DRG® DHEA ELISA (EIA-3415)

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Introduction

Intended Use

The **DRG DHEA ELISA** is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of Dehydroepiandrosterone (DHEA) in serum.

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.

PRINCIPLE of the test

The DRG 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 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.

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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.

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 H_2SO_4. 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 and microtiterplate readers.
- 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.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
- Safety Data Sheets for this product are available upon request directly from DRG.
  The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

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Kit Components

Contents of the Kit

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with a anti-DHEA antibody (polyclonal).
2. ***Standard (Standard 0-5)*****,** 6 vials, 1 mL, ready to use;
   Concentrations: 0 – 0.37 – 1.1 – 3.3 – 10 – 30 ng/mL
   Conversion: ng/mL x 3.467 = nmol/L
   contain 0.03% Proclin 300 + 0.005% gentamicin sulfate as preservative.
3. ***Enzyme Conjugate***, 1 vial, 14 mL, ready to use;
   DHEA conjugated to horseradish Peroxidase;
   * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservative.
4. ***Substrate Solution***, 1 vial, 14 mL, ready to use;
   Tetramethylbenzidine (TMB).
5. ***Stop Solution***, 1 vial, 14 mL, ready to use;
   contains 0.5M H_2SO_4
   Avoid contact with the stop solution. It may cause skin irritations and burns.
6. ***Wash Solution***, 1 vial, 30 mL (40X concentrated);
   see „Preparation of Reagents“.

* BND = 5-bromo-5-nitro-1,3-dioxane
MIT = 2-methyl-2H-isothiazol-3-one

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

Equipment and material required but not provided

- A microtiter plate calibrated reader (450±10 nm), (e.g. the DRG Instruments Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Aqua dest.

Storage and stability of the Kit

When stored at 2-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for six weeks if stored as described above.

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Preparation of Reagents

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

Wash Solution

Add deionized water to the 40X concentrated 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.

Disposal of the Kit

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 Sheets (see chapter 13).

Damaged Test Kits

In case of any severe damage of the test kit or components, DRG 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.

SPECIMEN

Serum can be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), 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 increased clotting time.

Specimen Storage

Specimens should be capped and may be stored for up to 24 hours at 2-8°C prior to assaying.
Specimens 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.

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Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) Dilution 1:10: 10 µL Serum + 90 µL *Standard 0* (mix thoroughly)
b) Dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly).

test procedure

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.

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Assay Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense **20 µL** of each *Standard, Control* and samples with new disposable tips into appropriate wells.
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 **60 minutes** 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 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 microtiter plate reader **within 10 minutes** after adding the *Stop Solution*.

Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. 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 standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

Example of Typical Standard Curve

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

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Standard	Optical Units (450 nm)
Standard 0 (0 ng/mL)	2.23
Standard 1 (0.37 ng/mL)	1.83
Standard 2 (1.1 ng/mL)	1.35
Standard 3 (3.3 ng/mL)	0.81
Standard 4 (10 ng/mL)	0.44
Standard 5 (30 ng/mL)	0.22

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 DRG 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

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 QC certificate added to the kit. The values and ranges 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 or DRG directly.

Assay Characteristics

Assay Dynamic Range
The range of the assay is between 0 – 30 ng/mL.

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Specificity of Antibodies (Cross Reactivity)

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

Steroid	% Crossreactivity
DHEA	100
17-OH Pregnenolone	0.072
Androsterone	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
17α-Hydroxyprogesterone	0.0004
Aldosterone	0.0003
Estradiol 17ß	n.d.
Estradiol 17α	n.d.
Estrone	n.d.
Estriol	n.d.

* n.d. = non detectable

Analytical Sensitivity

The analytical sensitivity was calculated from the mean minus two standard deviations of twenty (20) replicate analyses of *Standard 0* and was found to be 0.108 ng/mL.

Precision

Intra Assay Variation

The within assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	20	0.58	6.92
2	20	2.83	4.57
3	20	3.79	3.84

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Inter Assay Variation

The between assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1		0,51	9,96
2		2,83	3,75
3		4,01	6,86

Recovery

Recovery was determined by adding increasing amounts of the analyt to three different sera containing different amounts of endogenous analyt. The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

Sample	Endogenus DHEA ng/mL	Added DHEA ng/mL	Measured Conc. DHEA ng/mL	Expected Conc DHEA ng/mL	Recovery (%)
1	2.79	0	2.79		
		15.0	18.74	16.40	114.3
		5.0	6.54	6.40	102.2
		1.65	2.87	3.05	94.1
		0.55	2.00	1.95	102.9
2	6.47	0	6.47		
		15.0	19.93	18.23	109.3
		5.0	8.02	8.23	97.5
		1.65	4.83	4.88	99.0
		0.55	3.69	3.78	97.4
3	13.76	0	13.76		
		15.0	22.28	21.88	101.8
		5.0	12.38	11.88	104.3
		1.65	8.48	8.53	99.5
		0.55	7.15	7.43	96.2

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Linearity

Sample	Dilution	Measured Conc. DHEA ng/mL	Expected Conc. DHEA ng/mL	Recovery (%)
3	undil	13.76	13.76	
	1 : 2	6.75	6.88	98.1
	1 : 4	3.30	3.44	96.0
	1 : 8	1.57	1.72	91.3
	1:16	0.94	0.86	109.0
2	undil	6.47	6.47	
	1 : 2	3.19	3.23	98.8
	1 : 4	1.75	1.62	108.2
	1 : 8	0.90	0.81	111.0
	1:16	0.44	0.40	108.2
1	undil	2.79	2.79	
	1 : 2	1.40	1.40	100.1
	1 : 4	0.79	0.70	113.1
	1 : 8	0.39	0.35	110.7
	1:16	0.20	0.17	112.4

Limitations of Use

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

Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of DHEA in a sample.

High-Dose-Hook Effect

No hook effect was observed in this test.

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Legal Aspects

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 DRG.

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

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.

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