DHEA ELISA

For the quantitative determination of Dehydroepiandrosterone in human serum and plasma (EDTA and Citrate).

For “In Vitro Diagnostic” use within the United States of America.

This product is for “Research Use Only” outside of the United States of America

Catalog Number: 20-DHEHU-E01
Size: 96 wells
Version: 6.0_US 2023/07 vk-ALPCO 3.1

1. Introduction

1.1. Intended Use

The DHEA ELISA is an enzyme immunoassay for the quantitative measurement of dehydroepiandrosterone (DHEA) in human serum and plasma (EDTA or citrate).

The device is intended to be used as an aid to diagnosis for individuals with signs of hypoadrenalism, virilization, 21-hydroxylase deficiency, or hirsutism.

The device is not intended for detection of adrenal adenoma and carcinoma. For in vitro diagnostic use within the United States of America. This product is for Research Use Only outside the United States of America.

1.2. Summary and Explanation

DHEA (androstenolone; 3β-hydroxy-5-androsten-17-one) is a C_{19} steroid produced in the adrenal cortex and, to a lesser extent, gonads. DHEA serves as a precursor in testosterone and estrogen synthesis. DHEA has a rapid metabolic clearance rate as compared to its sulfated conjugate, DHEA-S. Because of this, serum DHEA levels are 100-to 1000-fold lower than DHEA-S levels.

Serum DHEA levels are relatively high in the fetus and neonate, low during childhood, and increase during puberty. Serum DHEA levels then progressively decline after the third decade of life. Serum DHEA levels increase in response to corticotropin and ACTH. No consistent changes in serum DHEA levels occur during the menstrual cycle or pregnancy.

The physiologic role of DHEA has not been conclusively defined. DHEA has relatively weak androgenic activity, which has been estimated at ~10% that of testosterone. However, in neonates, peripubertal children, and in adult women, circulating DHEA levels may be several-fold higher than testosterone concentrations, and rapid peripheral tissue conversion to more potent androgens (androstenedione and testosterone) and estrogens may occur. Moreover, DHEA has relatively low affinity for sex-hormone binding globulin. These factors may enhance the physiologic biopotency of DHEA. A variety of in vitro effects, including anti-proliferative effects in different cell lines and effects on enzyme-mediated cell metabolism, have been reported. In vivo studies suggest that DHEA may affect cholesterol and lipid metabolism, insulin sensitivity and secretion, and immune function.

Measurement of serum DHEA is a useful marker of adrenal androgen synthesis. Abnormally low levels may occur in hypoadrenalism, and elevated levels occur in several conditions including virilizing adrenal adenoma and carcinoma, 21-hydroxylase and 3β-hydroxysteroid dehydrogenase deficiencies, and in female hirsutism. Since very little DHEA is produced by the gonads, measurement of DHEA levels may aid in the localization of androgen source in virilizing conditions. Abnormal DHEA levels have been reported in schizophrenia, obesity, and stress. Therapeutic administration of DHEA has been proposed for several conditions, including obesity and cardiovascular disease.

2. Principle of the Assay

The DHEA 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 (rabbit) antibody directed towards antigenic sites on the DHEA molecule. During the first incubation, endogenous DHEA of a sample competes with a DHEA-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off, and the solid phase is incubated with substrate solution. The colorimetric reaction is stopped by addition of stop solution, and the optical density (OD) of the resulting yellow product is measured. The intensity of color is inversely proportional to the concentration of the analyte in the sample. A standard curve is

constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

3. Warnings and Precautions

1. This kit is for in vitro diagnostic use only within the United States of America and Research Use Only outside the United States of America. For professional use only. Follow good laboratory practices and safety guidelines.
2. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may be slightly differently.
3. Do not use reagents beyond expiry date as shown on the kit labels.
4. Do not reuse microwells.
5. Reagents of other manufacturers must not be used together with the reagents of this test kit.
6. Microbial contamination of reagents or samples may give false results.
7. Allow the reagents to reach room temperature (20°C – 26°C) before starting the test. Temperature will affect the optical density readings of the assay.
8. All indicated volumes must be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
9. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn the solution colored. Do not pour reagents back into vials as reagent contamination may occur.
10. 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.
11. The device contains material of animal origin, which is certified apparently free of infectious or contagious diseases and injurious parasites.
12. Bovine components originate from countries where BSE has not been reported.
13. Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure everything is understood.
14. All reagents in this kit are clear liquids, substrate solution is clear and colorless. Changes in its appearance may affect the performance of the test. In that case, contact ALPCO.
15. Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
16. Do not smoke, eat, drink, or apply cosmetics in areas where samples or kit reagents are handled.
17. Wear lab coats and disposable latex gloves when handling samples and reagents and wear necessary safety glasses.
18. Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation. Waste must be discarded according to local rules and regulations.
19. Avoid contact with Stop Solution containing 0.5M H_2SO_4. It may cause skin irritation and burns.
20. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes or skin with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
21. Some reagents contain preservatives in non-declarable concentrations. Nevertheless, in case of contact with eyes or skin, flush immediately with water.
22. Chemicals and prepared or used reagents must be treated as hazardous waste according to the national safety guideline or regulation.
23. This product does not contain substances which have carcinogenic, mutagenic, or toxic for

reproduction (CMR) properties.

24. All reagents of this test kit do NOT contain hazardous substances in concentrations to be declared, and classification and labelling is not required.
25. For information on hazardous substances included in the kit please refer to Safety Data Sheets.

4. Reagents

4.1. Reagents Provided

Component	Quantity	Description	Preparation
Microtiter wells	12 x 8 wells (separable)	**Microtiter plate** Coated with anti-DHEA antibody (polyclonal)	Ready to use
Standard (Standard 0 - 5)	6 x 1 mL	**Standards *** Concentrations 0.0, 0.37, 1.10, 3.30, 10.0, 30.0 ng/mL Calibrated against certified reference material, Cerilliant # D-063 Conversion: ng/mL x 3.467 = nmol/L	Ready to use
Control Low **Control High**	2 x 1 mL	**Controls*** For control values and ranges refer to vial label or Certificate of Analysis	Ready to use
Enzyme Conjugate	1 x 14 mL	**Enzyme Conjugate *** DHEA conjugated to horseradish peroxidase	Ready to use
Substrate Solution	1 x 14 mL	**Substrate Solution** Contains 3,3',5,5'-tetramethylbenzidine (TMB) *Keep away from light.*	Ready to use
Stop Solution	1 x 14 mL	**Stop Solution** Contains 0.5 M H_2SO_4. Avoid contact with the stop solution. It may cause skin irritation and burns.	Ready to use
Wash Solution	1 x 30 mL	**Wash Solution***, 40 X Concentrate	See *"Reagent Preparation"*
	1 x	Instructions for Use	
	1 x	Certificate of Analysis (CoA)	
		*Contains non-mercury preservative	

4.2. Materials Required but Not Provided

- A microtiter plate calibrated reader (450 nm, with reference wavelength at 620 to 630 nm)
- Calibrated variable precision micropipettes
- Manual or automatic equipment for rinsing microtiter well plates
- Absorbent paper
- Distilled water
- Timer
- Semi-logarithmic graph paper or software for data reduction

4.3. Storage Conditions

Unopened kits and reagents as well as opened reagents must be stored at 2°C to 8°C.

The microtiter plate contains snap-off strips. Do not open the pouch of the wells until it reaches

room temperature. Unused wells must be stored at 2°C to 8°C in the sealed foil pouch including the desiccant and used in the plate frame provided. Once the foil bag has been opened, care must be taken to close it tightly again.

Once opened, reagent vials must be closed tightly again.

	Storage Temperature	Stability
Unopened kits and unopened reagents	2°C to 8°C.	Until expiration date printed on the label. Do not use reagents beyond this date!
Opened Kit	2°C to 8°C.	8 weeks

4.4. Reagent Preparation

Bring all reagents and required number of strips to room temperature (20°C to 26°C) prior to use.

Wash Solution
Add distilled water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated Wash Solution with 1170 mL distilled water to a final volume of 1200 mL.
The 1x working Wash Solution is stable for 1 week at room temperature (20°C to 26°C).

4.5. Disposal of the Kit

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

4.6. Damaged Test Kits

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

5. Sample Collection and Preparation

Human serum or plasma (EDTA or citrate) can be used in this assay.

Notes: Samples containing sodium azide should not be used in the assay. In general, do not use hemolyzed, icteric, or lipemic samples. For further information refer to “Interfering Substances.”

