Human Androstenedione ELISA Kit

Enzyme Immunoassay for the quantification of Human Androstenedione in saliva.

Catalog number: ARG82782

Package: 96 wells

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

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INTRODUCTION

Androstenedione, or 4-androstenedione (abbreviated as A4 or Δ4-dione), also known as androst-4-ene-3,17-dione, is an endogenous weak androgen steroid hormone and intermediate in the biosynthesis of estrone and of testosterone from dehydroepiandrosterone (DHEA). It is closely related to androstenediol (androst-5-ene-3β,17β-diol).

Androstenedione is a precursor of testosterone and other androgens, as well as of estrogens like estrone, in the body. In addition to functioning as an endogenous prohormone, androstenedione also has weak androgenic activity in its own right.

Androstenedione has been found to possess some estrogenic activity, similarly to other DHEA metabolites. However, in contrast to androstenediol, its affinity for the estrogen receptors is very low, with less than 0.01% of the affinity of estradiol for both the ERα and ERβ. [Provided by Wikipedia: Androstenedione]

PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative enzyme immunoassay technique. A highly specific antibody for Androstenedione has been pre-coated onto a microplate. Androstenedione containing samples, Controls 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, the wells are washed with diluted Wash Buffer to remove unbound material. Then TMB substrate is added to the wells and color develops in inversely proportion to the amount of Androstenedione present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of Androstenedione in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C in the dark. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated microplate	8 X 12 strips	4°C
Standards A to E (0, 20, 100, 400, 1000 pg/mL)	1 mL each (ready to use)	4°C
Control 1 & 2	1 mL each (ready to use)	4°C
Diluent Buffer	30 mL (ready to use)	4°C
Androstenedione-HRP Conjugate	1 mL	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 reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Saliva collection device
- Mixer or Ultra-Turrax
- Microplate shaker
- Pipettes and pipette tips
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (22-28°C).
- Remove the number of strips required and return unused strips to the pack and reseal.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not

become completely dry during the assay.

- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Saliva:

1. Collect saliva sample with a centrifuge glass tube (contain a plastic straw).
2. Centrifuge the sample at 3000 rpm for 15 minutes.
3. Store at-20°C for at least 1 hour.
4. Centrifuge again at 3000 rpm for 15 minutes.
5. Use supernatant for the assay.

Note:

1. Samples containing sodium azide should not be used in the assay.
2. If saliva collection is carried out in the morning ensure that this is carried out prior to brushing teeth.
3. During the day allow 1 hour after a meal, oral intake of pharmaceutical drugs or tooth cleaning before collecting saliva samples.
4. It is very important that a good clear sample is received. No contamination with food, lipstick, blood (bleeding gums) or other extraneous materials.
5. Specimens should be capped and may be stored for up to one week at 2-8°C prior to assaying. Specimens stored for a longer time (up to 3 months) should be frozen only once at-20°C prior to assay. Thawed samples should be inverted several times prior to testing.
6. For samples with Androstenedione concentration greater than 1000 pg/ml dilute the sample (1:2) with Standard A.

REAGENT PREPARATION

- **1X Wash Buffer:** Dilute 50X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 20 mL of 50X Wash Buffer into 980 mL of distilled water to a final volume of 1000 mL) The 1X Wash Buffer is stable for up to 4 weeks at 2-8°C.
- **1X Androstenedione-HRP Conjugate:** Dilute 1:100 in Diluent Buffer before use (E.g. 10 µL of Androstenedione-HRP Conjugate in 1 mL of Diluent Buffer). If the whole plate is to be used dilute 120 µL of HRP in 12 mL of Diluent buffer. Discard any that is left over. **Stable at RT for 3 hours.**

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 22-28°C) before use. Standards and samples should be assayed in duplicates.

1. Add **50 µL** of **Standards, Controls and prepared samples** into the appropriate wells of the Antibody Coated Microplate.
2. Add **150 µL** of **1X Androstenedione-HRP Conjugate** into all wells.
3. Incubate at **37°C** for **1 hour** on a microplate shaker.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 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.
5. Add **100 µL** of **TMB Substrate** to each well, including the blank wells.

Incubate in the dark for **15 minutes** at **RT** on a microplate shaker.

6. Immediately Add **100 µL** of **Stop Solution** to each well, including the blank wells. The color of the solution should change from blue to yellow.
7. Read the OD with a microplate reader at **450 nm** immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance **within 5 minutes** after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of Controls, standards 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. 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.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. The clinical significance of Androstenedione determination can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.
6. For SI UNITS: pg/mL x 3.487 = pmol/L
7. Reference value:

		pg / mL
Women	Normal	20-160
	P.C.C.-Hirsute	120-300
Men		20-150

QUALITY ASSURANCE

Sensitivity

The sensitivity of the Human Androstenedione ELISA kit is 5 pg/mL.

Specificity

Substance	Cross Reactivity (%)
Androstenedione	100
Testosterone	1.2
Epitestosterone	0.2
5α-dihydrotestosterone	0.1
DHEA	0.1
Progesterone	$1x10^{-3}$
Estrone	$1x10^{-3}$
Cortisol	$1x10^{-3}$

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was $\leq$ 8.5% and CV value of inter-assay precision was $\leq$ 11%.

Recovery

81.7-115.5%