Human Androstenedione ELISA Kit

Enzyme Immunoassay for the quantification of human Androstenedione in serum and plasma

Catalog number: ARG80834

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

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INTRODUCTION

The steroid hormone Androstenedione is one of the main androgens, besides Testosterone and Dehydroepiandrosterone. Testosterone, the most important biological active androgen, is derived from peripheral enzymatic conversion of Androstenedione. In males, androgens are secreted primarily by the Leydig cells of the testes, to some degree also in the adrenal cortex. In females, the androgens are secreted mainly in the adrenal glands and in the ovary.

Around 10% of the androgens are derived from peripheral conversion, mainly of DHEA. Androstenedione and Testosterone show high diurnal variability. The highest levels are measured in the morning. At the age of puberty serum androstenedione levels rise, after menopause they decline again. High androstenedione levels are measured during pregnancy.

In women, high levels of androstenedione (47-100% above normal) are generally found in hirsutism, mostly in combination with other androgens as testosterone and DHEA-S. Androstenedione overproduction is due to ovarian dysfunction or may be of adrenal origin. High circulating androstenedione levels are found in women with polycystic ovaries and 21-hydroxylase effect. Significant lower androstenedione levels are found in postmenopausal osteoporosis.

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. A highly specific Androstenedione antibody has been pre-coated onto a microtiter plate.

Androstenedione containing samples or standards and a Androstenedione-HRP conjugate are given into the wells of the microtiter plate. Enzyme labeled and free Androstenedione 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 Androstenedione 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	1 plate	4°C
Standards 0-5 (0, 0.1, 0.3, 1, 3, 10 ng/ml)	6 X 1 ml (ready to use)	4°C
Control Low	1 ml (ready to use)	4°C
Control High	1 ml (ready to use)	4°C
HRP-Androstenedione conjugate	25 ml (ready to use)	4°C
40X Wash buffer	30 ml	4°C
TMB substrate	25 ml	4°C (Protect from light)
STOP solution	14 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- 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.
- Briefly spin down the HRP-Androstenedione Conjugate before use.
- If crystals are observed in the 40X Wash buffer, warm to RT (not more than 50°C) 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 40X Wash buffer into distilled water to yield 1X Wash buffer.
- Samples: Dilute 1:100 with standard zero.

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 20 µl of each Standard, Control and samples into appropriate wells.
3. Add 200 µl of HRP-Androstenedione conjugate into each well.
4. Cover wells and incubate for 60 minutes at RT.
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 (350 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 for 30 minutes at room

temperature in dark.

7. Add 100 µl of Stop Solution to each well.
8. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

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

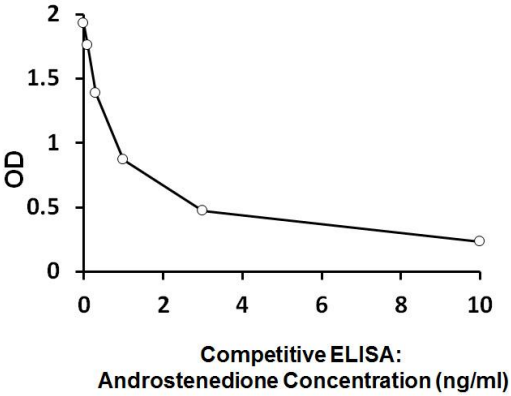
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

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.

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.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Androstenedione was 0.021 ng/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 7.13% and inter-assay precision was 8.37%.

Specificity

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

Compound	Cross reactivity %
Androstenedione	100.0
Androsterone	< 0.01
Aldosterone	0.0
Cortisol	0.2
Dihydrotestosterone	< 0.01
Dihydroepiandrosterone	0.01
Estriol	1.8
16-Epiestriol	< 0.01
Estradiol	< 0.01
Estriol-3-glucuronide	< 0.01
Estriol-16-glucuronide	< 0.01
Estriol-16-sulfate	< 0.01
Estrone	< 0.01
17a-Pregnenolone	< 0.01
17 OH-Progesterone	0.3
Progesterone	< 0.01
Testosterone	0.6

Recovery

93.3-114%