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Instructions for use

DHEA ELISA

REF **AA E-1600**

IVD CE

DHEA ELISA

1. INTRODUCTION

1.1 Intended Use

Enzyme immunoassay for the quantitative determination of Dehydroepiandrosterone (DHEA) in human serum and plasma (EDTA or citrate plasma).
The assay is intended for in vitro diagnostic use by professional users only. Manual processing is recommended. The usage of laboratory automats is the user's sole responsibility.
The kit is intended for single use only.

1.2 Description of the analyte

Dehydroepiandrosterone (DHEA) is a steroid hormone that is produced in the adrenal glands. Together with its sulfate ester DHEA-S, it is quantitatively the main secretion product of the adrenal glands and serves as a precursor of androgenic and estrogenic steroids. DHEA and DHEA-S are in balance. The concentration of DHEA-S is about 1,000 times higher than of DHEA (1, 4).
The fetal adrenal gland produces large amounts of DHEA and DHEA-S during fetal development and decreases significantly in the first months of life. The adrenal secretion of DHEA and DHEA-S then increases again during the adrenal phase in children aged 6 – 8 years. Maximum levels of circulating DHEA-S and DHEA are reached between 20 and 30 years of age. After that, the levels of serum DHEA and DHEA-S decrease (1, 2, 3).
The measurement of DHEA in serum is a useful marker of adrenal androgen synthesis. Elevated levels occur under various conditions, including 11β-hydroxylase and 3-hydroxysteroid dehydrogenase deficiencies, and in some cases female hirsutism (4). Since very little DHEA is produced by the gonads, measurement of DHEA levels can help to locate the source of androgen under virilizing conditions.
Abnormal DHEA levels have been reported in schizophrenia and obesity. Therapeutic administration of DHEA has been proposed for several conditions, including obesity and cardiovascular disease.

2. PRINCIPLE

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 an anti DHEA antibody. An unknown amount of DHEA present in the sample competes with a DHEA-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 in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to the concentration of DHEA 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. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the reagents should be handled in the same manner as potentially infectious material.
4. The microplate contains break apart strips. Unused wells must be stored at 2 – 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing substrate solution that had previously been used for conjugate solution may turn solution coloured. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the washing steps.
9. Allow the reagents to reach room temperature (18 – 25 °C) before starting the test. Temperature will affect the absorbance readings of the assay.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable protective gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.

13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. 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 different.
17. Avoid contact with Stop Solution. It may cause skin irritation and burns.
18. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
19. For information please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from the manufacturer.
20. All serious incidents occurring in relation to products made available on the EU market in accordance with Article 2(61) of Regulation (EU) 2017/746 shall be notified to the manufacturer and to the competent authority of the Member State where the user or patient is established in accordance with Article 82 of Regulation (EU) 2017/746.
21. If product information, including labeling, is incorrect or inaccurate, please contact the kit manufacturer or supplier.

4. REAGENTS

4.1 Reagents provided

AA E-1631 96 **Microtiterplate**

Content: 12 x 8 (break apart) strips with 96 wells; wells coated with anti-DHEA antibody.

Standards and **Controls** - Ready to use

Cat. no.	Component	Standard	Concentration	Volume/ Vial
AA E-1601	STANDARD A	Standard A	0 ng/ml	0.6 ml
AA E-1602	STANDARD B	Standard B	0.3 ng/ml	0.6 ml
AA E-1603	STANDARD C	Standard C	1 ng/ml	0.6 ml
AA E-1604	STANDARD D	Standard D	3 ng/ml	0.6 ml
AA E-1605	STANDARD E	Standard E	10 ng/ml	0.6 ml
AA E-1606	STANDARD F	Standard F	30 ng/ml	0.6 ml
AA E-1651	CONTROL 1	Control 1	For control values and ranges please refer to QC-Report.	0.6 ml
AA E-1652	CONTROL 2	Control 2		0.6 ml

Content: Standards: Serum matrix spiked with defined quantity of DHEA.
Controls: Serum matrix with defined quantity of DHEA.

AA E-1640 CONJUGATE **Enzyme Conjugate** - Ready to use

Content: Horseradish peroxidase-labelled DHEA in buffered matrix.

Volume: 1 x 13 ml/vial

AA E-1655 SUBSTRATE **Substrate Solution** - Ready to use

Content: Contains Tetramethyl-benzidine (TMB).

Volume: 1 x 26 ml/vial

AA E-1680 STOP-SOLN **Stop Solution** - Ready to use

Content: Contains 2 N hydrochloric acid solution. Avoid contact with the stop solution. It may cause skin irritations and burns.

Volume: 1 x 9 ml/vial

Hazards identification:

H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.
H335 May cause respiratory irritation.

