

Species crossreactivity

ADI's human DHEA-S ELISA kit has not been validated by ADI for animals or other species. However, the kit may be optimized for species (mouse, rat etc) where the DHEA-S are within the detectable range of the kit.

Samples requirements

This kit is optimized for human serum samples. Other biological fluids such as culture medium, plasma, CSF can be tested as well.

Related Steroid Hormone ELISA kits available from ADI (Details and complete listing is posted at the web site)

ItemName	Cat #
Human Cortisol ELISA Kit	1850
Human Progesterone ELISA Kit	1860
Human Pregnlone	1865
Human Progesterone (saliva) ELISA	1870
Human Aldosterone ELISA Kit	1875
Human Testosterone ELISA Kit	1880
Human free Testosterone ELISA Kit	1885
Human Androstenedione ELISA Kit	1910
Human Androstenedione (saliva) ELISA	1915
Human Estradiol ELISA Kit	1920
Human Estrone ELISA Kit	1925
Human Dihydrotestosterone (DHT) ELISA Kit	1940
Human DHEA-sulphate (DHEA-S) ELISA Kit	1950

KIT PROFILE

Date received: **Cat #** 1950 **Lot #** _____ **Exp.** _____

Date kit opened _____ **Technician:** _____

Date used: **# Strips used** _ **# Remaining** _____

Date used: **# Strips used** _ **# Remaining** _____

Remarks _____

Instruction Manual No. M-1950

Dehydroepiandrosterone Sulfate (DHEA-S)

ELISA KIT Cat. #. 1950

For Quantitative Determination of DHEA-S
In Human Serum

For In Vitro Research Use Only



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DHEA-S ELISA Kit Cat. #. 1950

Kit Components	96 tests
Anti-DHEA-S coated strip plate (96 wells), Cat. 1951	1 plate
DHEA-S Std. A (0 ug/ml), 2 ml , Cat # 1952A	1 vial
DHEA-S Std. B (0.005 ug/ml), 0.5 ml , Cat # 1952B	1 vial
DHEA-S Std. C (0.02 ug/ml), 0.5 ml , Cat # 1952C	1 vial
DHEA-S Std. D (0.1 ug/ml), 0.5 ml , Cat # 1952D	1 vial
DHEA-S Std. E (0.5 ug/ml), 0.5 ml , Cat # 1952E	1 vial
DHEA-S Std. F (2.5 ug/ml), 0.5 ml , Cat # 1952F	1 vial
DHEA-S Std. G (10 ug/ml), 0.5 ml , Cat # 1952G	1 vial
DHEA-S HIGH Control Serum, 0.5 ml; Cat # 1953 (lot specific values are specified on the vial) Ready to use	1 vial
DHEA-S LOW Control Serum, 0.5 ml; Cat # 1953L, Ready to use	1 vial
Assay buffer, 30 ml; Cat # 1 9 5 4	1 bottle
DHEA-S-HRP Conjugate, 50X (800 ul) Dilute with the assay buffer, Cat # 1 9 5 5	1 bottle
TMB substrate, 16 ml, Cat # 1956	1 bottle
Stop solution (1N H ₂ SO ₄), 6 ml; Cat. # 1957	1 bottle
Wash buffer (10X), 50 ml, Dilute 1:10, #1958	1 bottle
Instruction Manual, M - 1 9 5 0	1

Introduction

Dehydroepiandrosterone sulfate (DHEAS) is produced by the adrenals and gonads. As a result, the determination of the level of DHEA-S in serum is important in the evaluation of the functional state of these glands. DHEAS is a precursor of testosterone and estrone. Besides the adrenals in females, the ovaries have been shown to be an important source of DHEAS. It has been reported that there is a fluctuation day by day of DHEAS in women during the ovulatory cycle.

