Human DHEA sulfate form ELISA Kit

Competitive Enzyme Immunoassay for the quantification of human DHEA sulfate form (DHEA-S) in Serum and Plasma (EDTA).

Catalog number: ARG80837

TABLE OF CONTENTS

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INTRODUCTION

Dehydroepiandrosterone (5-Androstene-3 beta-oL-17-one, Androstenolone, Dehydroisoandrosterone, Transdehydroandrosterone, DHEA) is a steroid hormone present in blood mostly in its sulfate form (DHEA-S).

DHEA-S is a more specific product of the adrenals and measurements of this steroid are widely used in clinical practice. The clinical importance of plasma assays of DHEA-S is associated with the diagnosis of adrenal hyperplasia and differential diagnosis of hirsutism.

Dehydroepiandrosterone sulfate (DHEA-SO4) is almost exclusively synthesized by the adrenal cortex, and it is the most abundant steroid hormone in the peripheral circulation. It is the main source of the urinary 17-ketosteroids. The metabolic clearance of DHEA-SO4 is slow, and it is converted mostly to oestrogens. The hormone has a maximum level from puberty until 20-30 years of age, then there is a gradual decrease in the blood DHEA-SO4 concentration mainly in the menopause of women. Although the physiological role of DHEA-SO4 is not well established the serum level of this steroid hormone has an informative pathophysiological value.

1. The serum DHEA-SO4 radioimmunoassay seems to be a reliable tool to assess adrenal androgen function and the glandular overproduction of androgens.

2. High DHEA-SO4 values indicate a virilizing disorder of adrenal origin in women. This includes mainly adrenal neoplasms or early or late onset of congenital adrenal hyperplasia.

3. Monitoring the DHEA-SO4 concentration may be useful to control the

adrenal suppressive therapy (dexamethasone).

4. Low DHEA-SO4 levels can be an indicator of hormone-dependent immunological disorders.

5. Low levels of DHEA-SO4 may be related to the development of diseases that increases with age such as cancer and atherosclerosis. In these circumstances a systematically repeated assessment of the blood DHEA-SO4 values is recommended.

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. A highly specific human DHEA sulfate form (DHEA-S) antibody has been pre-coated onto a microtiter plate. DHEA-S containing samples or standards and a DHEA-S-HRP conjugate are given into the wells of the microtiter plate. Enzyme labeled and free DHEA-S compete for the antibody binding sites. After incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of DHEA-S is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C
Standards 0-6 (0, 0.03, 0.1, 0.3, 1, 3, 10 μg/ml)	7 X 0.3 ml (ready to use)	4°C
Control 1 (low)	1 x 0.3 ml (ready to use)	4°C
Control 2 (high)	1 x 0.3 ml (ready to use)	4°C
HRP-DHEA-S conjugate	11 ml (ready to use)	4°C
10X Wash buffer	50 ml	4°C
TMB substrate	22 ml (ready to use)	4°C (Protect from light)
STOP solution	7 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (620 nm as optional reference wave length)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times. Opened reagents must be stored at 2°-8°C. After first opening the reagents are stable for 30 days if used and stored properly.

- 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.
- 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.
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- 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.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- 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.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C for 15min or until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer.
- Samples: If the initial assay found samples contain cortisol higher than the highest standard, or samples are expected to contain DHEA-S concentrations higher than the highest calibrator (10 µg/ml) should be diluted with the zero calibrator before assay. For the calculation of the concentrations this dilution factor has to be taken into account.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 10 µl of each Standard, Control and sample with new disposable tips into appropriate wells.
3. Add 100 µl of HRP-DHEA-S conjugate into each well.
4. Cover wells and incubate for 60 minutes at RT on a microplate shaker (> 600 rpm) or alternatively without shaking but thoroughly mix for 10 seconds. It is important to have a complete mixing in this step. Rotating on a plate shaker increases OD values and improves precision.
5. Aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add 200 µl of TMB Reagent to each well. Incubate without shaking for 30 minutes at room temperature in dark.
7. Add 50 µl of Stop Solution to each well.
8. Read the OD with a microplate reader at 450 nm immediately. (620 nm as optional reference wave length). It is recommended to read the wells within 15 minutes.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient 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.

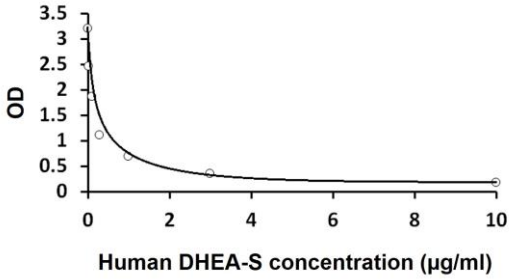
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

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 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 STANDARD CURVE

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



EXPECTED VALUES

Each laboratory should determine its own normal and abnormal values.

The following values are from a study conducted with apparently normal healthy adults, using this Human DHEA sulfate form ELISA Kit for reference.

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 therapeutically consequences. They have to be correlated to other clinical observations and diagnostic tests.

QUALITY ASSURANCE

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 0.002 μg/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 7.4% and inter-assay precision was 11.1%.

Specificity

The following materials have been evaluated for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to DHEA-S.

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

Recovery

84-106%

Linearity

99-118%

Interfering Substances

Minimal or mild hemolysis does not influence the assay results while severe hemolysis can influence the assay minimally.

No interference has been observed with bilirubin (up to 200 mg/l) containing sera.

Drug Interferences

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