Useŕs Manual

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DHEA ELISA

Enzyme immunoassay for the in-vitro diagnostic quantitative determination of DHEA in human serum and plasma

IVD

CE

REF **DEM-DEH3344**

Σ **96 Wells**

Version 3 05/16

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1 INTRODUCTION

1.1 Intended Use

The **DHEA ELISA** is a competitive immunoassay for the quantitative *in vitro diagnostic* measurement of Dehydroepiandrosterone (DHEA) in serum and plasma (EDTA).

1.2 Summary and explanation

Dehydroepiandrosterone (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. Due to the presence of a 17-oxo (rather than hydroxyl) group, 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.
The physiologic role of DHEA has not been conclusively defined. A variety of in vitro effects, including antiproliferative 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. 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.
Serum DHEA levels are relatively high in the fetus and neonate, low during childhood, and increase during puberty. Increased levels of DHEA during adrenarche may contribute to the development of secondary sexual hair. Serum DHEA levels progressively decline after the third decade of life. No consistent changes in serum DHEA levels occur during the menstrual cycle or pregnancy; however, parity may lower serum DHEA levels in premenopausal women.
DHEA has a rapid metabolic clearance rate as compared to its sulfated conjugate, DHEA-S. Because of this, serum DHEA levels are 100-1000 fold lower than DHEA-S levels. In addition, serum DHEA levels show significant diurnal variation which is dependent on adrenocorticotrophic hormone (ACTH). Serum DHEA levels increase in response to exogenous ACTH administration.
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 some cases of 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.

2 PRINCIPLE

The DEMEDITEC 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 an 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.

3 WARNINGS AND PRECAUTIONS

1. This kit is for in vitro 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. 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.
4. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
5. 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 solution colored. Do not pour reagents back into vials as reagent contamination may occur.
6. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
7. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
8. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
9. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
10. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
11. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
12. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
13. Do not use reagents beyond expiry date as shown on the kit labels.
14. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
15. 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 result slightly different.
16. Avoid contact with Stop Solution. It may cause skin irritation.
17. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
18. For information please refer to Material Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from Demeditec Diagnostics GmbH.

4 REAGENTS

4.1 Reagents provided

1. **SORB | MT Microtiterplate**, 12 x 8 (break apart) strips with 96 wells; Wells coated with anti-DHEA antibody.
2. **CAL | 0-5 Calibrators (Calibrator 0-5),** 6 vials, 0.3 mL each, ready to use; Concentrations: 0 – 0.3 – 1 – 3 – 10 – 30 ng/mL
3. **CONTROL | 1-2 Control low** / **Control high**, 2 vials, 0.3 ml each, ready to use; containing DHEA in serum. For control values and ranges please refer to QC-Datasheet.
4. **ENZ | CONJ Enzyme Conjugate**, 1 vial, 11 mL, ready to use; horseradish peroxidase labeled DHEA in buffered matrix.
5. **SUB | TMB Substrate Solution**, 1 vial, 22 mL, ready to use; contains tetramethylbenzidine (TMB)
6. **STOP | SOLN Stop Solution**, 1 vial, 7 mL, ready to use; contains 2 N hydrochloric acid solution.
7. **WASH | SOLN | 10x Wash Solution**, 1 vial, 50 mL (10X concentrated); see “Reagent Preparation”.

Note: Additional Calibrator 0 for sample dilution is available upon request.

4.2 Materials required but not provided

- A microtiter plate reader capable for endpoint measurement at 450 nm
- Calibrated variable precision micropipettes (25 µL, 100 µL, 200 µL, 300 µL).
- Microplate mixer operating more than 600 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°C to 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.
Microtiter wells must be stored at 2°C to 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 (21-26°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 (21-26°C).*

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 Material Safety Data Sheet.

4.6 Damaged test kits

In case of any severe damage of the test kit or components, DEMEDITEC DIAGNOSTICS GmbH 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

For determination of DHEA **serum or plasma (EDTA)** can be used. The procedure calls for 25 µL sample per well. The samples should be assayed immediately or aliquoted and stored at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples expected to contain DHEA concentrations higher than the highest calibrator (30 ng/mL) should be diluted with the zero calibrator before assay. The additional dilution step has to be taken into account for the calculation of the results. Do not use grossly haemolytic, icteric or grossly lipaemic specimens.

6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to 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 in order 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.
- 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.

6.2 Assay procedure

Each run must include a standard curve.

1. Prepare a sufficient number of microplate wells to accommodate calibrators and samples in duplicates.
2. Dispense **25 µL** of each **Calibrator, Sample and Control** with new disposable tips into appropriate wells.
3. Dispense **100 µL** of **Enzyme Conjugate** into each well.
4. Incubate for **60 minutes** at room temperature on a plate shaker (> 600 rpm).