The principle production of testosterone in females is from conversion of other related androgens, especially DHEAS. An abnormal testosterone level in women should be accompanied by the estimation of serum DHEAS. The use of serum testosterone determination in conjunction with Elisa of DHEAS can be used to determine if the source of excess androgen production is ovarian or adrenal.

ADI's DHEA-S ELISA is direct competitive ELISA kit for the measurement of DHEA-S in serum. It is not validated for other biological fluids.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on sixteen replicate determinations of the zero standards, the minimum DHEA-S concentration detectable using this assay is 0.005 ug/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision: Three serum samples (mean DHEA-S concentrations 0.24, 2.02, and 9.54 ug/ml) were run in 10 replicates. The samples showed good intra-assay precision with %CV of 7.5, 8.9, 11.5 with SD± 0.02, 0.18, and 0.11 respectively.

Inter-assay precision: Three serum samples were run in duplicate in sixteen independent assays. The samples showed good inter-assay precision (4-11 % CV). The actual values were: mean 0.13, 1.11, 6.38 ug/ml.

3. LINEARITY

Three samples (with original DHEA-S concentration of. 2.84, 6.32, and 7.12 ug/ml) were diluted (1:2, 1:4, and 1:8) with the assay buffer and their final DHEA-S values determined. The samples showed excellent mean recoveries of about 101% (range 90-104%).

4. Recovery

Three samples (with original DHEA-S concentration of. 0.67, 1.06, 1.73 ug/ml) were spiked with 0.1, 1.0, and 5.0 ug/ml and final DHEA-S values determined. The samples showed mean recoveries of 89-117%.

5. SPECIFICITY AND CROSSREACTIVITY

The following compounds were tested for cross-reactivity with the DHEA-S ELISA kit with DHEAS cross-reacting at 100%.

Steroid	%Cross Reactivity
DHEAS	100
Androsterone	16.0
Androstenedione	1.7
Testosterone	0.9
Progesterone	0.6
DHT	0.6
Cortisol	0.5

The following steroids were tested but cross-reacted at less than 0.001%: 17β-Estradiol, Estrone, Estrone-Sulfate and Pregnenolone.

References for ADI ELISA kti#1950

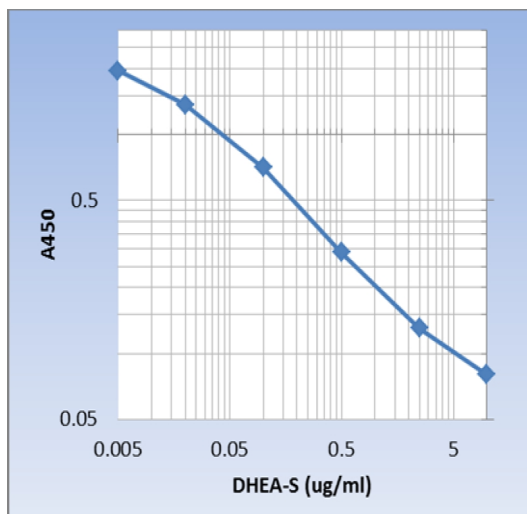
Graham CA 2007 Psychoneuroendocrinology, 32, 246-255
Greco T, 2007 Contraception, 76, 8-17

General References: Chasalow, F.I. (1988) Steroids 52/3: 205; Chasalow, F.I., (1989) Steroids 54/4: 373; de Peretti, E. (1978) Endocrinol. 47: 572-577. Holtzclaw, W.D. (1989) Steroids 54/4: 355-371; Koritnik, D.R., (1984) Steroids 42/6: 653-664; Orentreich, N., (1984) J. Clin. Endocr. 59: 551-555; Smith, M.R., (1975) Clin. Chim. Acta 65: 5; Check, J.H (1995) Gynecol Obstet Invest 40:139.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A ₄₅₀ nm	Calculated Conc'n (ng/ml)
A1, A2	Std. A (0 ug/ml)	2.23	
B1, B2	Std. B (0.005 ug/ml)	1.962	
C1, C2	Std. C (0.02 ug/ml)	1.368	
D1, D2	Std. D (0.1 ug/ml)	0.709	
E1, E2	Std. E (0.5 ug/ml)	0.289	
F1, F2	Std. F (2.5 ug/ml)	0.130	
G1, G2	Std. G (10.0 ug/ml)	0.080	
H1, H2	Sample 1	0.186	1.3 ug/ml

