Product information

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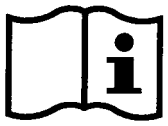

Useŕs Manual

DHEA-S ELISA

Enzyme immunoassay for the determination of DHEA-S in human serum and plasma

IVD

REF **IB79342**

Σ **96 Wells**

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1 INTRODUCTION

1.1 Intended Use

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

1.2 Summary and explanation

Dehydroepiandrosterone (DHEA) and its sulfate ester, Dehydroepiandrosterone-Sulfate (DHEA-S) are among the most abundant circulating hormones produced by the adrenal glands and serve as precursors for androgen and estrogen steroids. The concentration of circulating DHEA-S is around 1,000 times higher than DHEA.
Adrenal secretion of DHEA and DHEA-S increases during adrenarche in children at the age of 6-8 years. Maximal values are reached between the ages of 20 and 30 years. Thereafter, serum DHEA and DHEA-S levels decrease.
DHEA-S is a specific product of the adrenals and measurement of this steroid is widely used in clinical practice. The clinical importance of immunoassays for DHEA-S is associated with the diagnosis of adrenal hyperplasia and insufficiency as well as differential diagnosis of hirsutism and virilization. Further studies showed an indication of attenuated DHEA-S response during an acute stress situation in patients with clinical burnout.

2 PRINCIPLE

The IBL-AMERICA DHEA-S 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-S antibody. An unknown amount of DHEA-S present in the sample competes with a DHEA-S-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-S in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to the concentration of DHEA-S in the sample.

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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 IBL-AMERICA.

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4 REAGENTS

4.1 Reagents provided

1. **SORB | MT Microtiterplate**, 12 x 8 (break apart) strips with 96 wells;
   wells coated with anti-DHEA-S antibody.
2. **CAL | 0-6 Calibrators (Calibrator 0-6),** 7 vials, 0.3 ml each, ready to use;
   contain DHEA-S in serum.
   concentrations: 0 - 0.03 - 0.1 - 0.3 - 1 - 3 - 10 µg/ml.
3. **CONTROL | 1-2 Control low / Control high**, 2 vials, 0.3 ml each, ready to use;
   contain DHEA-S 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-S 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 (10 µl, 50 µl, 100 µl, 200 µl, 300 µl).
- Microplate mixer operating at more than 600 rpm (optional)
- 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.

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4.6 Damaged test kits

In case of any severe damage of the test kit or components, IBL-AMERICA 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-S **serum and plasma (EDTA)** can be used. The procedure calls for 10 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-S concentrations higher than the highest calibrator (10 µg/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.

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 **10 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) or alternatively without shaking. It is important to have a complete mixing in this step, thus thoroughly mix for 10 seconds.
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 ±10 nm. It is recommended to read the wells within 15 minutes.

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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 µg/ml)	3.209
Calibrator 1 (0.03 µg/ml)	2.404
Calibrator 2 (0.1 µg/ml)	1.743
Calibrator 3 (0.3 µg/ml)	1.080
Calibrator 4 (1 µg/ml)	0.576
Calibrator 5 (3 µg/ml)	0.303
Calibrator 6 (10 µg/ml)	0.185

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 IBL-AMERICA DHEA-S ELISA, the following values are observed:

Population	Age	5%-95% Percentile
Males	< 50 years	0.73 – 4.74 µg/ml
	> 50 years	0.40 – 2.52 µg/ml
Females	< 50 years	0.43 – 2.68 µg/ml
	> 50 years	0.43 – 2.13 µg/ml

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

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8 PERFORMANCE CHARACTERISTICS

8.1 Analytical Sensitivity

The lowest analytical detectable level of DHEA-S that can be distinguished from the Zero Calibrator is 0.002 µg/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-S.

Steroid	% Cross reaction
Cortisol	<0.1%
Androstenedione	30%
17-Hydroxyprogesterone	0.4%
Androsterone	20%
β-Estradiol	<0.1%
Estriol	1.3%
Testosterone	0.7%
Progesterone	0.9%

8.3 Assay dynamic range

The range of the assay is between 0.03 - 10 µg/ml.

8.4 Reproducibility

8.4.1 Intra-Assay

The intra-assay variation was determined by 18 replicate measurements of three serum samples within one run. The within-assay variability is shown below:

	Serum 1	Serum 2	Serum 3
Mean (µg/ml)	0.28	0.96	2.60
SD	0.02	0.07	0.18
CV (%)	8.6	6.9	6.8
n =	18	18	18

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 (µg/ml)	0.28	0.81	2.26
SD	0.03	0.09	0.28
CV (%)	9.7	11.5	12.2
n =	11	11	11

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8.5 Recovery

Using the calibrator matrix a spiking solution of 100 µg DHEA-S/ml was prepared. 500 µl of three sera were spiked with 5, 10 and 15 µl of the spiking solution leaving the serum matrices relatively intact. All samples were measured by the IBL-AMERICA DHEA-S ELISA procedure.

Sample	Spiking (µg/ml)	Measured (µg/ml)	Expected (µg/ml)	Recovery (%)
1	-	0.08	-	-
	1	0.91	1.08	84%
	2	1.93	2.08	93%
	3	2.72	3.08	88%
2	-	0.24	-	-
	1	1.04	1.24	84%
	2	2.30	2.24	103%
	3	3.07	3.24	106%
3	-	0.27	-	-
	1	1.10	1.27	87%
	2	2.33	2.27	103%
	3	3.15	3.27	96%

8.6 Linearity

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

Serum	Dilution	Measured (µg/ml)	Expected (µg/ml)	Linearity (%)
1	-	2.55	./.	./.
	1 in 2	1.30	1.27	102%
	1 in 4	0.70	0.64	110%
	1 in 8	0.36	0.32	113%
2	-	2.21	./.	./.
	1 in 2	1.09	1.11	98%
	1 in 4	0.58	0.55	105%
	1 in 8	0.33	0.28	118%
3	-	1.70	./.	./.
	1 in 2	0.84	0.85	99%
	1 in 4	0.43	0.42	100%
	1 in 8	0.21	0.21	100%

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9 LIMITATIONS OF PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is performed with complete understanding of the package insert instruction and adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

9.1 Drug Interferences

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

9.2 Interfering Substances

Minimal or mild haemolysis does not influence the assay results while severe haemolysis can have an effect on the assay results.
No interference has been observed with bilirubin (up to 200 mg/L) containing sera.

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 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 IBL-AMERICA.

10.2 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 IBL-AMERICA ELISA'S

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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