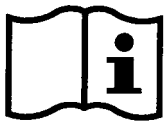
Product information



User's Manual

DHEA-S in Saliva ELISA

Enzyme Immunoassay for the determination of DHEA-S in Saliva

REF IB79308

Σ 96 Wells

RUO **For Research Use Only – Not for Use in Diagnostic Procedures**

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Version 08/13 / DMC
Updated 140715

INTENDED USE

Competitive immunoenzymatic colorimetric method for determination of DHEA-S concentration in saliva. DHEA-S Saliva kit is intended for research use only, not for use in diagnostic procedures.

1. SIGNIFICANCE

5-Dehydroepiandrosterone (DHEA-5) is a endogenous natural steroid hormone with 19 carbon atoms. It is the principal steroid hormone produced by the secretion of the adrenal glands, but it is also produced in the gonads and brain. DHEA is the most abundant circulating steroid in human beings.

DHEA-S is a natural steroid hormone found primarily in the kidneys and it is derived from the enzymatic conversion of DHEA in the adrenal and extra-adrenal tissues. It is the most abundant hormone in the human body and is a 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: for example its production in the brain suggests a role as neurosteroid.

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.

2. 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 (H_2O_2) and the TMB Substrate and develops a blu color that changes into yellow when the Stop Solution (H_2SO_4) is added.

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

DHEA-S concentration in the sample is calculated through a calibration curve.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. **Calibrators** (5 vials, 1 mL each)
   CAL0 – CAL4
2. **Controls** (2 vials, 1 mL each)
   Low Control
   High Control
3. **Incubation Buffer** (1 vial, 30 mL)
   Phosphate buffer pH 7.5, BSA 1 g/L
4. **Conjugate** (1 vial, 1 mL)
   DHEA-S conjugated with horseradish peroxidase (HRP)
5. **Coated Microplate** (1 microplate breakable)
   Anti DHEA-S antibody adsorbed on microplate
6. **TMB Substrate** (1 vial, 15 mL)
   H_2O_2-TMB 0.26 g/L *(avoid any skin contact)*
7. **Stop Solution** (1 vial, 15 mL)
   0.3 N acidic solution *(avoid any skin contact)*
8. **50X Conc. Wash Solution** (1 vial, 20 mL)
   NaCl 45 g/L, Tween-20 55 g/L

3.2. Reagents necessary not supplied
Distilled water.

3.3. Auxiliary materials and instrumentation
- Automatic dispenser.
- Microplates reader (450 nm, 620-630)
- Saliva Collection Device

Note

Store all reagents at 2÷8°C in the dark.

Open the bag of reagent 5 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, the microplate is stable until the expiry date of the kit.

4. WARNINGS
- This kit is intended for research use only 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^{R} 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 acidic solution. Acidic solution 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/H_2O_2 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.
- The significance of the determination DHEA-S can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.

5. PRECAUTIONS
- 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 2-8°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 (22-28°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 saliva samples.
- Maximum precision is required for reconstitution and dispensation of reagents.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of the Calibrators (C_0…C_4)

Before use, mix for 5 minutes with a rotating mixer.
The Calibrators are ready to use and have the following concentrations of DHEA-S:

	C_0	C_1	C_2	C_3	C_4
ng/mL	0	0.2	1.0	3.0	12.0

Dilute 1:2 the samples with concentration greater than 12.0 ng/mL with Calibrator 0.
Once opened, the Calibrators are stable at 2-8°C for 6 months.
For SI UNITS: ng/mL x 2,71 = nmol/L

6.2. Preparation of Diluted Conjugate

Prepare immediately before use.
Add 10 µL of Conjugate (reagent 4) to 1.0 mL of Incubation Buffer (reagent 3). Mix gently. Stable 3 hours at room temperature (22÷28°C).

6.3. Preparation of Wash Solution

Dilute the content of each vial of "50X Conc. Wash Solution" with distilled water to a final volume of 1000 mL prior to use. For smaller volumes respect the 1:50 dilution ratio. The diluted wash solution is stable for 30 days at 2÷8°C.

6.4. Preparation of the Sample

The determination of DHEA-S with this kit should be performed in saliva.

6.4.1.Sample Collection

For the correct collection of saliva we are recommending to only use appropriate devices made from ultra-pure polypropylene. Do not use any PE devices or Salivettes for sampling; this in most cases 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 IBL-America 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 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. The typical timing for a morning collection period would be as follows. Wake-up at 6:00 AM, drinking water and brushing teeth, 1st sample at 6:15 AM, followed by samples at 6:45 AM, 7:15 AM, 7:45 AM, and 8:15 AM, followed by breakfast at 8:25 AM. The typical timing for an afternoon collection period would be like: 1st sample at 5:00 PM, followed by samples at 5:30 PM, 6:00 PM, 6:30 PM, 7:00 PM, followed by dinner at 7:10 PM. Modest variation in the collection timing will not be critical, and the collection time-frame can be extended up to 3 hours.

6.4.2. 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 week. 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 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. Now the clear colorless supernatant is easy to pipette. If the sample should show even a slight reddish tinge 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.

6.5. Procedure

- **Allow all reagents to reach room temperature (22-28°C).** At the end of the assay, store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°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 (C_0-C_4), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Controls	Blank
Sample/ Controls		**50 µL**	
Calibrators C_0-C_4	**50 µL**		
Diluted conjugate	**150 µL**	**150 µL**	
Incubate at 37°C for 15 minutes. Remove the contents from each well; wash the wells 3 times with 0.3 mL of diluted wash solution. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate at room temperature (22÷28°C) for 15 minutes in the dark.			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

7. 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 calibration 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.

8. RESULTS

8.1. Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the calibration curve (C_0-C_4) and of each sample

8.2. Calibration curve

Plot the mean value of absorbance (Em) of the Calibrators (C_0-C_4) against concentration. Draw the best-fit curve through the plotted points. (es: Four Parameter Logistic).

8.3. Results

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

9. REFERENCE VALUES

As the values of salivary DHEA-S have a cicardian 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.

	ng/mL
WOMEN	0,2 – 2,5
MEN	0,2 – 2,7

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 Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

10. PERFORMANCE AND CHARACTERISTICS

10.1. Precision

10.1.1. Intra Assay Variation

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

10.1.2. Inter Assay Variation

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

10.2. 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.

10.3. Sensitivity

The lowest detectable concentration of DHEA-S that can be distinguished from the Calibrator 0 is 0.05 ng/mL at the 95% confidence limit.

10.4. 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 %
Colesterolo	0.001 %

10.5. Correlation

IBL-America DHEA-S Saliva kit was compared to an analogous commercially available kit. 31 saliva samples were analysed according to both test systems.
The linear regression curve was calculated:
$y = 0.37x + 1.10$
$r^2 = 0.826$
y = DHEA-S Saliva IBL-America Kit
x = DHEA-S Saliva Salimetrics Kit

11. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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TROUBLE SHOOTING
ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction
- no conjugate pipetted reaction after addition
- 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
- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)
  too high between-run - incubation conditions not constant (time, CV % temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

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