IBL INTERNATIONAL

Instructions for Use

DHEA-S Saliva ELISA

Enzyme immunoassay for the *in-vitro diagnostic* quantitative determination of DHEA-S in human saliva.

REF **RE52661 / RE52669**

Σ **96** **960**

EU: IVD CE U.S.: *For research use only.*
Not for use in diagnostic procedures.

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1. INTENDED USE

Enzyme immunoassay for the *in-vitro diagnostic* quantitative determination of DHEA-S in human saliva. DHEA-S Saliva kit is intended for laboratory use only.

2. SUMMARY AND EXPLANATION

Dehydroepiandrosterone sulfate (DHEA-S), a C-19 steroid hormone, is the most abundant adrenal androgen in the circulation. Most of the circulating DHEA-S originates from either direct adrenal secretion or by peripheral sulphatation of DHEA secreted by the adrenal cortex. Because it is sulphated, it has a long half-life and hence lacks a circadian rhythm. DHEA-S does not circulate bound to specific proteins. It circulates at much higher levels than other androgens or related steroids. Due to quantity produced, DHEA and DHEA-S can be considered as the main products of human steroid biosynthesis. DHEA-S circulates in blood in 20-fold higher concentrations than any other hormones. The concentration of DHEA-S exceeds the DHEA levels by approximately 300 to 500 times. It serves as a kind of repository form of DHEA. The conversion of DHEA-S into DHEA takes place very quickly and in almost all body tissues because the corresponding enzymes are available ubiquitously. Levels of DHEA-S increase from about the seventh year of life, peak in the third decade and decrease gradually thereafter.
The measurements of DHEA-S are widely used in clinical practice. Elevated concentrations of this steroid are found in patients with adrenal hyperplasia, adrenocortical carcinoma, or hirsutism. Low levels of DHEA-S are detectable in patients suffering from adrenal dysfunction or hypopituitarism.
Because of the hydrophilic structure of DHEA-S, only a small fraction is found in saliva. It is of utmost importance to check any blood contamination when measuring the DHEA-S in saliva. Furthermore DHEA-S does not diffuse into saliva as rapidly as the other free steroids, its passage into saliva is flow rate dependent and therefore flow stimulants such as gum chewing are not advised prior to saliva collection. It is recommended to note the flow rate (time needed for collecting the needed quantity of saliva) for interpreting the results.

3. TEST 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 microplates (solid phase). After incubation, the bound/free separation is performed by a simple solid-phase washing. The enzyme substrate (H2O2) and the TMB-Substrate (TMB) are added. After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the OD 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.

4. WARNINGS AND PRECAUTIONS

1. For in-vitro diagnostic 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. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Some reagents contain ProClin 300 as preservative. In case of contact with eyes or skin, flush immediately with water. When disposing reagents, flush with a large volume of water to avoid built-up.
9. Avoid contact with Stop solution. It may cause skin irritations and burns.

10. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2–8°C.

6. SPECIMEN COLLECTION AND STORAGE

Saliva

The patient should not eat, drink, chew gums or brush teeth for 60 min 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). Saliva can be collected in a suitable sampling device. A minimum of 0.5 mL liquid should be collected. It is recommended to freeze samples at –20°C prior to laboratory testing. After thawing, mix and centrifuge 15 min at 3000 x g to remove particulate material.		
⚠	**Take care that the saliva samples are visually okay (no reddish color indicating blood contamination).**	
Storage:	2-8°C	≤ -20°C (Aliquots)
Stability:	< 1 w	≥ 6 mon

7. MATERIALS SUPPLIED

Quantity RE52661	Quantity RE52669	Symbol	Component
1 x 12x8	10 x 12x8	MTP	**Microtiter Plate** Break apart strips. Coated with Anti-DHEA-S IgG.
5 x 1.0 mL	5 x 10 mL	CAL 0-4	**Standard 0-4** 0; 0.2; 1.0; 3.0; 12.0 ng/mL Ready to use. Contains: DHEA-S, ProClin 300.
1 x 30 mL	1 x 300 mL	DILBUF	**Dilution Buffer** Ready to use. Contains: Phosphate buffer, BSA, ProClin 300.
1 x 1.0 mL	1 x 10 mL	ENZCONJ CONC	**Enzyme Conjugate** Concentrate (100x) Contains: DHEA-S conjugated to HRP, stabilizers.
1 x 15 mL	1 x 150 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains: TMB, H_2O_2.
1 x 15 mL	1 x 150 mL	TMB STOP	**TMB Stop Solution** Ready to use. 0.15mol/L H_2SO_4.
1 x 20 mL	1 x 200 mL	WASHBUF CONC	**Wash Buffer** Concentrate (50x) Contains: Tween20.