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 more clotting time.

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

Whole blood should not be frozen before centrifugation.

5.2. Sample Storage and Preparation

Samples should be tightly capped and may be stored for up to 7 days at 2-8°C prior to assaying. Samples held for a longer time (up to 12 months) should be frozen only once at -20°C prior to

assay. Thawed samples should be inverted several times prior to testing.

Samples can be assayed without additional preparation.

6. Assay Procedure

6.1. General Remarks

- All reagents and samples must be allowed to come to room temperature (20°C to 26°C) before use.
- All reagents must be mixed without foaming.
- Do not interchange caps of reagent vials to avoid cross-contamination.
- Use new disposable plastic pipette tips for each standard, control, or sample to avoid cross-contamination.
- To avoid cross-contamination and falsely elevated results, pipette samples and dispense conjugate without splashing accurately to the bottom of wells.
- Mix the contents of the microtiter plate wells thoroughly to ensure good test results.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Once the test has been started, all steps should be completed without interruption and in the same sequence for each step.
- The enzymatic reaction is linearly proportional to time and temperature.
- Optical density is a function of the incubation time and temperature. Respect the incubation times and temperatures as given in chapter “Test Procedure”.
- 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.

Important note to wash procedure:
Washing is critical. Improperly washed wells will give erroneous results. The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

Test performance using fully automated analysis devices:
Automated test performance using fully automated, open-system analysis devices is possible. However, the combination must be validated by the user.

6.2. Assay Procedure

Each run must include a standard curve.
The controls serve as internal controls for the reliability of the test procedure. They must be assayed with each test run.
The given test procedure describes manual processing.

1. Secure the desired number of microtiter wells in the frame holder.
2. Dispense 10 µL of each Standard, Control, and sample with new disposable tips into appropriate wells.
3. Dispense 100 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. Wash the wells as follows:

   If an automated plate washer is used, rinse the wells 4 times with 400 µL of 1x working Wash

Solution per well.

For manual washing, rinse the wells 4 times with 300 µL of 1x working Wash Solution per well.

At the end of the washing step, always strike the wells sharply on absorbent paper to remove residual droplets.

Important note: the sensitivity and precision of this assay are markedly influenced by the correct performance of the washing procedure!

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 100 µL of Stop Solution to each well.
9. Read the OD at 450 nm and at 620 to 630 nm (background subtraction recommended) with a microtiter plate reader. It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

6.3. Calculations of Results

1. The concentration of the samples can be read directly from the standard curve.
2. For duplicate determinations, the mean of the two optical density (OD) values for each standard, control, and sample must be taken. If the two values deviate substantially from one another, ALPCO recommends retesting the samples.
3. Samples with concentrations exceeding the highest standard can be further diluted with Standard 0 and re-assayed as described in "Test Procedure" or must be reported as > 30 ng/mL. For the calculation of the concentrations, this dilution factor must be considered.

(Example: dilution 1:10: 10 µL sample + 90 µL Standard 0)

4. Automated method:
The results in the instructions for use have been calculated automatically using a four-parameter logistic (4PL) curve fit. (4PL Rodbard or 4PL Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.

5. Manual method:
Using semi-logarithmic graph paper, construct a standard curve by plotting the (mean) OD obtained from each standard against its concentration with OD value on the vertical (Y) axis and concentration on the horizontal (X) axis. Determine the corresponding sample concentration from the standard curve by using the (mean) OD value for each sample.

6.3.1. Example of Typical Standard Curve

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

Standard	OD (450 nm)
Standard 0 (0 ng/mL)	2.27
Standard 1 (0.37 ng/mL)	1.95
Standard 2 (1.1 ng/mL)	1.59
Standard 3 (3.3 ng/mL)	1.04
Standard 4 (10 ng/mL)	0.58
Standard 5 (30 ng/mL)	0.29

7. Reference Values

It is strongly recommended that each laboratory determine its own reference values.
In a study conducted with apparently healthy subjects, using the DHEA ELISA the following values were observed:

Population	n	Mean (ng/mL)	Median (ng/mL)	2.5th-97.5th Percentile (ng/mL)	Range (min. – max.) Percentile (ng/mL)
Males	40	3.51	2.81	0.87 – 9.56	0.83 – 9.92
Females	40	3.94	4.10	1.01 – 10.30	0.49 – 10.30

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

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 Certificate of Analyses (CoA) added to the kit. The values and ranges stated on the CoA always refer to the current kit lot and must 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 analyzing control values and trends. If the results of the assay do not agree with the established acceptable ranges of control materials, 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, aspiration and washing methods. After checking the above-mentioned items without finding any error, please contact ALPCO.

