



DHEA Elisa

BL-47-E

IN VITRO DIAGNOSTIC USE



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1 INTRODUCTION

Dehydroepiandrosterone (DHEA; androstenedione; 3 β -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 3 β -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 OF THE TEST


The **Bio-Line DHEA ELISA** procedure follows the basic principle of enzyme immunoassay where there is competition between in unlabelled antigen and an enzyme-labeled antigen for a fixed number of antibody binding sites. The amount of enzyme-labeled antigen bound to the antibody is inversely proportional to the concentration of the unlabelled analyte present. Unbound materials are removed by decanting and washing the wells.

3 PRECAUTIONS

- This kit is for in vitro diagnostic use only.
- For information on hazardous substances included in the kit please refer to Material 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.
- Avoid contact with Stop Solution containing 0.5 M H₂SO₄. It may cause skin irritation and burns.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even if 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.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
- Safety Data Sheets for this product are available upon request.
The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

4 KIT COMPONENTS

4.1 Contents of the Kit

1.  12x8 (break apart) strips, 96 wells
Wells coated with anti-DHEA antibody
2.

CAL	N
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 N=1 to 5
5 vials, 1 ml, ready to use
See exact values on the vial labels
Conversion factor from ng/ml in nmol/l: ng/ml x 3.46 = nmol/l
3.

CAL	0
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 1 vial, 1 ml, ready to use
0 ng/ml
4.

Ab	HRP
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 1 vial, 14 ml, ready to use
Anti-DHEA antiserum conjugated to horseradish peroxidase
5.

SUB	BUF
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 1 vial, 14 ml, ready to use
TMB
6.

STOP	SOLN
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 1 vial, 14 ml, ready to use
contains 0.5M H₂SO₄
Avoid contact with the stop solution. It may cause skin irritations and burns.
7.

WASH	SOLN	CONC
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 1 vial, 30 ml (40X concentrated)
see „Preparation of Reagents“

Note: Additional Zero Calibrator for Sample dilution available on request.

4.2 Equipment and material required but not provided

1. A microtiterplate calibrated reader (450±10 nm).
2. Calibrated variable precision micropipettes (Varipette Eppendorf), Multipette Eppendorf or similar products.
3. Absorbent paper.
4. Distilled water.

4.3 Storage and stability of the Kit

- When stored at 2° to 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
- Enzyme-Conjugate, Substrate Solution, Calibrators and Zero Calibrator must be stored at 2° to 8°C.
- Microtiter wells must be stored at 2° to 8°C. Once the foilbag has been open care should be taken to close it tightly again.

4.4 Preparation of Reagents

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

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 2 weeks at room temperature.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national official regulations. Special information for this product are given in the Material Safety Data Sheets (see chapter 13).

4.6 Damaged Test Kits

In case of any severe damage of the test kit or components, Bio-Line Europe have to be informed written, latest 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

5.1 Specimen collection

Serum or EDTA plasma should be used in the assay. No special pretreatment of sample is necessary. Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not use haemolytic, icteric or lipaemic serum. This kit is for use with samples without additives (like Na N3) only .

5.2 Specimen storage

Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying. Specimen held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen dilution

If in an initial assay, a serum specimen is found to contain more than the highest calibrator, the specimens can be diluted 10-fold with Zero Calibrator and reassayed as described in Assay Procedure.

6 TEST 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 pipet tips for each calibrator, control of sample in order to avoid crosscontamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents be ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.

6.2 Procedural Notes

- All calibrators, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
- The concentration of the samples can be read directly from this calibrator curve. Samples with a concentration higher than that of the highest calibrator have to be diluted 1 : 10 with Zero Calibrator. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3 Assay Procedure

1. Secure the desired number of Microtiterwells in the holder.
2. Dispense **20 µl** DHEA Calibrators, Controls and samples **with new disposable tips** into appropriate wells. Time between distribution of first Calibrator and last sample can be up to 10 minutes without affecting the results.
3. Dispense **100 µl** Enzyme Conjugate into each well.
4. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for **1 hour** at room temperature.
6. Briskly shake out the contents of the wells.
Rinse the wells 3 times with diluted Wash Solution (400 µl per well). Strike the wells sharply on absorbent paper to remove residual water droplets.
Important note:
The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7. Add **100 µl** of Substrate Solution to each well.
8. Incubate for **15 minutes** at room temperature.
9. Stop the enzymatic reaction by adding **100 µl** of Stop Solution to each well.
10. Read the OD at **450±10 nm** with a microtiterplate reader **within 10 minutes** after adding the Stop Solution.

