Human Androstenedione ELISA Kit

Enzyme Immunoassay for the quantitative determination of human Androstenedione in saliva

Catalog number: ARG80858

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

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INTRODUCTION

Androstenedione (also known as 4-androstenedione) is a 19-carbon steroid hormone produced in the adrenal glands and the gonads as an intermediate step in the biochemical pathway that produces the androgen testosterone and the estrogens estrone and estradiol. It is the common precursor of male and female sex hormones. Some androstenedione is also secreted into the plasma, and may be converted in peripheral tissues to testosterone and estrogens.

Androstenedione originates either from the conversion of dehydro-epiandrosterone or from 17-hydroxyprogesterone. It is further converted to either testosterone or estrone.

The production of adrenal androstenedione is governed by ACTH, while production of gonadal androstenedione is under control by gonadotropins. In premenopausal women the adrenal glands and ovaries each produce about half of the total androstendione (about 3 mg/day). After menopause androstenedione production is about halved, primarily due to the reduction of steroid secreted by the ovary. Nevertheless, androstenedione is the principal steroid produced by the postmenopausal ovary. Increased Androstenedione levels often are seen in PCOS.

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 an 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 in dark. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
Standards 0-4 (0, 20, 100, 400, 1000 pg/ml)	5 X 1 ml (ready to use)	4°C
Control 1 (low) (89.7 pg/ml; acc. range: 59.2-120.2 pg/ml)	1 x 1 ml (ready to use)	4°C
Control 2 (high) (466.4 pg/ml; acc. range: 312.5-620.3 pg/ml)	1 x 1 ml (ready to use)	4°C
HRP-Androstenedione Conjugate	1 ml	4°C
Incubation Buffer	30 ml (ready to use)	4°C
50X Wash Buffer	20 ml	4°C
TMB substrate	15 ml (ready to use)	4°C (Protect from light)
STOP solution	15 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 620-620 nm as reference wavelength)
- 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 in dark. All reagents should be stored refrigerated at 2°C - 8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Open the bag of antibody-coated Microplate only when it is at room temperature and close immediately after use; once it opened, the microplate is stable until the expiry date of kit. Do not remove the adhesive sheets on the unused strips.
- If crystals are observed in the 50X Wash buffer and incubation buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Allow all kit components and specimens to reach room temperature (22 °C- 28 °C) and mix well prior to use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Briefly spin down the HRP-Androstenedione conjugate before 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.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it

is recommended to repeat the dose response curve in each plate.

- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Samples contain azide cannot be assayed. Samples microbiologically contaminated, highly lipaemic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

SAMPLE COLLECTION & STORAGE INFORMATION

Samples containing sodium azide should not be used in the assay. The saliva samples should be completely colorless. Even the slightest red color shows blood contamination. Such blood contamination will give falsely elevated concentration values. In case of visible blood contamination the patient should discard the sample, rinse the sampling device with tap water, also rinse the mouth with (preferably) cold water, wait for 10 minutes and take a new sample. Do not chew anything during the sampling period. Any pressure on the teeth may result in falsely elevated measurements due to an elevated content of gingival liquid in the saliva sample.

Collection –

1. For the correct collection of saliva we are recommending to only use appropriate devices with a centrifuge glass tube and a plastic straw. Other commercially available sample collectors have not been tested.
2. Collect saliva samples at the times indicated. If no specific instructions

have been given, saliva samples may be collected at any time, paying attention to the following indications:

a. If saliva collection is carried out in the morning ensure that this is carried out prior to brushing teeth.
b. During the day allow 1 hour after a meal, oral intake of pharmaceutical drugs or tooth cleaning before collecting saliva samples.
c. It is very important that a good clear sample is received – i.e. no contamination with food, lipstick, blood (bleeding gums) or other extraneous materials.

