



Human DHEA ELISA Kit

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

Catalog number: ARG80949

For research use only. Not for use in diagnostic procedures.

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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.

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PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. An anti-DHEA (DHEA) antibody has been pre-coated onto a microtiter plate. DHEA containing samples or standards and a DHEA-HRP conjugate are given into the wells of the microtiter plate. Enzyme labeled and free DHEA 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 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 0-5 (0, 0.3, 1, 3, 10, 30 ng/ml)	6 X 0.3 ml (ready to use)	4°C
Control 1 (low) (1.3 ng/ml; Acc. Range: 0.8-1.7 ng/ml)	1 x 0.3 ml (ready to use)	4°C
Control 2 (high) (3.7 ng/ml; Acc. Range: 2.4-4.9 ng/ml)	1 x 0.3 ml (ready to use)	4°C
HRP-DHEA 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
- Pipettes and pipette tips
- Deionized or distilled water
- Microplate shaker (~600 rpm)
- 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.
- 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. Take care that the foil bag is sealed tightly.
- 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.
- 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.

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- 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 until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Once the test has been started, all steps should be completed without interruption.
- 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.
- 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.
- 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.
- 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.

Note: Do not use grossly haemolytic, icteric or grossly lipaemic specimens.

REAGENT PREPARATION

- **1X Wash buffer:** Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 50 ml of 10X Wash buffer + 450 ml of distilled water.) The diluted Wash Solution is stable for at least 12 weeks at room temperature (21-26°C).
- **Samples:** If the initial assay found samples contain cortisol higher than the highest standard, or samples are expected to contain DHEA concentrations higher than the highest calibrator (30 ng/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

Allow the reagents and the required number of wells to reach room temperature (21-26°C) before starting the test. Standards, samples and controls should be assayed in duplicates.

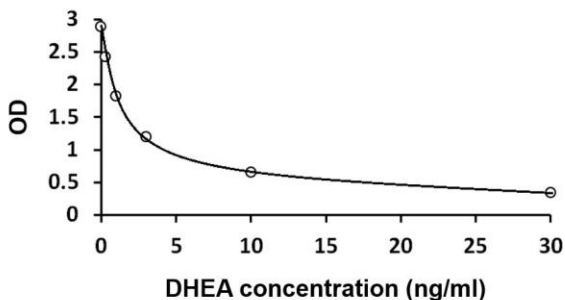
1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **25 µl** of each **Standard, Control and sample** with new disposable tips into appropriate wells.
3. Add **100 µl** of **HRP-DHEA conjugate** into each well.
4. Cover wells and incubate for **60 minutes at RT** on a microplate shaker (> 600 rpm). 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 Substrate** 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. 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 ELISA Kit for reference.

Population	Range
Adult Males	1.8 – 12.5 ng/ml
Adult Females	1.3 – 9.8 ng/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 lowest analytical detectable level of DHEA that can be distinguished from the Zero Calibrator is 0.07 ng/mL at the 2SD confidence limit.

Assay dynamic range

The range of the assay is between 0.3 – 30 ng/mL.

Specificity

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

Steroid	% of Cross-reactivity
DHEA-S	< 0.01
Testosterone	< 0.01
5 α -Dihydrotestosterone	< 0.01
Androstenedione	0.06
Progesterone	0.23
17 α -Hydroxyprogesterone	< 0.01
Pregnenolone	0.01
17-Hydroxy-Pregnenolone	0.07
Desoxycorticosterone	0.05
Corticosterone	< 0.01
Cortisol	< 0.01

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 7.5% and inter-assay precision was 5.67%.

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Recovery

81-100%

Linearity

82-120%

Drug Interferences

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