AR E-0030 WASH-CONC 10x **Wash Solution** - 10X concentrated

Volume: 1 x 50 ml/vial
see "Reagent Preparation" (4.4).

4.2 Materials required but not provided

- A microtiter plate reader capable for endpoint measurement at 450 nm
- Calibrated variable precision micropipettes
- Microplate mixer operating at 900 rpm
- Absorbent paper
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

4.3 Storage conditions

When stored at 2 – 8 °C unopened reagents will be stable until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2 – 8 °C. After first opening the reagents are stable for 30 days if used and stored properly. Keep away from heat and direct sunlight.
Microtiter wells must be stored at 2 – 8 °C. Take care that the foil bag is sealed tightly.

4.4 Reagent preparation

Allow the reagents and the required number of wells to reach room temperature (18 – 25 °C) before starting the test.

Wash Solution:
Dilute 50 ml of 10x concentrated Wash Solution with 450 ml deionized water to a final volume of 500 ml. The diluted Wash Solution is stable for at least 12 weeks at room temperature (18 – 25 °C). Precipitates may form when stored at 2 – 8 °C, which should dissolve again by swirling at room temperature (18 – 25 °C). The wash solution should only be used when the precipitates have completely dissolved.

4.5 Disposal of the kits

The disposal of the kit must be made according to the 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, the manufacturer has to be informed in writing within one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5. SPECIMEN COLLECTION AND STORAGE

For determination of DHEA **serum or plasma (EDTA, citrate plasma)** can be used.
The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use hemolytic, icteric or lipemic specimens. Furthermore, we recommend special caution when using serum gel collection systems, as an influence on the measurement results cannot be excluded in case of improper handling. Samples containing sodium azide should not be used in the assay.
The procedure calls for 25 µl sample per well. The samples should be assayed immediately or aliquoted and stored at ≤ -20 °C up to 12 months. Avoid repeated freeze-thaw cycles. Samples expected to contain DHEA concentrations higher than the highest standard (30 ng/ml) should be diluted with the Standard A before assayed. The additional dilution step has to be taken into account for the calculation of the results.

6. ASSAY PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature (18 – 25 °C) 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 in order to avoid cross contamination.
- Optical density 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.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Respect the incubation times as stated in this instructions for use.
- Standards, controls, and samples should at least be assayed in double determinations.
- A standard curve must be established for every run

6.2 Assay procedure

1.	Prepare a sufficient number of microplate wells to accommodate standards, controls and samples in duplicates.
2.	Dispense **25 µl** of each **Standard, Sample, and Controls** with new disposable tips in duplicates into appropriate wells.
3.	Dispense **100 µl** of **Enzyme Conjugate** into each well.
4.	Incubate for **60 minutes** at room temperature (18 – 25 °C) on a plate shaker (900 rpm).
5.	Discard the content of the wells and rinse the wells **4 times** with diluted **Wash Solution** (300 µl per well). Remove as much Wash Solution as possible by beating the microplate on absorbent paper.
6.	Add **200 µl** of **Substrate Solution** to each well.
7.	Incubate without shaking for **30 minutes** at room temperature (18 – 25 °C) in the dark.
8.	Stop the reaction by adding **50 µl** of **Stop Solution** to each well.
9.	Determine the optical density of each well at 450 nm and read the wells within 15 minutes.

6.3 Calculation of results

1. Calculate the average optical density (OD) values for each set of standards, controls, and samples.
2. The obtained optical densities of the standards (y-axis, linear) are plotted against their corresponding concentrations (x-axis, logarithmic) either on semi-logarithmic paper or using an automated method.
3. Using the mean OD value for each sample, determine the corresponding concentration from the standard curve.
4. Automated method: The results in the package insert have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred calculation method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be determined directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted with Standard A and assayed again. For the calculation of the concentrations, this dilution factor has to be taken into account.

Example of typical standard curve
Following data are intended for illustration only and must not be used to calculate results from another run.

Standard	Optical Density (450nm)
Standard A (0 ng/ml)	3.003
Standard B (0.3 ng/ml)	2.501
Standard C (1 ng/ml)	1.912
Standard D (3 ng/ml)	1.220
Standard E (10 ng/ml)	0.647
Standard F (30 ng/ml)	0.341

7. EXPECTED VALUES

It is strongly recommended that each laboratory should determine its own normal and pathological values. Samples from apparently normal healthy adults, collected in the morning, were analyzed using the DHEA ELISA and the following values were observed:

		ng/ml			
Population	**n**	**Range**	**Median**	**2.5 percentile**	**97.5 percentile**
Female < 50 years	39	1.9 - 12.6	4.9	2.2	12.0
Female ≥ 50 years	20	1.1 - 5.4	2.4	1.1	4.7
Male < 50 years	20	2.4 - 15.8	5.7	2.7	15.3
Male ≥ 50 years	22	0.3 - 5.6	2.0	0.7	4.8

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

8. QUALITY CONTROL

Good laboratory practice requires that controls are run with each standard 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 included in the kit. The values and ranges stated on the QC certificate 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 in order 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, microtiter plate reader, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or the manufacturer directly.

