



DCM005-10
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DHEA-S ELISA

for routine analysis

Direct immunoenzymatic determination of Dehydroepiandrosterone sulfate (DHEA-S) in human serum or plasma

IVD



LOT

See external label

2°C 8°C



Σ = 96 tests

REF DKO005

INTENDED USE

The [DHEA-S ELISA Assay Kit](#) is a competitive immunoenzymatic colorimetric method for quantitative determination of Dehydroepiandrosterone sulfate (DHEA-S) concentration in human serum or plasma. DHEA-S ELISA Assay Kit is intended for research use only and not to be used in diagnostic procedures.

1. 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.

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.

2. PRINCIPLE OF THE METHOD

In the DHEA-S ELISA Assay Kit, the (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

the 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 color 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. [DHEA-S Calibrators](#)

(6 vials, 1 mL each, ready to use)

CAL0	REF DCE002/0506-0
CAL1	REF DCE002/0507-0
CAL2	REF DCE002/0508-0
CAL3	REF DCE002/0509-0
CAL4	REF DCE002/0510-0
CAL5	REF DCE002/0511-0

2. [DHEA-S Control](#) (1 vial, 1 mL)

Concentration of Control is Lot-specific and is indicated on the Certificate of Analysis

REF DCE045/0503-0

3. [5X Conc. Serum diluent](#) (1 vial, 20 mL)

HEPES 187 mM pH 7.5; BSA 0.5 g/L

REF DCE049/0549-0

4. [Conjugate](#) (1 vial, 12 mL)

DHEA-S conjugate with horseradish peroxidase (HRP)

REF DCE002/0502-0

5. [Coated Microplate](#) (1 breakable microplate)

Anticorpo anti DHEA-S adsorbed on microplate

REF DCE002/0503-0

6. [TMB Substrate](#) (1 vial, 15 mL)

H_2O_2 -TMB 0.26 g/L (avoid any skin contact)

REF DCE004-0

7. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15 mol/L (*avoid any skin contact*)
REF DCE005-0

3.2. Reagents necessary not supplied

Distilled water.

3.3. Auxiliary materials and instrumentation

Automatic dispenser.

Microplates reader (450 nm)

Notes

Store all reagents between 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.

Do not remove the adhesive sheets on the unused strips.

4. WARNINGS

- This DHEA-S ELISA Assay 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 of DHEA-S ELISA Assay Kit contain small amounts of Proclin 300^R as preservatives. 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.
- This DHEA-S ELISA Assay Kit allows the determination of Dehydroepiandrosterone Sulphate from 0.1 µg/mL to 10 µg/mL.
- The clinical significance of the determination Dehydroepiandrosterone Sulphate can be invalidated if the sample 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 of the DHEA-S ELISA Assay Kit 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 DHEA-S ELISA Assay 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 the 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.

6. PROCEDURE

6.1. Preparation of the Calibrators (C₀...C₅)

The Calibrators are ready to use and have the following concentrations:

	C ₀	C ₁	C ₂	C ₃	C ₄	C ₅
µg/mL	0	0.1	0.4	1.0	4.0	10.0

The Calibrators are stable until the expiry date printed on the label. Once opened, the Calibrators are stable for 6 months at 2±8°C.

6.2. Preparation of Serum diluent

Dilute the content of 5X Conc. Serum Diluent to 100 mL with distilled or deionized water in a suitable storage container. Store at room $2\pm 8^{\circ}\text{C}$ until the expiry date printed on the label.

6.3. Preparation of the Sample

The determination of Dehydroepiandrosterone Sulphate can be performed in human plasma as well as in serum of patients.

Store the sample at -20°C if the determination is not performed on the same day of the sample collection. Avoid repetitive freezing and thawing of samples.

Immediately before use, dilute each sample 1:50 with diluted Serum Diluent (i.e. add to 980 μL of diluted Serum Diluent 20 μL of sample). Mix well.

The Control is ready for use.

6.4. PROCEDURE

- Allow all reagents to reach room temperature ($22-28^{\circ}\text{C}$).
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at $2-8^{\circ}\text{C}$.
- 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/ Control	Blank
Diluted Sample/ Control		30 μL	
Calibrators C_0-C_5	30 μL		
Conjugate	100 μL	100 μL	
Incubate at 37°C for 1 hour. Remove the contents from each well. Wash the wells 2 times with 300 μL of distilled water.			
TMB Substrate	100 μL	100 μL	100 μL
Incubate at room temperature ($22\pm 28^{\circ}\text{C}$) 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 Blank.			

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 absorbances (E_m) for each point of the calibration curve (C_0-C_5) and of each sample.

8.2. Calibration Curve

Plot the values of absorbance (E_m) of the Calibrators (C_0-C_5) against concentration.

Draw the best-fit curve through the plotted points (es: Four Parameter Logistic).

8.3. Calculation of Results

Interpolate the values of the samples on the calibration curve to obtain the corresponding values of the concentrations expressed in $\mu\text{g/mL}$.

9. REFERENCE VALUES

The serum or plasma Dehydroepiandrosterone Sulphate reference values are:

	WOMAN $\mu\text{g/mL}$	MAN $\mu\text{g/mL}$
Newborns	0.9 - 1.8	0.9 - 1.8
Before puberty	0.25 - 1.0	0.25 - 1.0
Adults	0.9 - 3.6	0.9 - 3.6
After menopause	< 0.25 - 1.0	---
Pregnancy	0.25 - 1.8	---

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.

10. PERFORMANCE AND CHARACTERISTICS

10.1. Precision

10.1.1. Intra-Assay Variation

Within run variation was determined by replicate the measurements of four different control sera in one assay. The within assay variability is $\leq 5.7\%$.

10.1.2. Inter-Assay Variation

Between run variation was determined by replicate (16x) the measurements of three different control sera in different lots. The between assay variability is $\leq 9.6\%$.

10.2. Accuracy

The recovery of 5 – 2.5 – 1.25 – 0.6 $\mu\text{g/mL}$ of DHEA-S added to sample gave an average value ($\pm\text{SD}$) of $96.71\% \pm 12.02\%$ with reference to the original concentrations.

The dilution test performed on three sera diluted 2 - 4 - 8 times gave an average value ($\pm\text{SD}$) of $98.19\% \pm 5.70\%$.

10.3. Sensitivity

The lowest detectable concentration of DHEA-S that can be distinguished from the Calibrator 0 is 0.03 $\mu\text{g/mL}$ 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 %
Androstenedione	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 %

10.5. Correlation with RIA

Diametra DHEA-S kit was compared to another commercially available DHEA-S assay. Serum samples of 29 females and 20 males were analysed according in both test systems.

The linear regression curve was calculated:

$$(\text{DHEA-S Diametra}) = 0.94 * (\text{DHEA-S RIA}) - 0.01$$
$$r^2 = 0.906$$

11. WASTE MENAGEMANT

Reagents must be disposed off in accordance with local regulations.

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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