Storage and Preparation-

1. Let the saliva flow down through the straw into the centrifuge glass tube.
2. Centrifuge the sample for 15 minutes at 3000 rpm.
3. Store at-20 °C for at least 1 hour.
4. Centrifuge again for 15 minutes at 3000 rpm.
5. The saliva sample is now ready to be tested.
6. Store the sample at 2 °C- 8 °C for one week or at-20 °C for longer time.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 20 ml of 50X Wash buffer + 980 ml of distilled water.) The diluted Wash Solution is stable for 30 days at 2 °C- 8 °C.
- Standards: The standards are ready to use. Before use, mix for 5 minutes with rotating mixer. Once opened, the standards are stable 6 months at 2°C - 8°C. For SI UNITS: pg/mL x 3.487 = pmol/L.
- 1X HRP-Androstenedione Conjugate working solution: Prepare immediately before use. Dilute 1:100 with incubation buffer. Add 10 µL of HRP-Androstenedione Conjugate to 1.0 mL of Incubation Buffer, mix gently. The diluted 1X HRP-Androstenedione Conjugate working solution is stable for 3 hours at RT (22°C - 28°C).
- Sample: If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted 1:2 with Standard 0 and re-assayed as described in Assay Procedure.

  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; (22°C- 28°C) 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 **50 µl** of **Standard, controls and samples** in duplicate into the appropriate wells. Leave one well empty as blank.
3. Add **150 µl** of **1X HRP-Androstenedione Conjugate working solution** into each well (except the Blank well) Incubate for **60 minutes at 37°C**.
4. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes.** Wash by filling each well with **1X Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. During each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel.
5. Add **100 µl** of **TMB substrate mixture** to each well (including blank well). Incubate for **15 minutes at room temperature in dark.**
6. Add **100 µl** of **Stop Solution** to each well (including blank well). The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing.
7. Read the OD with a microplate reader at **450 nm** immediately. (Optional: read at 620-620 nm as reference wavelength) It is recommended read the absorbance within 5 minutes after adding the stop solution.

CALCULATION OF RESULTS

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

2. Using log-log, semi-log or 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. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

5. If samples have been diluted prior to the assay, the measured concentration must be multiplied by their respective dilution factors.

6. Conversion factor: SI UNITS: pg/mL x 3.487 = pmol/L.

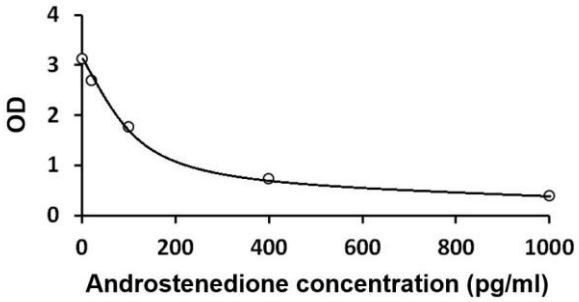
7. REFERENCE VALUE: As the values of salivary Androstenedione have a circadian pattern we suggest collecting the samples at the same hour (8 A.M.). The following values can be used as preliminary guideline until each laboratory established its own normal range.

Samples		pg/mL
WOMEN	Normal	20 – 160
	P.C.O.- Hirsute	120 – 300
MEN		20- 150

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the manufacturer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

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.



The OD of Standard 0 should ≧ 1.250

Standard 1 should ≧ 1.075

Standard 2 should ≧ 0.705

Standard 3 should ≧ 0.295

Standard 4 should ≧0.155

If the tested ODs do not meet the criteria above, please re-test it.

QUALITY ASSURANCE

Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of Standard 0 is 5 pg/ml.

Specificity

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

Steroid	Cross Reaction (%)
Testosterone	1.2
Epitestosterone	0.2
5α-dihydrotestosterone	0.1
DHEA	0.1
Progesterone	0.001
Estrone	0.001
Cortisol	0.001

Intra-assay and inter-assay precision

The CV value of intra-assay precision was ≤ 8.5% and the CV value of inter-assay precision was ≤ 11 %.

Recovery

89.4-115.8%