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Product Information

Human Androstenedione ELISA kit

Catalog Number: EA100852
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The Androstenedione ELISA Kit is intended for the measurement of Androstenedione in serum or plasma.

Background

Androstenedione is the primary precursor of testosterone in women. It is synthesized in the adrenal gland. Measurement of Androstenedione may be used as an indicator of androgenic activity in women. The steroid hormone Androstenedione is one of the main androgens, besides Testosterone and Dehydroepiandrosterone. 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 maybe 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 test

The Androstenedione ELISA kit is based on the principle of competitive binding between Androstenedione in the test specimen and Androstenedione-HRP conjugate for a constant amount of rabbit anti-Androstenedione. In the first incubation, goat anti-rabbit IgG-coated wells are incubated with 25µl of Androstenedione standards, patient samples, 50µl Androstenedione-HRP conjugate reagent and 50µl rabbit anti-Androstenedione reagent at room temperature for 60 minutes. During the incubation, HRP labeled Androstenedione competes with the endogenous Androstenedione in the standard and sample, for a fixed number of binding sites of the specific Androstenedione antibody. Thus, the amount of Androstenedione peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of Androstenedione in the specimen increases. Unbound Androstenedione peroxidase conjugate is then removed and the wells washed. Next, a solution of TMB Reagent is added and incubated at room temperature for 15 minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450nm. A standard curve is prepared relating color intensity to the concentration of Androstenedione.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Goat anti-rabbit IgG	12x8x1
2. Standard : 6 vials (ready to use)	0.5 ml
3. Enzyme Conjugate (ready to use)	7 ml
4. Rabbit Anti- Androstenedione Reagent (ready to	7 ml
5. TMB substrate (ready to use)	12 ml
6. Stop solution (ready to use)	12 ml
7. Wash Solution 20x Concentrated	25ml

Materials and Equipment Required but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection Handling

1. Collect blood specimens and separate the serum immediately.
2. Plasma should be treated with EDTA.
3. Plasma samples may be stored at 2-8°C for up to 8 hours, and should be frozen at -20°C or lower for up to 4 months.
4. Avoid multiple freeze-thaw cycles.
5. Prior to assay, frozen sera should be completely thawed and mixed well.
6. Do not use grossly hemolyzed or lipemic specimens.

Reagent Preparation

20x Wash Buffer: Prepare 1X Wash Buffer by adding the contents of the bottle (25ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26 °C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- It is recommended that standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

1. Secure the desired number of microwells strips in the holder.
2. Dispense 25 µl Androstenedione Standards, controls and samples into appropriate wells.
3. Dispense 50 µl Enzyme Conjugate into each well.
4. Dispense 50 µl anti- Androstenedione reagent into each well.
5. Incubate for 60 minutes at room temperature with shaking.
6. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted wash solution. Strike the wells sharply on absorbent paper to remove residual water droplets.
   - NOTE: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure.
7. Add 100 µl of Substrate Solution to each well.
8. Incubate for 15 minutes at room temperature.
9. Stop the enzymatic reaction by adding 50µl of Stop Solution into each well.
10. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stop solution.

Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration in ng/ml 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 of Androstenedione from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log can generally give a good fit.
5. The concentration of the samples can be read directly from this standard curve. Samples with Androstenedione concentration higher than the concentration of the highest standard have to be diluted with zero standard. For the calculation of the concentrations this dilution factor has to be taken into account.

Example of a standard Curve

	OD 450 nm	Conc. ng/mL
Std 1	2.132	0
Std 2	1.705	0.12
Std 3	1.324	0.37
Std 4	0.811	1.11
Std 5	0.314	3.33
Std 6	0.171	10

Expected Values

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values may be used as initial guideline ranges only:

	Age	Conc. Range
Male and Pre-Menopausal	Adult	0.25 – 3.0
Post-menopausal women	Adult	0.12 – 1.5

Limitation of the Test

Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Performance Characteristics

1. Sensitivity

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean ng/ml	Standard Deviation	Mean + 2SD (Sensitivity) ng/ml
Zero Standard	24	0.008	0.008	0.024

The cross-reaction of the antibody calculated at 50% method, according to Abraham, are shown in the table:

Analyte	% Cross reactivity
Androstenedione	100
Testosterone	0.3486
5 alpha-Dihydrotestosterone	<0.0001
Androsterone	0.009
DHEA-S	0.0007
Cortisol	<0.0001
17 Estradiol	<0.0001
Estrone	0.0167
Androsterone-SO4	0.0017
Progesterone	0.1091
Desoxycorticosterone	0.200

References

1. Horton R., Tait J., Androstenedione production and interconversion rates measured in peripheral blood and studies on the possible site of its conversion to testosterone.. J.Endocrinol Invest. 45: 301-313, 1966.
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3. Erickson GF 1993 Normal regulation of ovarian androgen production. Seminars in Reproductive Endocrinology 11:307-312.
4. Kicman, A. T., Bassindale, T., Cowan, D. A., Dale, S., Hutt, A. J., and Leeds, A. R., Effect of androstenedione ingestion on plasma testosterone in young women; a dietary supplement with potential health risks Clin.Chemistry 2003; 49:167-169.
5. Brown, G.A., Vukovich, M.D., Martini, E.R., Kohut, M.L., Franke, W.D., Jackson, D.A., and King, D.S. Endocrine responses to chronic androstenedione intake in 30- to 56-year-old men. J Clin Endocrinol Metab 2000, 85:4074- 4080.