



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



96 +2 *8



CE

INTENDED USE

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

DHEA-S Saliva kit is intended for laboratory use only.

CLINICAL SIGNIFICANCE

Dehydroepiandrosterone sulfate (DHEA-S), is a natural steroid hormone found atop of the kidneys in the human body. DHEA-S derived from enzymatic conversion of DHEA in adrenal and extradrenal tissues. DHEA-S is also produced in the gonads, adipose tissue and the brain. It is the most abundant hormone in the human body and it is precursor of all sex steroids.

As most DHEA-S is produced by the zona reticularis of the adrenal, it is argued that there is a role in the immune and stress response. DHEA-S may have more biologic roles. Its production in the brain suggests that is also has a role as a <u>neurosteroid</u>.

The majority of DHEA-S in saliva is non-protein bound and enters the saliva via intracellular mechanisms. Salivary DHEA-S levels are unaffected by salivary flow rate or salivary enzymes.

Measurement of serum DHEA-S is a useful marker of adrenal androgen synthesis. Abnormally low levels may occur in have been reported in hypoadrenalism, while elevated levels occur in several conditions, e.g. virilizing adrenal adenoma and carcinoma, 21-hydroxylase and 3β -hydroxysteroid dehydrogenase deficiencies and in some cases of female hirsutism. Women with polycystic ovary syndrome tend to have normal or mildly elevated levels of DHEAS. As very little DHEA-S is produced by the gonads, measurement of DHEA-S levels may aid in the localization of androgen source in virilizing conditions. DHEA-S levels show no diurnal variation.

PRINCIPLE

The DHEA-S (antigen) in the sample competes with the antigenic DHEA-S conjugated with horseradish peroxidase (HRP) for binding to the limited number of antibodies anti DHEA-S coated on the microplate (solid phase).

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

Then, the enzyme HRP in the bound-fraction reacts with the Substrate (H2O2) and the TMB Substrate and develops a blu color that changes into yellow when the Stop Solution (H2SO4) is added.

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

Reagent, material and instrumentation

Reagent and material supplied in the kit

Standards

	Cat. no.	Standard	Concentration	Volume/Vial
STANDARD A	SA E-6501	Standard 0	0 ng/ml	1 ml
STANDARD B	SA E-6502	Standard 1	0.2 ng/ml	1 ml
STANDARD C	SA E-6503	Standard 2	1 ng/ml	1 ml
STANDARD D	SA E-6504	Standard 3	3 ng/ml	1 ml
STANDARD E	SA E-6505	Standard 4	12 ng/ml	1 ml

INC-BUFF

SA E-6513 Incubation Buffer

(1 bottle) 30 mL; Phosphate buffer

CONJUGATE-CONC SA E-6540 Conjugate

(1 bottle) 1 mL; DHEA-S-HRP conjugate

III 96 SA E-6531 Coated Microplate

(1 microplate breakable); Anti-DHEA-S IgG adsorbed on microplate

WASH-CONC 50x

SA E-0030 Conc. Wash Solution 50X

(1 bottle) 20 mL; NaCl 45 g/L; Tween 20, 55 g/L

SUBSTRATE MS E-0055 TMB-Substrate

(1 bottle) 15 mL; H_2O_2 -TMB 0.26 g/L, (avoid any skin contact)

STOP-SOLN MS E-0080 Stop Solution

(1 bottle) 15 mL; Sulphuric acid 0.15 mol/L (avoid any skin contact)

Version 5.0

Reagents necessary not supplied

Distilled water.

Auxiliary materials and instrumentation

Automatic dispenser. Microplates reader Saliva Collection Device : e.g. SALI SET 100 REF SA D-6100 available from LDN

Note

Store all reagents at 2- 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 microplate, once opened, it stable until the expiry date of kit. Do not remove the adhesive sheets on the unused strips

PRECAUTION AND WARNINGS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2 °C 8 °C in their original container. Any exceptions are clearly
 indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22 °C 28 °C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- 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 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of reagents.
- Samples microbiologically contaminated, highly lipemic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells
- This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Some reagents contain small amounts of Proclin 300 as preservative. Avoid the contact with skin or mucosa.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H₂O₂ to directed sunlight, metals or oxidants. Do not freeze the solution.
 This method allows the determination of DHEA-S from 0.2 ng/mL to 12 ng/mL.
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- The clinical significance of the determination DHEA-S can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.

PROCEDURE

Preparation of the Standard (S₀,S₁,S₂,S₃,S₄)

Before use, mix for 5 min. with rotating mixer

The standard has the following concentration of DHEA-S:

Preparation of Conjugate

Prepare immediately before use. Add 10 μ l of Conjugate (reagent 3) to 1.0 mL of Incubation Buffer (reagent 2). Mix gently. Stable 3 hours at 22-28°C.

