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Labor Diagnostika Nord GmbH & Co.KG

Instructions for use

DHEA-S Saliva ELISA Free

REF SA E-6500

96

+2 +8 °C

IVD

CE

INTENDED USE

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

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 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. 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

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 (H_2O_2) and the TMB-Substrate (TMB) are added. After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the absorbances 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.

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) 0.4 mL; DHEA-S-HRP conjugate

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 9 gr/L; Tween20 1gr/

SUBSTRATE **MS E-0055 TMB-Substrate**

(1 bottle) 12 mL; H_2O_2-TMB 0.25gr/L (avoid any skin contact)

STOP-SOLN **MS E-0080 Stop Solution**

(1 bottle) 12 mL; Sulphuric acid 0.15 mol/L (avoid any skin contact)

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

- Maximum precision is required for reconstitution and dispensation of the reagents.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants.
- This method allows the determination of DHEA-S from 0.2 ng/mL to 12 ng/mL.
- 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 (S_0,S_1,S_2,S_3,S_4)

Before use, mix for 5 min. with rotating mixer
The standard has the following concentration of DHEA-S:

	S_0	S_1	S_2	S_3	S_4
ng/ml	0	0.2	1.0	3.0	12.0

Once open is stable at +4°C until the expiration date of kit.
For SI UNITS: ng/mL x 2,71 = nmol/L

Preparation of Conjugate

Prepare immediately before use.
Add 10 µl 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 LDN *Saliva Collection Device*.
Do not use sample collector commercially available as "SALIVETTE". 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

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.

Procedure

As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the standard curve (S_0-S_4), two for each sample, one for Blank.

Pipette:

	Standard	Sample	Blank
Sample	---	50 µl	---
Standards S_0 -S_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 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.

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.

LIMITATION OF PROCEDURE

Assay Performance

Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemeic or haemolysed specimen(s) 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 step(s) may result in poor replication and spurious results.

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.

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 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

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% ± 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.

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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)

<u>too high within-run CV%</u>

- reagents and/or strips not pre-warmed to room temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

<u>too high between-run CV %</u>

- 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:

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