   Important Note:
   Optimal reaction in this assay is markedly dependent on shaking of the microplate!
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** in the dark.
8. Stop the reaction by adding **50 µL** of **Stop Solution** to each well.
9. Determine the absorbance of each well at 450 nm. It is recommended to read the wells within 15 minutes.

6.3 Calculation of results

1. Calculate the average absorbance values for each set of calibrators, controls and 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 calibration 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 calibrator curve. Samples with concentrations higher than that of the highest calibrator have to be further diluted. For the calculation of the concentrations, this dilution factor has to be taken into account.

Example of typical calibrator curve

Following data are intended for illustration only and should not be used to calculate results from another run.

Standard	Optical Units (450nm)
Calibrator 0 (0 ng/mL)	3.003
Calibrator 1 (0.3 ng/mL)	2.501
Calibrator 2 (1 ng/mL)	1.912
Calibrator 3 (3 ng/mL)	1.220
Calibrator 4 (10 ng/mL)	0.647
Calibrator 5 (30 ng/mL)	0.341

7 EXPECTED VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.
In a study conducted with apparently normal healthy adults, using the DEMEDITEC DHEA ELISA the following values are observed:

Population	Range
Adult Males	1.8 – 12.5 ng/mL
Adult Woman	1.3 – 9.8 ng/mL

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

8 PERFORMANCE CHARACTERISTICS

8.1 Analytical Sensitivity

The lowest analytical detectable level of DHEA that can be distinguished from the Zero Calibrator is 0.07 ng/mL at the 2SD confidence limit.

8.2 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	< 0,01
Testosterone	< 0,01
5α-Dihydrotestosterone	< 0,01
Androstendione	0,06
Progesterone	0,23
17α-Hydroxyprogesterone	< 0,01
Pregnenolone	0,01
17-Hydroxy-Pregnenolone	0,07
Desoxycorticosterone	0,05
Corticosterone	< 0,01
Cortisol	< 0,01

8.3 Assay dynamic range

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

8.4 Reproducibility

8.4.1 Intra-Assay

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

	Serum 1	Serum 2	Serum 3
Mean (ng/mL)	2.08	5.34	20.84
SD	0.16	0.39	1.511
CV (%)	7.9	7.3	7.3
n =	20	20	20

8.4.2 Inter-Assay

The inter-assay (between-run) variation was determined by duplicate measurements of three serum samples in 11 different tests.

	Serum 1	Serum 2	Serum 3
Mean (ng/mL)	2.14	5.26	20.63
SD	0.15	0.27	1.03
CV (%)	6.9	5.1	5.0
n =	11	11	11

8.5 Recovery

Using the calibrator matrix a spiking solution of 1000 ng DHEA/mL was prepared. 500 µL of three sera were spiked with 1.5, 3 and 5 µL of the spiking solution leaving the serum matrices relatively intact. All samples were measured by the DEMEDITEC DHEA ELISA procedure.

Sample	Spiking (ng/mL)	Measured (ng/mL)	Expected (ng/mL)	Recovery (%)
1	-	0	-	-
	3	2.63	3	88%
	6	5.52	6	92%
	10	10.04	10	100%
2	-	0.96	-	-
	3	3.36	3.96	85%
	6	5.67	6.96	81%
	10	8.73	10.96	80%
3	-	1.69	-	-
	3	4.05	4.69	86%
	6	7.11	7.69	92%
	10	10.24	11.69	88%

8.6 Linearity

Three serum samples were assayed undiluted and diluted with the zero calibrator.

Serum	Dilution	Measured (ng/mL)	Expected (ng/mL)	Linearity (%)
1	-	11.34	./.	./.
	1 in 2	5.91	5.67	104%
	1 in 4	3.24	2.84	114%
	1 in 8	1.55	1.48	105%
2	-	4.78	./.	./.
	1 in 2	2.57	2.39	108%
	1 in 4	1.23	1.2	103%
	1 in 8	0.49	0.6	82%
3	-	12.08	./.	./.
	1 in 2	6.57	6.04	109%
	1 in 4	3.57	3.02	118%
	1 in 8	1.82	1.51	120%

9 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.

Drug Interferences

Any medication (cream, oil, pill etc.) containing DHEA will significantly influence the measurement of this analyte.

10 LEGAL ASPECTS

10.1 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 test 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. In case of any doubt or concern please contact DEMEDITEC.

10.2 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 10.1. 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.

10.3 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 10.2. 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.

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SYMBOLS USED WITH ELISA

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
Σ	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributtore

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