9. Performance Characteristics

9.1. Specificity of Antibodies (Cross-Reactivity)

The following substances were tested for cross-reactivity of the assay:

Substance	Concentration Range of Spiked Substance (ng/mL)	Mean Cross-Reactivity (%)
DHEA-S	1120 - 11200	0.03
Testosterone	0.8 - 80	0.00
Cortisol	23 - 2300	0.00
Estrone	0.01 - 1	0.00
Estriol	1.5 - 150	2.61
Corticosterone	0.5 - 50	0.00
Estradiol	0.02 - 2	0.00
Aldrosterone	0.01 - 1	0.00
Androstenedione	0.22 - 22	1.40
Progesterone	42.2 - 4220	0.05
17-OH Progesterone	1.2 - 120	0.00
Cortisone	1.6 - 160	0.02

9.2. Sensitivity and Range

The Limit of Blank (LoB) is 0.252 ng/mL.
The Limit of Detection (LoD) is 0.453 ng/mL.
The Limit of Quantification (LoQ) is 0.634 ng/mL.
Measuring Range is 0.453 – 30 ng/mL.
Linear Range is 0.900 – 30 ng/mL.

9.3. Reproducibility

9.3.1. Within-Run Precision

The within-run precision was determined with 4 samples covering the complete range in 5 independent runs within 5 days in 5 replicates per run. CV was calculated as mean CV of 5 runs.

Sample	n	Mean (ng/mL)	CV (%)
1	5	1.37	3.5
2	5	3.99	5.2
3	5	6.19	4.7
4	5	18.06	6.9

9.3.2. Between-run Precision

The between-run variation was determined with 4 samples. The 4 samples are measured in 5 days with 5 replicates per run. 25 data points are generated per sample (5 replicates x 5 runs = 25 data points).

Sample	n	Mean (ng/mL)	CV (%)
1	25	1.37	12.7
2	25	3.99	12.5
3	25	6.19	8.8
4	25	18.06	14.4

9.3.3. Between Lot Precision

The between-lot variation was determined by 6 measurements of different samples with 3 different kit lots.

Sample	n	Mean (ng/mL)	CV (%)
1	18	1.42	2.8
2	18	4.36	6.3
3	18	6.84	4.9
4	18	20.70	7.1

9.4. Recovery

Recovery was determined by adding increasing amounts of the analyte to different samples containing different amounts of endogenous analyte. The percentage recoveries were determined by comparing expected and measured values of the samples.

	Sample 1	Sample 2	Sample 3	Sample 4
Concentration (ng/mL)	1.21	1.96	4.11	11.16
Average Recovery (%)	104.5	111.3	93.0	106.0

Range of Recovery (%)	**from**	98.2	106.3	90.2	103.9
	to	109.4	114.0	96.1	109.1

9.5. Linearity

Samples containing different amounts of analyte were serially diluted with Standard 0. The percentage recovery was calculated by comparing the expected and measured values for the analyte.

		Sample 1	**Sample 2**	**Sample 3**	**Sample 4**
Concentration (ng/mL)		9.82	15.95	16.20	17.40
Average Recovery (%)		111.2	108.2	103.3	94.5
Range of Recovery (%)		110.0	102.7	96.3	86.2
	from				
	to	114.1	110.3	112.3	101.1

10. Limitations of Use

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the instructions for use and with adherence to good laboratory practice. 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) have no influence on the assay results.

10.2. Drug Interferences

Currently no substances (drugs) are known for this assay, which have an influence on the measurement of DHEA in a sample.

10.3. High-Dose-Hook Effect

No hook effect was observed in this test up to 600 ng/mL of DHEA.

11. Legal Aspects

11.1. Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover, 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 assay procedure, a sufficient number of controls for validating 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. If there is any doubt or concern regarding a result, please contact ALPCO.

11.2. Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results agree 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.

11.4 Reporting of Serious Incident

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent regulatory authorities in which the user and/or the patient is established.

12. References

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