

**BioVendor**

Research  
and Diagnostic Products



## **DHEA FREE IN SALIVA ELISA**

Product Data Sheet

Cat. No.: RTC012R

For Research Use Only

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**➤➤ This kit is manufactured by:  
BioVendor – Laboratorní medicína a.s.**

**➤➤ Use only the current version of Product Data Sheet enclosed with the kit!**

## 1. INTENDED USE

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An enzyme immunoassay for the quantitative in vitro diagnostic measurement of active free DHEA in saliva.

**For research use only.**

## 2. STORAGE, EXPIRATION

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When stored at 2°C to 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. Microtiter wells must be stored at 2°C to 8°C. Take care that the foil bag is sealed tightly.

## 3. INTRODUCTION

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Dehydroepiandrosterone (DHEA; androstenedione; 3 $\beta$ -hydroxy-5-androsten-17-one) 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 (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.

## 4. TEST PRINCIPLE

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The BioVendor DHEA free in Saliva ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with an antibody directed against 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 color developed is inversely proportional to the concentration of DHEA in the patient sample.

## 5. PRECAUTIONS

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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 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.
4. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
5. 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.
6. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
7. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
8. 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.
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 latex 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 result slightly different.
16. Avoid contact with Stop Solution. It may cause skin irritation and burns.
17. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
18. 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 BIOVENDOR.

## 6. REAGENT SUPPLIED

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1. **Microtiterwells**, 12x8 (break apart) strips, 96 wells.  
Wells coated with an anti-DHEA antiserum (polyclonal).
2. **Calibrator 0**, 1 vial, 3.0 ml, ready to use.
3. **Calibrator (Calibrator 1-5)**, 5 vials, 1.0 ml each, ready to use.  
Concentrations: 10 - 40 - 160 – 640 – 2560 pg/ml
3. **Quality Control HIGH and LOW** 2 vials, 1.0 ml each, ready to use;  
For control values and ranges please refer to Quality Control Sheet.
4. **Enzyme Conjugate**, 1 vial, 11 ml, ready to use;  
DHEA conjugated to horseradish peroxidase;
5. **Substrate Solution**, 1 vial, 22 ml, ready to use;  
Tetramethylbenzidine (TMB).
6. **Stop Solution**, 1 vial, 7 ml, ready to use;  
contains 2 N hydrochlorid acid solution. Avoid contact with the stop solution. It may cause skin irritations and burns.
7. **Wash Solution**, 1 vial, 50 ml (10X concentrated).  
see „Preparation of Reagents“.

All reagents contain azide-free and mercury-free preservatives.

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

## 7. MATERIAL REQUIRED BUT NOT SUPPLIED

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- Microcentrifuge
- A microtiter plate reader capable for endpoint measurement at  $450\pm 10\text{nm}$
- Microplate mixer operating at about 600-900 rpm
- Vortex mixer
- Calibrated variable precision micropipettes (50  $\mu\text{l}$ , 100  $\mu\text{l}$ , 200  $\mu\text{l}$ )
- Absorbent paper.
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

## 8. PREPARATION OF REAGENTS

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Allow the reagents and the required number of wells to reach room temperature (21-26°C) before starting the test.

### **Preparation of Wash Solution**

Add deionized water to the 10X concentrated Wash Solution. Dilute 50 ml of concentrated Wash Solution with 450 ml deionized water to a final volume of 500 ml. The diluted Wash Solution is stable for 3 months at room temperature.

### **8.1 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.

### **8.2 Damaged Test Kits**

In case of any severe damage of the test kit or components, BioVendor 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.

## 9. SPECIMEN COLLECTION AND STORAGE INSTRUCTION

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### 9.1 Specimen

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 falsely elevated concentration values. In case of visible blood contamination the patient 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. Do not chew anything during the sampling period. Any pressure on the teeth may result in falsely elevated measurements due to an elevated content of gingival liquid in the saliva sample.

### 9.2 Specimen Collection

For the correct collection of saliva we are recommending to use only appropriate devices made from ultrapure polypropylene. Do not use any PE devices or cotton based Salivettes for sampling. False readings will result. 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 BioVendor for more details. As food might contain significant amounts of steroid hormones samples preferably should be taken while fasting. If fasting should be a problem at least any food of animal origin (meat or dairy products) should be avoided prior to finalizing the collection. In the morning breakfast should be done only after finalizing the collection procedure. During the day the collection period should be timed just before an anticipated meal. 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 5 samples during a 2 hour period; this means every 30 minutes one sample. If possible the volume of each single sample should be a minimum of 0.5 ml (better 1 ml). Saliva flow may be stimulated by drinking water. This is allowed and even recommended before and during the collection period. Drinking of water is not allowed during the last 5 minutes before taking the single samples.

