please contact the manufacturer for more details.

As the steroid hormone secretion in saliva as well as in serum shows an obvious dynamic secretion pattern throughout the day it is important to always collect five samples during a two hour period; this means every 30 minutes one sample. It is recommended to collect the samples within two hours after awakening time. If possible the volume of each single sample should be a minimum of 0.5 ml (better 1 ml). Rinse mouth with water 10 minutes prior to specimen collection.

The donor should not eat a major meal, brush teeth or chew gum for 60 minutes before sampling. Do not take a sample within 12 hours after drinking alcohol.

## 5.2 Specimen Storage and Preparation

Saliva samples may be stored at 2 – 8 °C for up to one week. For longer storage, it is recommended to store the samples at ≤-20 °C. Repeated thawing and freezing should be minimized. Each sample has to be frozen, thawed, and centrifuged at least once anyhow in order to separate the mucins by centrifugation. Upon arrival of the samples at the lab, the samples have to be kept frozen at least overnight. Next morning the samples are thawed and mixed carefully. The samples have to be centrifuged for 5 to 10 minutes. The clear colorless supernatant is easy to pipette. If the sample should show even a slight red colour, it might be contaminated with blood and should be discarded. Blood contamination influences the results and leads to false results. Due to the episodic variations of the steroid secretion the strategy of multiple sampling is highly recommended. If such a set of multiple samples has to be tested the staff of lab (after at least one freezing, thawing, and centrifugation cycle) should mix aliquots of the five single samples and perform the determination using the mixture.

## 5.3 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 A (STD A) and re-assayed as described in Assay Procedure. For the calculation of the concentrations this dilution factor has to be taken into account.

## 6. ASSAY PROCEDURE

### 6.1 General remarks

- All reagents and specimens must be allowed to come to room temperature (18 – 25 °C) 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.
- Optical density 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.
- Respect the incubation times as stated in this instructions for use.
- Standards, controls and samples should at least be assayed in duplicates.
- Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or a multipipette, respectively, or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with wash solution, and that there are no residues in the wells.
- A standard curve must be established for every run.

### 6.2 Assay Procedure

<b>1.</b> Prepare a sufficient number of microtiter plate wells to accommodate standards, controls and samples in duplicates.
<b>2.</b> Dispense <b>100 µl</b> of each <b>standard, control and sample</b> <u>with new disposable tips</u> in duplicates into appropriate wells.
<b>3.</b> Dispense <b>100 µl</b> of <b>Enzyme Conjugate</b> into each well.
<b>4.</b> Incubate for <b>60 minutes</b> at room temperature (18 – 25 °C) on a microtiter plate shaker at 900 rpm. <b>Important note:</b> Optimal reaction in this assay is markedly dependent on shaking of the microtiter plate!
<b>5.</b> Briskly empty the contents of the wells by aspiration or by decanting. Rinse the wells 4 times with diluted Wash Solution (300 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets. <b>Important note:</b> The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
<b>6.</b> Add <b>200 µl</b> of <b>Substrate Solution</b> to each well.
<b>7.</b> Incubate without shaking for <b>30 minutes</b> in the dark at room temperature (18 – 25 °C).
<b>8.</b> Stop the enzymatic reaction by adding <b>50 µl</b> of <b>Stop Solution</b> to each well.
<b>9.</b> Determine the absorbance of each well at <b>450 nm</b> . It is recommended to read the wells <u>within 15 minutes</u> .

### 6.3 Calculation of results

- Calculate the average absorbance values for each set of standards, controls and samples.
- The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic paper or using an automated method.
- Using the mean optical density value for each sample determine the corresponding concentration from the standard curve.
- Automated method: The results in the package insert 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.
- The concentration of the samples can be determined directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

#### 6.3.1 Example of typical Standard Curve

The following data are intended for demonstration only and must not be used to calculate results from another run.

Standard		Optical Density (450 nm)
Standard A	0 pg/ml	2.940
Standard B	10 pg/ml	2.701
Standard C	40 pg/ml	2.290
Standard D	160 pg/ml	1.657
Standard E	640 pg/ml	0.890
Standard F	2560 pg/ml	0.426

### 7. EXPECTED NORMAL VALUES

Because of differences, which may exist between laboratories and location with respect to population, laboratory technique and selection of reference group, it is important for each laboratory to determine its own normal and pathological values. Samples were collected in the morning.

Age Group [Years]	Men			Women		
	5. - 95. Percentile [pg/ml]	Median [pg/ml]	n	5. - 95 Percentile [pg/ml]	Median [pg/ml]	n
<21	30.4 - 537.7	200.7	7	27.2 - 564.5	215.7	24
21 - 30	291.4 - 826.7	464.4	10	73.5 - 780.7	605.2	50
31 - 40	306.7 - 892.3	514.2	10	124.5 - 745.1	335.0	50
41 - 50	86.8 - 713.7	285.2	25	85.7 - 480.8	222.3	50
51 - 60	79.1 - 525.3	228.4	23	76.7 - 620.2	217.7	50
>60	39.4 - 694.9	171.2	28	34.7 - 467.1	170.8	50

### 8. QUALITY CONTROL

Good laboratory practice requires that controls need to be run with each standard 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 kit-controls and the corresponding results 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 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 manufacturer directly.

## 9. PERFORMANCE CHARACTERISTICS

### 9.1 Analytical Sensitivity

The analytical sensitivity of the DHEA Saliva ELISA was calculated by subtracting 2 standard deviations from the mean of at least twenty (20) replicate analyses of Standard A (STD A). The analytical sensitivity of the assay is 6.4 pg/ml.

