Instructions for Use

DHEA ELISA

Enzyme Immunoassay for the in-vitro-diagnostic quantitative determination of DHEA in human serum.

REF **RE52221**

Σ **96**

EU: IVD CE

U.S.: *For research use only.*
Not for use in diagnostic procedures.

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1. INTENDED USE

Enzyme immunoassay for the *in-vitro diagnostic* quantitative determination of Dehydroepiandrosterone (DHEA) in human serum.

2. SUMMARY AND EXPLANATION

Dehydroepiandrosterone (DHEA; androstenolone; 3b-hydroxy-5-androsten-17-one) is a C19 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 3b-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.

3. TEST PRINCIPLE

The DHEA ELISA 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 molecule. Endogenous DHEA of a patient 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 colour developed is inversely proportional to the concentration of DHEA in the patient sample.

4. WARNINGS AND PRECAUTIONS

1. 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. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
9. Avoid contact with Stop solution. It may cause skin irritations and burns.
10. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely. For this reason reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the indicated expiry after the kit is broken. Make sure that the broken bag is tightly closed when stored at 2-8 °C.

6. SPECIMEN COLLECTION AND STORAGE

Serum

<table>
<tr><td colspan="4">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 grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.</td></tr>
<tr><td>Storage:</td><td>2-8°C</td><td>≤ -20°C (Aliquots)</td><td rowspan="2">Keep away from heat or direct sunlight.
Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Stability:</td><td>4 days</td><td>3 months</td></tr>
</table>

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Coated with anti-DHEA antibody (polyclonal, rabbit).
1 x 6 x 1 mL	CAL A-F	**Standard A-F** 0; 0.37; 1.1; 3.3; 10.0; 30.0 ng/mL Ready to use. Contains: DHEA, Human serum, stabilizers.
1 x 2 x 1 mL	CONTROL 1+2	**Control 1+2** Ready to use. Contains: DHEA, low and high, Human serum, stabilizers. Exact concentrations see vial labels or QC certficate.
1 x 13 mL	ENZCONJ	**Enzyme Conjugate** Ready to use. Contains: DHEA conjugated to HRP, stabilizers.
1 x 100 mL	WASHBUF CONC	**Wash Buffer Concentrate** (10x) Contains: phosphate buffer, Tween.
1 x 15 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains: TMB, Buffer, stabilizers.
1 x 15 mL	TMB STOP	**TMB Stop Solution** Ready to use. 1 M H_2SO_4.

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 20; 100; 250 µL
2. Vortex mixer
3. 8-Channel Micropipettor with reagent reservoirs
4. Wash bottle, automated or semi-automated microtiter plate washing system
5. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
6. Bidistilled or deionised water
7. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of concentrated components

Dilute / dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
100 mL	**WASHBUF CONC**	ad 1000 mL	bidist. water	1:10	Mix vigorously.	2-8 °C	4 weeks

10.2. Dilution of Samples

Samples suspected to contain concentrations higher than the highest standard have to be diluted with Standard A. Measured results must be multiplied with the dilution factor to obtain corrected results.

11. TEST PROCEDURE

1.	Pipette **20 µL** of each **Standard, Control and sample** into the respective wells of the Microtiter Plate.
2.	Pipette **100 µL** of **Enzyme Conjugate** into each well.
3.	Thoroughly mix for 10 seconds.
4.	**Incubate 60 min** at **RT (18-25°C)**.
5.	Discard incubation solution. Wash plate **3 x** with **250 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
6.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
7.	**Incubate 30 min** at **RT (18-25°C).**
8.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate. Colour changes from blue to yellow.
9.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **15 min** after pipetting of the Stop Solution.

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistics or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read directly from the standard curve.

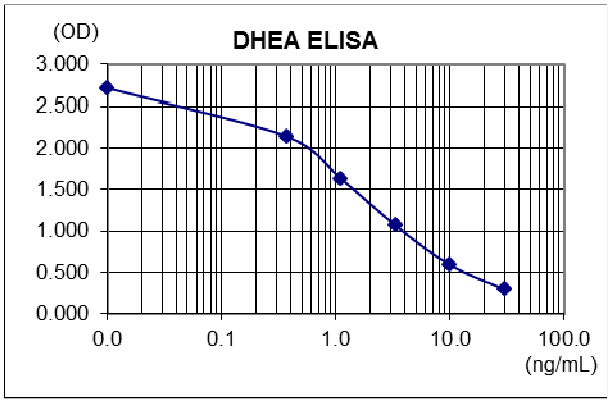
Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

Conversion:

DHEA (ng/mL) x 3.467 = nmol/L

Typical Calibration Curve
(Example. Do not use for calculation!)

Standard	DHEA (ng/mL)	OD_{Mean}	OD/OD_{max} (%)
A	0.0	2.715	100
B	0.37	2.141	79
C	1.10	1.633	60
D	3.30	1.068	39
E	10.0	0.586	22
F	30.0	0.291	11



14. EXPECTED VALUES

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

Apparently healthy subjects show the following values:

DHEA	n	Median (ng/mL)	95% percentile (ng/mL)	total Range (ng/mL)
Males	100	2.30	0.52 – 5.18	0.06 – 13.37
Females	100	2.05	0.36 – 7.82	0.06 – 12.21

It is recommended that each laboratory establishes its own range of normal values.

15. LIMITATIONS OF THE PROCEDURE

The following substances do not have a significant effect on the test results up to the below stated concentrations (+/- 20%).

Hemoglobin	5 mg/mL	NaN_3	0.10 %
Bilirubin	3 mg/mL	Thimerosal	0.01 %
Triglyceride	46 mg/mL	ProClin 300	0.10 %

16. PERFORMANCE

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)	Cross-reactivity of other substances tested ≤ 0.001 %
	17α-OH-Pregnenolone	0.287	
	Androstenedione	0.194	
	Pregnenolone	0.072	
	11-Desoxy-Cortisol	0.010	
	DHEA-S	0.008	
	Corticosterone	0.006	
	19-Nortestosterone	0.006	
	Testosterone	0.003	
	Estrone	0.002	
Analytical Sensitivity (Limit of Detection)	0.03 ng/mL	Mean signal (Zero-Standard) - 2SD	
Functional Sensitivity	0.13 ng/mL	Mean Conc. < 20 % CV	
Precision	Range (ng/mL)	CV (%)	n
Intra-Assay	0.61 – 10.59	3.9 – 7.6	20
Inter-Assay	0.88 – 10.55	5.1 – 10.4	10
Linearity	Range (ng/mL)	Serial dilution up to	Range (%)
	0.5 – 13.1	1:8	86 - 119
Recovery (n=5)	Mean (%)	Range (%)	% Recovery after spiking (Added concentration 0.5-10 ng/mL)
	103	92 - 116	
Method Comparison versus LCMS	IBL-Assay = 0.96 x LCMS +0.22		r = 0.99; n = 70

17. PRODUCT LITERATURE REFERENCES

1. Thomas Lothar; Labor und Diagnose: Indikation und Bewertung von Laborbefunden für die medizinische Diagnostik, 8. Auflage, 2012
2. Tietz, N.W. Textbook of Clinical Chemistry. Saunders, 1986.

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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