### 9.3 Specimen Storage and Preparation

Saliva samples in general are stable at ambient temperature for several days. Therefore mailing of such samples by ordinary mail without cooling will not create a problem. Storage at 4°C can be done for a period of up to one month. Whenever possible samples preferable should be kept at a temperature of -20°C. Even repeated thawing and freezing is no problem. 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 in the lab the samples have to be kept frozen 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. Now the clear colorless supernatant is easy to pipette. If the sample should show even a slightly red color it should be discarded. Otherwise the concentration value most probably will be falsely elevated. Due to the episodic variations of the steroid secretion we highly recommend the strategy of multiple sampling. If such a set of multiple samples has to be tested the lab (after at least one freezing, thawing, and centrifugation cycle) has to mix the aliquots of the 5 single samples in a separate sampling device and perform the testing from this mixture.

## 9.4 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Calibrator 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 *Calibrator 0* (mix thoroughly)
- b) Dilution 1:100: 10 µl of dilution a) + 90 µl *Calibrator 0* (mix thoroughly).

## 10. ASSAY PROCEDURE

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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.

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **100 µL** of each **Calibrator, Control and samples** with new disposable tips into appropriate wells.
3. Dispense **100 µL Enzyme Conjugate** into each well.
4. Incubate for **60 minutes** at room temperature on a Microplate mixer ( $\geq 600$  rpm).  
**Important note:** Optimal reaction in this assay is markedly dependent on shaking of the microplate!
5. 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.  
**Important note:** The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add **200 µL** of **Substrate Solution** to each well.
7. Incubate for **30 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **50 µL** of **Stop Solution** to each well.
9. Determine the absorbance (OD) of each well at **450 ± 10 nm**.  
It is recommended that the wells be read **within 15 minutes**.



## 11. CALCULATION

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1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each calibrator 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 calibrator have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

### Example of Typical Standard Curve

Following data are intended for illustration only and should not be used to calculate results from another run.

<b>Calibrator</b>	<b>Optical Units (450 nm)</b>
Calibrator 0 0 pg/ml	2.940
Calibrator 1 10 pg/ml	2.701
Calibrator 2 40 pg/ml	2.290
Calibrator 3 160 pg/ml	1.657
Calibrator 4 640 pg/ml	0.890
Calibrator 5 2560 pg/ml	0.426

## 12. QUALITY CONTROL

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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 BIOVENDOR directly.

## 13. LIMITATIONS

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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. The patient should not eat, drink, chew gum or brush teeth for 30 minutes before sampling. Otherwise rinse mouth thoroughly with cold water 5 min prior to sample collection. Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).

### 13.1 Drug Interferences

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

### 13.2 High-Dose-Hook Effect

No hook effect was observed in this test.

## 14. REFERENCE VALUE

In order to determine the normal range of DHEA free in Saliva samples from adult male and female apparently healthy subjects were collected and analyzed using the BioVendor DHEA free in Saliva ELISA kit. The following range was calculated from this study.

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

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

Furthermore, we recommend that each laboratory determines its own range for the population tested.

## 15. PERFORMANCE CHARACTERISTICS

### 15.1 Assay Range

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

### 15.2 Analytical Sensitivity

The analytical sensitivity of the DHEA free in Saliva ELISA was calculated by subtracting 2 standard deviations from the mean of twenty-two (22) replicate analyses of Calibrator 0. The analytical sensitivity of the assay is 3.7 pg/ml.

### 15.3 Reproducibility

#### 15.3.1 Intra-Assay

The intra-assay variation was determined by replicate measurements of 3 saliva samples within one run using the BioVendor DHEA free in Saliva ELISA. The within-assay variation is shown below:

	<b>Sample 1</b>	<b>Sample 2</b>	<b>Sample 3</b>
Mean (pg/ml)	255.1	672.8	822.8
SD (pg/ml)	13.3	60.7	39.9
CV (%)	5.2	9.0	4.9
n =	19	19	19

### 15.3.2 Inter-Assay

The inter-assay variation was determined by duplicate measurements of 3 saliva samples in 10 different runs using the BioVendor DHEA free in Saliva ELISA. The inter-assay variation is shown below:

	<b>Sample 1</b>	<b>Sample 2</b>	<b>Sample 3</b>
Mean (pg/ml)	238.6	648.8	797.5
SD (pg/ml)	10.6	44.2	51.2
CV (%)	4.5	6.8	6.4
n =	10	10	10

### 15.4 Specificity

The following materials have been evaluated for cross reactivity.