Preparation of Wash Solution

Dilute the whole contents of Concentrate Wash Solution bottle to 1 L with distilled or deionized water in a suitable storage container.

Store at room temperature until expiration date printed on concentrate label.

Preparation of the Sample

The determination of DHEA-S can be performed in saliva.

It is recommended to collect saliva samples with a centrifuge glass tube and a plastic straw or **Sali Set** (catalogue no. SA D-6100, 100 pieces) or with the "Salivette" (Sarstedt, Ref. 51.1534.500). Other sample collector commercially available has not been tested.

Method and Limitations

Collect saliva samples at the times indicated.

If no specific instructions have been given oral fluid (saliva) samples may be collected at any time for saliva collection, the following should be noted:

- a. If saliva collection is to be carried out in the morning ensure that this is carried out prior to brushing teeth
- b. During the day allow 1 hour after any food or drink before collecting saliva samples
- c. It is very important that a good clear sample is received i.e. no contamination with food, lipstick, blood (bleeding gums) or other such extraneous materials.

Saliva Processing Instructions with SaliSet

Let the saliva flow down through the straw into the centrifuge glass tube

- 1. Centrifuge the sample for 15 minutes at 3000 rpm
- 2. Store at 20°C for at least 1 hour
- 3. Centrifuge again for 15 minutes at 3000 rpm
- 4. The saliva sample is now ready to be tested.
- 5. Store the sample at 2- 8°C for one week or at 20°C for longer time.

Saliva Processing Instructions with Salivette (Sardstedt)

- 1. Remove the swab from the suspended insert of the Salivette
- 2. Gently chewing the swab for 1 minute produces a sufficient quantity of saliva.
- 3. Replace the swab into the Salivette and firmly close the tube using the stopper.
- 4. Centrifuge the Salivette for 2 minutes at 1000g (rcf) for saliva generation.
- 5. Remove the insert complete with the swab from the centrifuge vessel and discard. The clear saliva is now ready for analysis (at least 1 mL of saliva should be recovered with this method).

Procedure

- Allow all reagents to reach room temperature (22 °C 28 °C).
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored _ at 2 °C - 8 °C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (S0-S4), two for each Control, two for each sample, one for Blank. Pinette.

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	Standard	Sample	Blank	
Sample		50 µl		
Standards S ₀ -S ₄	50 µl			
Diluted Conjugate	150 µl	150 μl		
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Incubate at 37°C for 15 minutes.

Remove the contents from each well; wash the wells with 0.3 mL of diluted wash solution. Repeat the washing procedure two more times, for a total number of three washings, by draining the water completely.

Pipette					
	Standard	Sample	Blank		
TMB-Substrate	100 µl	100 µl	100 μl		
Incubate at room temperature 22+28°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			

the absorbance (E) at 450 nm against Blank.

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.

RESULTS

Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the standard curve and of each sample

Standard Curve

Plot the mean value of absorbance of the standards (Em) against concentration. Draw the best-fit curve through the plotted points. (es: Four Parameter Logistic).

Calculation of Results

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL.

Reference Value

As the values of salivary DHEA-S have a cicardian pattern we suggest collecting 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 Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation.

Therefore each laboratory should consider the range given by the manufacturer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works

Performance and Characteristics

Precision

Intra Assay Variation

Within run variation was determined by replicate determination (16x) of two different control sera in one assay. The within assay variability is 4.8%.

Inter Assay Variation

Between run variation was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is 8.9%.

Accuracy

The recovery of 1.25 - 2.5 - 5.0 ng/mL of DHEA-S added to sample gave an average value (±SD) of $102.7\% \pm 4.6\%$ with reference to the original concentrations.

Sensitivity

The lowest detectable concentration of DHEA-S that can be distinguished from the zero standard is 0.045 ng/ml at the 95 % confidence limit.

Specificity

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

DHEA-S	100%
DHEA	65.0%
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 %

Hook Effect

The DHEA-S ELISA, a competitive enzyme immunoassay, shows no Hook Effect up to 40 µg/ml.

WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

BIBLIOGRAPHY

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TROUBLESHOOTING ERRORS / POSSIBLE CAUSES / SUGGESTIONS No colorimetric reaction

- no conjugate pipetted
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers
- insufficient washing (conjugates not properly removed)
- too high within-run CV%
- reagents and/or strips not pre-warmed to room temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)
- too high between-run CV %
- incubation conditions not constant (time, temperature)

controls and samples not dispensed at the same time (with the same intervals) (check pipetting order) person-related variation

Symbols:

+2	Storage temperature	***	Manufacturer	Σ	Contains sufficient for <n> tests</n>
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
i	Consult instructions for use	CONT	Content	CE	CE labelled
Â	Caution	REF	Catalogue number	RUO	For research use only!