DHEA-S ELISA

For the quantitative determination of DHEA-S in serum and plasma.

For In Vitro Diagnostic use in the United States.
For Research Use Only outside of the United States.

Catalog Number: 20-DHSHU-E01
Size: 96 wells
Version: 8.0 2023-12-04 - fd - ALPCO 2.0

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1. Introduction

1.1 Intended Use

The **DHEA-S ELISA** is an enzyme immunoassay for the quantitative measurement of DHEA-S in serum and plasma (EDTA, heparin, or citrate). For *in vitro diagnostic* use in the United States. For Research Use Only outside of the United States.

1.2 Summary and Explanation

Dehydroepiandrosterone (5-Androstene-3β-0L-17-one, Androstenolone, Dehydroisoandrosterone, Transdehydroandrosterone, DHEA) is a steroid hormone present in blood mostly in its sulfate form (DHEA-S) (1). DHEA-S is a more specific product of the adrenals and measurements of this steroid are widely used in clinical practice. The clinical importance of plasma assays of DHEA-S is associated with the diagnosis of adrenal hyperplasia and differential diagnosis of hirsutism (2, 3).

2 PRINCIPLE OF THE TEST

The DHEA-S ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with a polyclonal antibody directed towards an antigenic site on the DHEA-S molecule. Endogenous DHEA-S of a patient sample competes with a DHEA-S-horseradish peroxidase conjugate for binding to the coated antibody. After incubation, the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of DHEA-S in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to the concentration of DHEA-S in the patient sample.

3 PRECAUTIONS

- This kit is for in vitro diagnostic use only. For professional use only.
- For information on hazardous substances included in the kit please refer to the Safety Data Sheets
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA-approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use. Be sure that everything is understood.
- The microplate contains snap-off strips. Unused wells must be stored at 2°C to 8°C in the sealed foil pouch and used in the frame provided.
- Pipet samples and reagents as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents, this applies especially to substrate reservoirs. Using a reservoir for dispensing a substrate solution that was also used for the conjugate solution may discolor the solution. Do not pour reagents back into vials as reagent contamination may occur.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Allow reagents to reach room temperature (21-26°C) before staring the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
- Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where samples or reagents are handled.
- Wear disposable latex gloves when handling samples and reagents. Microbial contamination of reagents or samples may give false results.
- Handle according to an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond the expiry date shown on the kit labels.
- All indicated volumes must be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.

- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even from the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may be slightly different.
- Chemicals and prepared or used reagents must be treated as hazardous waste according to national and local biohazard safety guidelines or regulations.
- Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
- Some reagents contain Proclin 300, BND, and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
- TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with plenty of water and skin with soap and water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- The Safety Data Sheet for this product is available upon request.

4 REAGENTS

4.1 Reagents provided

1. **Microtiterwells**, 12x8 (break apart) strips, 96 wells; Wells coated with anti-DHEA-S antibody (polyclonal).
2. ***Standards (Standards 0-6),** 7 vials, 1 mL, ready to use;
   Concentrations: 0, 0.1, 0.5, 1, 2.5, 5, 10 µg/mL
   Conversion: 1 µg/mL = 2.6 µmol/L
3. ***Enzyme Conjugate**, 1 vial, 25 mL, ready to use; DHEA-S conjugated to horseradish peroxidase
4. **Substrate Solution**, 1 vial, 14 mL, ready to use; Tetramethylbenzidine (TMB).
5. **Stop Solution**, 1 vial, 14 mL, ready to use; contains 0.5M H_2SO_4.
   Avoid contact with the stop solution. It may cause skin irritations and burns.
6. **Wash Solution**, 1 vial, 30 mL (40X concentrated); see ”Reagent Preparation“

*Standards and Enzyme Conjugate contain non-mercury preservative.

4.2 Materials required but not provided

- A microtiter plate calibrated reader (450±10 nm)
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled or deionized water
- Timer
- Semi-logarithmic graph paper or software for data reduction
- Centrifuge

4.3 Storage and stability of the Kit

When stored at 2-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, carefully close it tightly again. Opened kits retain activity for two months if stored as described.

4.4 Reagent Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.

Wash Solution

Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 1 week at room temperature.

