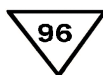


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**Instructions for use**  
**DHEA-S Saliva ELISA**

**REF**

**SA E-6500**



**IVD**

**CE**

## DHEA-S Saliva ELISA

### 1. **Intended Use**

Competitive immunoenzymatic colorimetric method for the quantitative determination of DHEA-S in saliva.

### 2. **Principle**

DHEA-S (antigen) in the sample competes with horseradish peroxidase DHEA-S (enzyme-labelled antigen) for binding onto the limited number of anti-DHEA-S (antibody) sites on the micro plate (solid phase).

After incubation, the bound/free separation is performed by a simple solid-phase washing.

The enzyme substrate (H<sub>2</sub>O<sub>2</sub>) and the TMB-Substrate (TMB) are added. After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the absorbencies are determined. DHEA-S concentration in the sample is calculated based on a series of standard.

The colour intensity is inversely proportional to the DHEA-S concentration of in the sample.

### 3. **Advice on handling the test**

#### 3.1 **Reliability of the test results**

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (e.g. GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions.

It is recommended that each laboratory establishes its own reference intervals. The values reported in this test instruction are only indicative.

The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.

#### 3.2 **Complaints**

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

#### 3.3 **Warranty**

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

#### 3.4 **Disposal**

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.

The appropriate safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

#### 3.5 **Interference**

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

#### 3.6 **Precautions**

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.

All reagents of this testkit which contain human or animal 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.

#### 4. Reagents, material and instrumentation

<u>STANDARD A</u>	
<u>STANDARD B</u>	
<u>STANDARD C</u>	
<u>STANDARD D</u>	
<u>STANDARD E</u>	DHEA-S Standards, 1 ml
<u>INC-BUFF</u>	Incubation buffer (1 bottle), 30 ml, Phosphate buffer
<u>CONJUGATE-CONC</u>	Conjugate (1 bottle), 0.4 ml, DHEA-S-HRP conjugate
<u>MI 96</u>	Microplate, Anti-DHEA-S-IgG adsorbed on microplate
<u>SUBSTRATE</u>	TMB Substrate (1 bottle), 12 ml, H <sub>2</sub> O <sub>2</sub> .TMB 0.25gr/L (avoid any skin contact)
<u>STOP-SOLN</u>	Stop Solution (1 bottle), 12 ml, Sulphuric acid 2 mol/L (corrosive:avoid any skin contact)
<u>WASH-CONC 50x</u>	Wash Concentrate, 20ml, 50x Conc.

#### Notes

Store all reagents between +2 °C and + 8 C°in the dark.

Open the bag of reagent 4 (Coated Microplate) only when it is at room temperature and close immediately after use.

The micro plate, once opened, it stable until expiry date of kit. Do not remove the adhesive sheets on the unused strips

#### Reagents necessary which are not supplied with the kit

Distilled water.

#### Auxiliary materials and instrumentation

Automatic dispenser.

Microplates reader

Sample tubesPreparation of reagents

#### Standards

Before use, mix for 5 min. with rotating mixer

The standards have the following concentration of DHEA-S:

	S <sub>A</sub>	S <sub>B</sub>	S <sub>C</sub>	S <sub>D</sub>	S <sub>E</sub>
ng/ml	0	0.2	1	3	12

After opening the standards are stable at +4 °C until the expiration date of the kit.

#### Diluted Conjugate (Prepare immediately before use!)

Add 10 µL stock solution (reagent 3) to 1.0 mL of Incubation Buffer (reagent 2).Mix gently.

Stable for 3 hours at 18 °C – 25°C.

#### Wash Buffer

Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1000 mL.

Storage: up to 6 months 2–8°C

## 5. **Sample collection, storage 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**

It is recommended to collect saliva samples with commercially available equipment (e.g. SALI SET 100, REF SA D-6100 available from LDN).

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.

## 6. Test procedure

As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the standard curve (S0-S4), two for each sample, and one for Blank.

### Pipette:

	Standard	Sample	Blank
Sample	-	50 µl	-
Standards (S <sub>A</sub> - S <sub>E</sub> )	50 µl	-	-
Diluted Conjugate	150 µl	150 µl	-

**Mix well.**

**Incubate at 37 °C for 15 minutes.**

Discard or aspirate the contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.

### Pipette:

	Standard	Sample	Blank
TMB- Substrate	100 µl	100 µl	100 µl

**Incubate at room temperature** (20-25°C) for **15 minutes** in the dark.

### Pipette:

	Standard	Sample	Blank
Stop Solution	100 µl	100 µl	100 µl

**Read** the absorbance (E) at **450 nm** against Blank.

## 7. Calculation of results

Calculate the mean absorbance (E<sub>m</sub>) corresponding to the single points to the standard curve and of each sample. Express data as the percentage of the mean absorbance of B0 (E<sub>m</sub>S0) with the following formula:

$$(B/B_0)\% = \frac{E_m}{(E_m S_0)} \times 100$$

Plot the values of the standards expressed as (B/B0)% on the enclosed logit-log paper.

Extrapolate the line passing through the points.

Interpolate the values of the samples expressed as (B/B0)% on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL.

## 8. **Assay characteristics**

### **Expected reference values**

As the values of salivary DHEA-S have a circadian pattern we suggest to collect the samples at the same hour (8 A.M.).

The following values can be used as preliminary guideline until each laboratory established its own normal range:

WOMAN 0.2 – 2.5 ng/mL  
MAN 0.2 – 2.7 ng/mL

### **Specificity**

The cross reaction of the assay calculated at 50% according to Abraham are shown in the table:

DHEA-S	100.0 %
DHEA	65.0 %
Androsterone-S-Na	48.0 %
Androstenedione	20.0 %
Etiocholanolone-S-Na	0.2 %
5 $\alpha$ Androstandione	0.01 %
Testosterone	0.01 %
Progesterone	0.01 %
17 $\alpha$ OH-Progesterone	0.01 %
Estrone	0.01 %
Cortisol	0.001 %
Cholesterol	0.001 %

### **Sensitivity**

The sensitivity of this method, calculated as two times the S.D. from B0, is 25 pg/mL when the value of (B/B0)% is approx 90%.

### **Precision**

The inter and intra-run precision had a coefficient of variation of 3.7% and 5.6% respectively.

### **Accuracy**

The recovery of 0.2- 1- 3- 12 ng/mL of Dehydroepiandrosterone Sulphate added to a sample gave an average value ( $\pm$  SE) of 102.1%  $\pm$  4.6% with reference to the original concentrations.







### **Correlation with serum sample**

Correlation with a serum sample of the same patient:

$r = 0.78$      $n = 43$      $p < 0.001$

 **For actual literature, information about clinical significance or any other information please contact your local supplier.**

**Symbols:**

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