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



/3-ADI_ELISA_Grpahs_Arif

A typical std. assay curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

DHEA-S ELISA kit is based on simultaneous binding of DHEA-S from samples and DHEA-S-HRP conjugate to anti-DHEA-S immobilized on the microtiter well plates. Therefore, free DHEA-S and enzyme-bound DHEA-S compete for limited antibody bound to the plate. In the absence of free DHEA-S, there is a maximum amount of DHEA-S-enzyme bound to the plate. At the end it will produce the maximum color. As the amount of free DHEA-S (samples or std) increases, it reduces the amount of enzyme bound to the plate. The color (blue) produced is inversely proportional to the concentration of DHEA-S in the sample. A std. curve is constructed by generating color from no DHEA-S (std. A, 0 ug/ml DHEA-S) to the highest std (Std G, 10 ug/ml DHEA-S). Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of DHEA-S in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS

ADI's DHEA-S ELISA kit is intended for *in vitro research* use only. The reagents contain proclin-300 (0.1%) as preservative; necessary care should be taken when disposing solutions. The stds/controls sera may contain human serum that has been shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H₂SO₄ (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera can not be immediately assayed , these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing. No preservatives should be added to the serum.

REAGENTS PREPARATION FOR THE ASSAY AND STORAGE

HRP Conjugate. Dilute 1:50 in assay buffer (prepare 20 ml for a full 96 well plate; 20 ul in 1 ml of the assay buffer). Do not store diluted solution. Prepare in required amounts only. Store stock solution at 4oC.

Wash Buffer Concentrate- Dilute 1:10 in distilled water before use.

Occasionally, some buffer components may crystallize that will dissolve at room temperature. Store at 4oC.

Limitations

1. All the reagents within the kit are calibrated for the direct determination of DHEAS in human serum. The kit is not calibrated for the determination of DHEAS in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is at least 6 months from the date of shipping under appropriate storage conditions. After opening the kit components, the shelf life is approx. 2 months.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

Dilute Wash buffer, DHEA-S-HRP conjugate. Label or mark the microtiter well strips to be used on the plate.

1. Pipet **25 ul** stds., controls, and samples into appropriate wells.
2. Pipet **200 ul of Diluted DHEA-S-HRP** conjugate into each well. Mix gently. Cover the plate and incubate for **45 minutes** at room temperature on a plate shaker (approx 200 rpm).
3. Aspirate and wash the wells **3 times** with 300 ul of diluted wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
4. Add **150 ul** of HRP-substrate soln. (TMB) into each well. Mix gently. Cover the plate and incubate for **15-20** minutes at room temperature until dark blue color develops in std A. The reaction can be stopped sooner or prolonged until desired color is obtained.
5. Stop the reaction by adding **50 ul** of stopping solution to all Mix gently. Blue color turns yellow. Measure the absorbance at **450 nm** using an ELISA reader. Color is stable for at least one 30 min after stopping.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision.

Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each wells the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Samples containing more than 10 ug/ml DHEA-S should be first diluted with the zero std and retested. The results obtained should be multiplied by the appropriate first dilution factor.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Draw the standard curve on semi-log graph paper by plotting net absorbance values of standards against appropriate DHEA-S concentrations. Read off the DHEA-S concentrations of the control and patient samples. If ELISA software is being used, a 4-parameter curve is recommended.

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (µg/mL)
Males	0.39-4.63
Females	0.46-2.75
Postmenopausal Females	0.48-2.08