9. PERFORMANCE CHARACTERISTICS

9.1 Analytical Sensitivity

The lowest analytical detectable level of DHEA that can be distinguished from the Zero Standard is 0.082 ng/ml at the 2SD confidence limit.

9.2 Specificity (Cross Reactivity)

The following materials have been evaluated for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to DHEA.

Steroid	**% Cross reaction**
DHEA-S	0.06
Testosterone	< 0.02
Androstendione	< 0.02
Progesterone	0.03
17α-Hydroxyprogesterone	< 0.02
Pregnenolone	0.03
Prednisone	< 0.02
Prednisolone	< 0.02
Corticosterone	< 0,02
11-Deoxycorticosterone	0.2
Cortisol	< 0.02
11-Deoxycortisol	< 0.02
Cortisone	< 0.02
Dexamethasone	< 0.02
17ß-Estradiol	< 0.02
Estrone	< 0.02
Estriol	< 0.02
Danazole	< 0.02

9.3 Assay dynamic range

The range of the assay is between 0.3 – 30 ng/ml.

9.4 Reproducibility

9.4.1 Intra-Assay

The intra-assay variation was determined by 20 replicate measurements of three serum samples within one run. The intra-assay variability is shown below:

	Serum 1	Serum 2	Serum 3
Mean (ng/ml)	2.01	6.02	27.63
SD	0.17	0.39	2.13
CV (%)	8.2	6.4	7.7
n =	20	20	20

9.4.2 Inter-Assay

The inter-assay variation was determined by duplicate measurements of three serum samples in ten different runs.

	Serum 1	Serum 2	Serum 3
Mean (ng/ml)	1.74	5.86	14.61
SD	0.18	0.35	0.69
CV (%)	10.3	6.0	4.7
n =	10	10	10

9.5 Recovery

Recovery was determined by adding increasing amounts of the analyte to three different samples containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) was measured by the DHEA ELISA. The percentage recoveries were determined by comparing expected and observed results of the samples.

Serum	Spiking (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
1	-	0.74	-	-
	3.0	3.74	3.7	100
	6.0	6.97	6.7	103
	9.0	9.57	9.7	98
2	-	3.71	-	-
	3.0	7.09	6.7	106
	6.0	9.49	9.7	98
	9.0	12.52	12.7	99
3	-	3.73	-	-
	3.0	6.69	6.7	99
	6.0	10.30	9.7	106
	9.0	13.75	12.7	108

9.6 Linearity

Three serum samples were assayed undiluted and diluted with the zero standard. The percentage linearity was calculated by comparing the expected and observed values.

Serum	Dilution	Observed (ng/ml)	Expected (ng/ml)	Linearity (%)
1	-	17.36	-	-
	1 : 2	8.35	8.7	96
	1 : 4	4.11	4.3	95
	1 : 8	1.86	2.2	86
2	-	15.18	-	-
	1 : 2	7.58	7.6	100
	1 : 4	3.81	3.8	100
	1 : 8	1.69	1.9	89
3	-	11.04	-	-
	1 : 2	5.57	5.5	101
	1 : 4	2.89	2.8	105
	1 : 8	1.51	1.4	109

10. LIMITATIONS OF PROCEDURE

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 practice. Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

- Hemolytic samples should not be used in the DHEA ELISA to exclude any interferences.
- Bilirubin (up to 0.2 mg/ml), and Lipids (up to 30 mg/ml) show no influence on the assay results. However, we recommend not to use any icteric or lipemic specimens to avoid any interferences.
- Samples containing sodium azide should not be used in the assay.
- The result of any immunological test system may be affected by heterophilic antibodies, anti-species antibodies or rheumatoid factors present in human samples. For example, the presence of heterophilic antibodies in patients who are regularly exposed to animals or animal products may interfere with immunological tests. Therefore, interference with this in vitro immunoassay cannot be excluded. If unplausible results are suspected, they should be considered invalid and verified by further testing. For diagnostic purposes, results should always be considered only in conjunction with the patient's clinical picture and further diagnostic tests.

10.2 Drug Interferences

Any medication (cream, oil, pill etc.) containing DHEA, or DHEA-S will significantly influence the measurement of this analyte. Any medication should be taken into account when assessing the results.

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 a sufficient number of controls within the test procedure 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. In case of any doubt or concern please contact the manufacturer.

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 therapeutic consequences should 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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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		