4.5 Kit Disposal

Kit disposal must be made according to national and local regulations. Special information for this product is given in the Safety Data Sheet.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, ALPCO must be informed in writing within one week of receiving the kit. Severely damaged single components should not be used for a test run. They must be stored until a final solution has been determined. After this, they should be disposed according to official regulations.

5 SAMPLE COLLECTION AND PREPARATION

Serum or plasma (EDTA-, Heparin- or citrate plasma) can be used in this assay.
Do not use hemolytic, icteric or lipemic samples.
Please note: Samples containing sodium azide should not be used in the assay.

5.1 Sample Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma:
Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

5.2 Sample Storage and Preparation

Samples should be capped and may be stored for up to 7 days at 2-8°C prior to assaying.
Samples held for a longer time should be frozen *only once* and can be stored for at least 12 months at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Sample Dilution

If in an initial assay, a sample is found to contain more than the highest standard, the samples can be diluted with *Standard 0* and re-assayed as described in the Assay Procedure.
For the calculation of the concentrations this dilution factor must be considered:.
Example:
a) Dilution 1:10 10 µL sample + 90 µL *Standard 0* (mix thoroughly)
b) Dilution 1:100 10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly)

6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and samples must come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control, or sample to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- Generally the enzymatic reaction is linearly proportional to time and temperature.

6.2 Assay Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **25 µL** of each *Standard, Control,* and **samples** with new disposable tips into appropriate wells.
3. Dispense **200 µL** *Enzyme Conjugate* into each well.
   Thoroughly mix for 10 seconds. It is important to completely mix in this step.
4. Incubate for **60 minutes** at room temperature.
5. Briskly shake out the contents of the wells.
   Rinse the wells **3 times** with 1x working *Wash Solution* (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note: The sensitivity and precision of this assay is markedly influenced by correct washing!
6. Add **100 µL** of *Substrate Solution* to each well.
7. Incubate for **15 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **50 µL** of *Stop Solution* to each well.
9. Determine the absorbance (OD) of each well at **450±10 nm** with a microtiter plate reader.
   It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls, and patient samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration from the standard curve.
4. Automated method: The results in this IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. (4 Parameter Robard or 4 Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results. The concentration of the samples can be read directly from this standard curve. Samples with concentrations above the highest standard must be further diluted or reported as > 10 µg/mL. For the calculation of the concentrations this dilution factor must be considered.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 µg/mL)	1.69
Standard 1 (0.1 µg/mL)	1.35
Standard 2 (0.5 µg/mL)	0.93
Standard 3 (1.0 µg/mL)	0.67
Standard 4 (2.5 µg/mL)	0.46
Standard 5 (5.0 µg/mL)	0.33
Standard 6 (10.0 µg/mL)	0.23

7 EXPECTED VALUES HEALTHY POPULATION

Each laboratory should determine its own normal and abnormal values. The reference values of the DHEA-S ELISA for healthy individuals were determined by measuring the values of apparently healthy subjects. 50 male and 49 female samples were measured. q-q plot (quantil-quantil plot) was performed to test normal distribution of values and give the chance to identify and exclude potentially false healthy subjects. Final calculation of 2.5th to 97.5th percentile was done with a data set which was cleared by q-q-plot analysis. In a study conducted with apparently normal healthy adults, using the DHEA-S ELISA the following values were observed:

Population	n	MEAN (µg/mL)	MEDIAN (µg/mL)	2.5th - 97.5th Percentile (µg/mL)	Range (min.-max.) (µg/mL)
Male < 50 years	41	1.73	1.67	0.59 – 3.15	0.34 – 4.02
Male > 50 years	9	1.01	1.01	0.49 – 1.63	0.46 – 1.69
Female < 50 years	42	1.13	1.00	0.40 – 2.22	0.27 – 2.31
Female > 50 years	7	0.63	0.69	0.21 – 1.05	0.17 – 1.11

The results of the DHEA-S ELISA alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests (4).

8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day-to-day validity of results. Use controls at both normal and pathological levels. The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results. It is also recommended to make use of national or international Quality Assessment programs to ensure the accuracy of the results. Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials, patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, and aspiration and wash methods. After checking these items without finding any error, contact ALPCO.