<b>Steroids</b>	<b>% Crossreactivity at 50% Binding</b>
DHEA-S	<0.01
Testosterone	<0.01
5 $\alpha$ -Dihydrotestosterone	<0.01
Androstendione	0.06
Progesterone	0.23
17 $\alpha$ -Hydroxyprogesterone	<0.01
Pregnenolone	0.01
17-Hydroxy-Pregnenolone	0.07
Deoxycorticosterone	0.05
Corticosterone	<0.01
Cortisol	<0.01
11-Desoxycortisol	0.01
Estradiol-17 $\beta$	<0.01
Estradiol-17 $\alpha$	<0.01
Estrone	<0.01
Estriol	<0.01

### 15.5 Recovery

Using the calibrator matrix a spiking solution of 20 ng DHEA/ml was prepared. 500 µl of three saliva were spiked with 5, 10 and 15 µl of the spiking solution leaving the saliva matrices relatively intact. All samples were measured by the BioVendor DHEA free in Saliva ELISA procedure.

Saliva	Spiking (pg/ml)	Observed (pg/ml)	Expected (pg/ml)	Recovery (%)
1	-	291	-	-
	200	517	491	105
	400	668	691	97
	600	859	891	96
2	-	64	-	-
	200	287	264	109
	400	520	464	112
	600	661	664	100
3	-	247	-	-
	200	490	447	110
	400	668	647	103
	600	847	847	100

### 15.6 Linearity

Four saliva samples containing different amounts of analyte were serially diluted with *Calibrator 0* and assayed with the BioVendor DHEA free in Saliva ELISA. The percentage recovery was calculated by comparing the expected and observed values for DHEA.

Saliva	Dilution	Observed (pg/ml)	Expected (pg/ml)	Linearity (%)
1	native	569	-	-
	1 in 2	284	285	100
	1 in 4	127	142	89
	1 in 8	77	71	108
2	native	384	-	-
	1 in 2	167	192	87
	1 in 4	78	96	81
	1 in 8	40	48	83
3	native	238	-	-
	1 in 2	86	119	72
	1 in 4	63	60	105
	1 in 8	30	30	100
4	native	292	-	-
	1 in 2	135	146	92
	1 in 4	73	73	100
	1 in 8	40	37	108

## 16. LEGAL ASPECTS

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### 16.1 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 BIOVENDOR.

### 16.2 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 16.1. 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 therapeutic consequences should be derived.

The test result itself should never be the sole determinant for deriving any therapeutic consequences.

### 16.3 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 16.2. 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.

## 17. REFERENCES

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*BioPsychoSocial Medicine* 2015, 9:4

## 18. EXPLANATION OF SYMBOLS

Symbol	English
	European Conformity
	Consult instructions for use
	In vitro diagnostic device
	Catalogue number
	Lot. No. / Batch code
	For research use only
	Contains sufficient for <n> tests/
	Storage Temperature
	Expiration Date
	Legal Manufacturer
<i>Distributed by</i>	Distributor

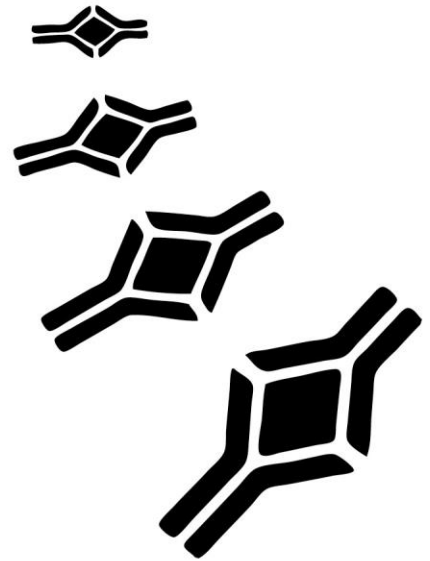


NOTES









**BioVendor – Laboratorní medicína a.s.**

Karasek 1767/1, 621 00 Brno, Czech Republic

Phone: +420-549-124-185, Fax: +420-549-211-460

E-mail: [info@biovendor.com](mailto:info@biovendor.com), [sales@biovendor.com](mailto:sales@biovendor.com)

Web: [www.biovendor.com](http://www.biovendor.com)

There are BioVendor branches and distributors near you.

To find the office closest to you, visit [www.biovendor.com/contact](http://www.biovendor.com/contact)

