DHEA-S ELISA Kit

Catalog Number KA1902

96 assays

Version: 04

Intended for research use only

Table of Contents

Introduction

Intended Use

Competitive immunoenzymatic colorimetric method for quantitative determination of DHEA-S concentration in saliva. For research use only.

Background

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 saliva 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.

Principle of the Assay

The DHEA-S (antigen) in the sample competes with the antigenic DHEA-S conjugated with horseradish peroxidase (HRP) for binding onto 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 blue 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.

General Information

Materials Supplied

List of component

Component	Detail	Amount
Standard A	Standard 0, 0 ng/mL	1 mL
Standard B	Standard 1, 0.2 ng/mL	1 mL
Standard C	Standard 2, 1 ng/mL	1 mL
Standard D	Standard 3, 3 ng/mL	1 mL
Standard E	Standard 4, 12 ng/mL	1 mL
Control 1	Control low, refer to vial labels for expected value and acceptable range.	1 mL
Control 2	Control high, refer to vial labels for expected value and acceptable range.	1 mL
Incubation buffer	Phosphate buffer pH 7.5, BSA 1g/L	30 mL
Conjugate	DHEA-S conjugated with horseradish peroxidase (HRP)	1 mL
Microtiterwells	Anti-DHEA-S antibody adsorbed on microplate	96 (8x12) wells
Conc. Wash Solution 50X	NaCl 45 g/L; Tween 20, 55 g/L	20mL
TMB Substrate	H_2O_2-TMB 0.26 g/L (avoid any skin contact)	15 mL
Stop Solution	Sulphuric acid 0.15 mol/L (avoid any skin contact)	15 mL

Storage Instruction

- ✓ Store all reagents between 2-8°C in the dark.
- ✓ Open the bag of the Coated Microplate only when it is at room temperature and close immediately after use; once opened the microplate is stable until the expiry date of the kit.

Materials Required but Not Supplied

- ✓ Distilled water.
- ✓ Automatic dispenser
- ✓ Microplates reader (450 nm, 620-630)
- ✓ Saliva Collection Device

Precautions for Use

- ✓ 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.
- To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- 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 research 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/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.

✓ Waste management

Reagents must be disposed off in accordance with local regulations.

Assay Protocol

Reagent Preparation

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

Before use, mix for 5 min. with rotating mixer

The Standards are ready to use and have the following concentrations of DHEA-S:

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

Samples with concentration greater than 12.0 ng/mL have to be diluted 1:2 with Standard 0. Once open, the Standards are stable at 2°C – 8°C for 6 months.

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

✓ Preparation of diluted Conjugate

Prepare immediately before use.

Add 10 µL Conjugate to 1.0 mL of Incubation Buffer. Mix gently.

Stable 3 hours at room temperature (22°C – 28°C).

✓ Preparation of Wash Solution

Dilute the content of each vial of Concentrate 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°C - 8°C.

Sample Preparation

✓ Preparation of the Sample

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

It is recommended to collect saliva samples with a centrifuge glass tube and a plastic straw, with the “Salivette” (Sarstedt, Ref. 51.1534.500). Other commercially available sample collector devices have not been tested.

✓ Method and Limitations

Collect saliva samples at the times indicated.

If no specific instructions have been given saliva samples may be collected at any time, paying attention to the following indications:

- If saliva collection is to be carried out in the morning ensure that this is carried out prior to brushing teeth.
- During the day allow 1 hour after a meal, oral intake of pharmaceutical drugs or tooth cleaning.
- 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 glass tubes

  Let the saliva flow down through the straw into the centrifuge glass tube.
- Centrifuge the sample for 15 minutes at 3000 rpm
- Store at -20 °C for at least 1 hour
- Centrifuge again for 15 minutes at 3000 rpm
- The saliva sample is now ready to be tested.
- Store the sample at 2°C - 8°C for one week or at -20 C for longer time.

- ✓ Saliva Processing Instructions with Salivette (Sardstedt)
- Remove the swab from the suspended insert of the Salivette
- Gently chewing the swab for 1 minute produces a sufficient quantity of saliva.
- Replace the swab into the Salivette and firmly close the tube using the stopper.
- Centrifuge the Salivette for 2 minutes at 1000 g (rcf) for saliva generation.
- 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).

Assay Procedure

- Allow all reagents to reach room temperature (22°C - 28°C). At the end of the assay, store immediately the reagents at 2 °C - 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°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.

<table>
<tr><td>Reagent</td><td>Standard</td><td>Sample/Controls</td><td>Blank</td></tr>
<tr><td>Sample/Controls</td><td>-</td><td>50 µL</td><td>-</td></tr>
<tr><td>Standards (S0 – S4)</td><td>50 µL</td><td>-</td><td>-</td></tr>
<tr><td>Diluted Conjugate</td><td>150 µL</td><td>150 µL</td><td>-</td></tr>
<tr><td colspan="4">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.
Automatic washer: if you use automated equipment, wash the wells at least 5 times.</td></tr>
<tr><td>TMB-Substrate</td><td>100 µL</td><td>100 µL</td><td>100 µL</td></tr>
<tr><td colspan="4">Incubate at room temperature 22-28°C for 15 minutes in the dark.</td></tr>
<tr><td>Stop Solution</td><td>100 µL</td><td>100 µL</td><td>100 µL</td></tr>
<tr><td colspan="4">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.</td></tr>
</table>

Data Analysis

Calculation of Results

- ✓ Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the calibration curve (S0 – S4) and of each sample.

- ✓ Calibration Curve

Plot the mean value of absorbance (Em) of the standards (S0 – S4) 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 calibration curve to obtain the corresponding values of the concentrations expressed in ng/mL.

- ✓ 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.

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.

- ✓ 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.

Performance Characteristics

✓ Precision

- 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%.

- 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%.

✓ 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.

✓ 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.

✓ 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%
Androstenedione	20%
Etiocholanolone-S-Na	0.2%
5α Androstandione	0.01%
Testosterone	0.01%
Progesterone	0.01%
17 OH-Progesterone	0.01%
Estrone	0.01%
Cortisol	0.001%
Cholesterol	0.001%

Resources

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

References

1. Joshi, U. M., et al. Steroids 34 (1) 35 (1979)
2. Turkes, A., et al. J Endocrinol. 81 (2) P165 (1979)
3. Ismail A.A, et al. J.Clin.Endocr.Metab. 34,177-184 (1972)
4. Rajkowski,K.M, et al. Steroids 29 no 5 (1977)
5. Widsdom G.B. Clin. Chem. 22/8, 1243 - 1255 (1976)

6. Abraham G.E, et al. Obstet.Gynecol.,47(4),395 (1976)
7. Abraham G.E, et al. Obstet. Gynecol.,53(1),111 (1979)
8. Hopper B.R, et al. J.Clin.Endoc.Metab.40(3),458 (1975)
9. Winter J.S.D,et al. Clin.Obste.and Gynec.,21(1),67 (1978)
10. D. Riad et al. Endocr. Reviews, 3 (4) 304 367 (1978)

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												