6.4 Calculation of Results

1. Calculate the average absorbance values for each set of calibrators, controls and patient samples.
2. Construct a calibrator curve by plotting the mean absorbance obtained from each calibrator against its concentration in IU/ml 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 calibrator curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log can generally give a good fit.
5. The concentration of the samples can be read directly from this calibrator curve. Samples with concentration higher than that of the highest calibrator have to be diluted 1 : 10 with zero calibrator. The dilution factor has to be taken into account.

7 ASSAY CHARACTERISTICS

7.1 Expected values

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

Adult Men	1.8 – 12.5 ng/ml
Adult Women	1.3 – 9.8 ng/ml

7.2 Specificity

The Specificity of the BIO-LINE DHEA EIA was assessed according to Abraham's method.

<u>Steroid</u>	<u>% Crossreactivity</u>
DHEA	100
17-OH Pregnenolone	0.072
Androstenedione	0.056
Desoxycorticosterone	0.052
Progesterone	0.023
Pregnenolone	0.013
11-Desoxycortisol	0.012
Corticosterone	0.004
DHEA-S	0.0037
Testosterone	0.002
5- α Dihydrotestosterone	0.0007
Cortisol	0.0007
17 α -Hydroxyprogesterone	0.0004
Aldosterone	0.0003
Estradiol 17 β	n.d.
Estradiol 17 α	n.d.
Estrone	n.d.
Estriol	n.d.

* n.d. = non detectable

7.3 Sensitivity

The lowest detectable level of DHEA – defined as the DHEA concentration given by the mean absorbance minus 2 standard deviations of 18 replicates of the zero calibrator - was assessed to be ≤ 0.1 ng/ml.

7.4 Accuracy

Quality Control

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 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 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; photometer, 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.

7.5 Precision

Intra and Inter Assay Variation

The within assay variability is shown below:

Intraassay				Interassay		
Serum	n	<X> \pm SD ng/ml	CV %	n	<X> \pm SD ng/ml	CV %
1	12	0.72 \pm 0.02	2.71	21	0.70 \pm 0.04	6.44
2	12	3.67 \pm 0.04	1.14	21	3.88 \pm 0.09	2.31
3	13	5.31 \pm 0.12	2.32	21	5.60 \pm 0.19	3.35

7.6 Recovery

Serum	Endogenous DHEA (ng/ml)	Added DHEA (ng/ml)	Measured Concentration (ng/ml)	Recovery%	
1	0.33	15.00	15.04	98.1	
		1.65	2.18	110.2	
		0.19	0.51	99.4	
2	1.87	15.00	16.30	96.6	
		1.65	3.35	95.1	
		0.19	2.19	106.4	
3	2.65	15.00	17.97	101.8	
		1.65	4.54	105.6	
		0.19	2.79	98.1	

7.7 Linearity

Dilution test

Serum	Dilution Factor	Measured Concentration (ng/ml)	Recovery %
1	Undiluted	17,97	
	1:2	9,15	101,8
	1:4	4,72	105,1
	1:8	2,28	101,3
	1:16	1,17	104,4
2	Undiluted	5,32	
	1:2	2,55	95,8
	1:4	1,25	94,2
	1:8	0,64	96,6
	1:16	0,34	101,1

8 LIMITATIONS OF USE

8.1 Interfering Substances

Any improper handling of samples or modification of this test might influence the results. Interferences caused by improper sample handling are explained in the chapters 'Specimen - Collection'.

8.2 High-Dose-Hook Effect

No hook effect was observed in this test.

9 LEGAL ASPECTS

9.1 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.

9.2 Therapeutical Consequences

Therapeutical consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 9.1. 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 therapeutical consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutical consequences.

9.3 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 9.2. 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.