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volume: 50, 100; 150. 1000 µL
2. A suitable sampling device should be used (can be ordered separately from IBL under REF RE69991). (Do not use sample collector commercially available as “SALIVETTE”).
3. Vortex mixer
4. 8-Channel Micropipettor with reagent reservoirs
5. Incubator, 37°C
6. Wash bottle, automated or semi-automated microtiter plate washing system
7. Bidistilled or deionised water

8. Paper towels, pipette tips and timer
9. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout. A pipetting scheme covering both sample pretreatment and assay is available at the IBL-Homepage.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate 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 evenly with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.
9. The clinical significance of the determination DHEA-S can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.

10. PRE-TEST SETUP INSTRUCTIONS

⚠	The contents of the kit for 96 determinations can be divided into 3 separate runs. **The volumes stated below are for one run with 4 strips (32 determinations).**

10.1. Preparation of concentrated components

Dilute/ dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
20 mL	**WASHBUF**	ad 1000 mL	bidist. water	1:50	Mix vigorously.	18-25°C	until exp. date
100 µL	**ENZCONJ**	with 10 mL	**DILBUF**	1:101	Mix without foaming.	Prepare freshly and use only once.	

11. TEST PROCEDURE

1.	Pipette **50 µL** of each **Standard and sample** into the respective wells of the microtiter plate.
2.	Pipette **150 µL** of freshly prepared ready to use **Enzyme Conjugate** into each well.
3.	**Incubate 15 minutes** at **37°C**.
4.	Discard incubation solution. Wash plate **3 x** with **300 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
5.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
6.	**Incubate 15 min** at **RT (18-25°C)** in the dark.
7.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Shake plate briefly. Color changes from blue to yellow.
8.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600 – 650 nm) within **15 min** after pipetting of the Stop Solution.

12. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of DHEA-S for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

13. CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistics or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read directly from the standard curve.

For SI UNITS: ng/mL x 2.71 = nmol/L

Saliva samples with remarkably elevated values should be reviewed for blood contamination.

14. EXPECTED VALUES

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

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

It is recommended to report the saliva flow rate (time needed for collecting the needed quantity of saliva) for interpreting the results.

15. LIMITATIONS OF THE PROCEDURE

15.1. Assay Performance

Samples which are contaminated microbiologically should not be used in the assay. Highly lipemic or haemolysed specimens should similarly not be used. It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells. Failure to remove adhering solution adequately in the aspiration or decantation wash steps may result in poor replication and spurious results.

15.2. Interpretation

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

16. PERFORMANCE AND CHARACTERISTICS

16.1. Precision

16.1.1. Intra Assay Variation

Within run variation was determined by replicate determination (14x) of two different control sera in one assay. The within assay variability is ≤ 7.8%.

16.1.2. Inter Assay Variation

Between run variation was determined by replicate measurements (9x) of three different control sera with different lots of kits. The between assay variability is ≤ 14.9%.

16.2. Accuracy

The recovery of 0.5 – 1.5 – 6.0 ng/mL of DHEA-S added to sample gave an average value (±SD) of 108.86% ± 3.27% with reference to the original concentrations.

16.3. Sensitivity

The lowest detectable concentration of DHEA-S that can be distinguished from the zero standard is 0.05 ng/mL at the 95 % confidence limit.

16.4. Specificity

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

DHEA-S	90%
DHEA	100%
Androsterone-S-Na	48 %
Androstendione	20 %
Etiocolanone-S-Na	0.2 %
5-Androstendione	0.01 %
Testosterone	0.01 %
Progesterone	0.01 %
17 OH Progesterone	0.01 %
Estrone	0.01 %
Cortisol	0.001 %
Cholesterol	0.001 %

16.5. Correlation

The IBL DHEA-S Saliva ELISA kit was compared to an analogous commercially available Kit. 31 saliva samples were analysed according in both test systems.
The linear regression curve was calculated:

$y = 0.37x + 1.10$
$r^2 = 0.826$
y = DHEA-S Saliva IBL Kit
x = DHEA-S Saliva Salimetrics Kit

17. PRODUCT LITERATURE REFERENCES

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Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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