### 9.2 Specificity (Cross-Reactivity)

The following materials have been evaluated for cross reactivity. The percentage indicates cross-reactivity at 50% displacement compared to DHEA.

<b>Steroids</b>	<b>% Cross-Reactivity</b>
Testosterone	<0.01
Androstendione	0.07
Progesterone	0.04
17 $\alpha$ -Hydroxyprogesterone	0.10
Pregnenolone	0.03
11-Deoxycorticosterone	0.09
Corticosterone	<0.01
Cortisol	<0.01
11-Desoxycortisol	<0.01
Estradiol-17 $\beta$	<0.01
Estrone	<0.01
Estriol	<0.01

### 9.3 Assay Dynamic Range

The range of the assay is between 10 – 2560 pg/ml.

### 9.4 Reproducibility

#### 9.4.1 Intra-Assay

The intra-assay variation was determined by 20 replicate measurements of three saliva samples within one run using the DHEA Saliva ELISA.

	<b>Sample 1</b>	<b>Sample 2</b>	<b>Sample 3</b>
<b>Mean (pg/ml)</b>	117.5	316.0	1018.3
<b>SD (pg/ml)</b>	12.9	25.1	82.8
<b>CV (%)</b>	11.0	7.9	8.1
<b>n =</b>	20	20	20

#### 9.4.2 Inter-Assay

The inter-assay variation was determined by duplicate measurements of three saliva samples in ten different runs using the DHEA Saliva ELISA.

	<b>Sample 1</b>	<b>Sample 2</b>	<b>Sample 3</b>
<b>Mean (pg/ml)</b>	250.6	891.3	143.7
<b>SD (pg/ml)</b>	19.0	88.9	16.7
<b>CV (%)</b>	7.6	10.0	11.6
<b>n =</b>	10	10	10

## 9.5 Recovery

Recovery was determined by adding increasing amounts of the analyte to three different saliva samples containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) was measured by the DHEA Saliva ELISA. The percentage recoveries were determined by comparing expected and observed results of the samples.

Saliva	Spiking (pg/ml)	Observed (pg/ml)	Expected (pg/ml)	Recovery (%)
1	native	242.9	-	-
	200	467.0	442.9	105
	400	714.2	642.9	111
	800	1158.3	1042.9	111
2	native	143.7	-	-
	200	417.7	343.7	122
	400	620.8	543.7	114
	800	1231.2	943.7	130
3	native	122.6	-	-
	200	338.4	322.6	105
	400	579.2	522.6	111
	800	1191.0	922.6	129

## 9.6 Linearity

Three saliva samples containing different amounts of analyte were serially diluted with Standard A (STD A) and assayed with the DHEA Saliva ELISA. The percentage linearity was calculated by comparing the expected and observed values for DHEA.

Saliva	Dilution	Observed (pg/ml)	Expected (pg/ml)	Linearity (%)
1	native	690.5	-	-
	1:2	290.4	345.3	84
	1:4	140.2	172.6	81
	1:8	70.7	86.3	82
2	native	643.6	-	-
	1:2	294.7	321.8	92
	1:4	150.1	160.9	93
	1:8	69.6	80.5	87
3	native	513.2	-	-
	1:2	209.2	256.6	82
	1:4	92.0	128.3	72
	1:8	51.2	64.2	80

## 10. LIMITATIONS OF PROCEDURE

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.

### 10.1 Interfering Substances

- Blood contamination in saliva samples will affect results and usually can be seen by eye. In case of visible blood contamination, the donor should discard the sample, rinse the sampling device with water, wait for ten minutes and take a new sample. Do not collect samples when oral diseases, inflammation, or lesions exist (blood contamination). Find more details about sample collection and preparation in chapter 5.
- Samples containing sodium azide should not be used in the assay. This can cause false results.
- The result of any immunological test system may be affected by heterophilic antibodies, anti-species antibodies or rheumatoid factors present in human samples [3 – 5]. For example, the presence of heterophilic antibodies in donors who are regularly exposed to animals or animal products may interfere with immunological tests. Therefore, interference with this *research* immunoassay cannot be excluded. If unplausible results are suspected, they should be considered invalid and verified by further testing.

## 10.2 Drug Interferences

Any medication (cream, oil, pill, etc.) containing DHEA of course will significantly influence the measurement of this analyte.

## 10.3 High Dose Hook Effect

Up to a tested concentration of 100 000 pg/ml DHEA, no High Dose Hook Effect was observed for the DHEA Saliva ELISA.

## 11. LEGAL ASPECTS

### 11.1 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 a sufficient number of controls within the test procedure 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 manufacturer.

### 11.2 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.

## 12. REVISION HISTORY OF INSTRUCTION FOR USE









Changes from the previous version 6.0c to actual version 7.0

General	Editorial changes
Chapter 1	Updated intended use and description of the analyte
Chapter 2	Updated; editorial changes
Chapter 3	Additional information
Chapter 4	Updated and additional information; plate shaker at 900 rpm required (before $\geq 600$ rpm) (4.2)
Chapter 5	Updated: collection and storage conditions of saliva samples
Chapter 6	Updated information (6.1; 6.3); shaking during incubation at 900 rpm (before $\geq 600$ rpm) (6.2)
Chapter 9	Updated assay characteristics
Chapter 10	Additional information, updates of interfering substances; High-Dose-Hook-Effect added (10.3)
Chapter 12	Added
Chapter 13	References updated

## 13. REFERENCES

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**Symbols:**

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date	<b>LOT</b>	Batch code		
	Consult instructions for use	<b>CONT</b>	Content		
	Caution	<b>REF</b>	Catalogue number		Distributor
	Date of manufacture			<b>RUO</b>	For research use only!