9 PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 0.04 – 10 µg/mL.
The linear range of the assay is between 0.080 – 10 µg/mL.

9.2 Specificity of Antibodies (Cross-Reactivity)

No substantial (>10%) cross-reactivity of the assay to structurally related substances is detected besides Androstenedione and Androsterone. Since physiological concentrations of Androstenedione and Androsterone are approx. 1000-fold lower than for DHEA-S, cross-reactivity will not substantially interfere with DHEA-S measurement.

The following substances were tested for cross-reactivity:

Substance	Conc. Range of Spiked Substance µg/mL	Mean Cross-Reactivity %
DHEA-S	0.50 – 1	100.0
Androstenedione	2 – 4	20.9
Androsterone	5 – 10	8.5
Androsterone - Sulfate	600	< 0.1
Progesterone	10 – 20	4.7
Testosterone	200 – 400	0.3
Estrone	600	0.9
Estriol	600	< 0.1
17β Estradiol	600	< 0.1
Estradiol - Sulfate	600	< 0.1
Cortisol	600	< 0.1

9.3 Analytical Sensitivity

The analytical sensitivity was calculated from the mean minus two standard deviations of twenty (20) replicate analyses of *Standard 0* and was found to be 0.044 µg/mL. The Limit of Blank (LoB) is 0.039 µg/mL.

9.4 Precision

9.4.1 Intra Assay Variation

The within-run variability is shown below:

Sample	n	Mean (µg/mL)	CV (%)
1	20	0.20	4.6
2	20	1.35	5.2
3	20	4.22	4.6

9.4.2 Inter Assay Variation

The between-run variability is shown below:

Sample	n	Mean (µg/mL)	CV (%)
1	32	0.2	6.4
2	32	1.24	10.8
3	32	4.45	5.5

9.4.3 Reproducibility

The between-lot variability is shown below:

Sample	n	Mean Conc. (µg/mL) Lot 1	Mean Conc. (µg/mL) Lot 2	Mean Conc. (µg/mL) Lot 3	Mean Conc. (µg/mL)	Between- Lot CV (%)
1	18	0.61	0.54	0.62	0.59	6.7
2	18	0.96	0.87	1.01	0.95	7.5
3	18	1.73	1.51	1.74	1.66	7.9
4	18	6.88	6.39	6.84	6.70	4.0

9.5 Recovery

Samples have been spiked by adding DHEA-S solutions with known concentrations in a 1:1 ratio.
The % Recovery has been calculated by multiplying the ratio of the measurements and the expected values with 100 (expected value = [(endogenous DHEA-S + added DHEA-S)/2]; because of a 1:2 dilution of serum with spiked material).

Sample	Added Concentration 1:1 (v/v) (µg/mL)	Measured Conc. (µg/mL)	Expected Conc. (µg/mL)	Recovery (%)
1	--	0.2	0.20	100
	1.0	0.7	0.60	109
	2.5	1.4	1.35	106
	5.0	2.8	2.60	108
2	--	1.1	1.10	100
	1.0	1.1	1.05	101
	2.5	2.1	1.80	114
	5.0	3.4	3.05	110
3	--	3.8	3.80	100
	2.5	2.8	3.15	91
	5.0	4.3	4.40	97
	10.0	7.0	6.90	102

9.6 Linearity

Sample	Dilution	Mean Conc. (µg/ml)	Recovery (%)
1	None	4.83	-
	1:2	2.51	104
	1:4	1.19	99
	1:8	0.64	107
	1:16	0.32	106
2	None	1.15	-
	1:2	0.62	108
	1:4	0.31	108
	1:8	0.15	107
	1:16	0.08	109
3	None	4.20	-
	1:2	2.12	101
	1:4	1.02	98
	1:8	0.49	93
	1:16	0.26	101

10 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practices. Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

Hemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 7.5 mg/mL) do not influence the assay results.

10.2 Drug Interferences

Currently, no substances (drugs) are known which influence the measurement of DHEA-S in a sample.

10.3 High-Dose-Hook Effect

No hook effect was observed in this test.

11 LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. The user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, enough controls to validate the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact ALPCO.

11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient. Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived. The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

12 REFERENCES

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