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CALBIOTECH
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ANDROSTENEDIONE ELISA

Catalog No. AD183E (96 Tests)

INTENDED USE

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

SUMMARY AND EXPLANATION

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.

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 use)	7 ml
5.	TMB substrate (ready to use)	12 ml
6.	Stop solution (ready to use)	12 ml
7.	Wash Solution 20x Concentrated	25 ml

MATERIAL 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

STORAGE AND STABILITY

1. Store the kit at 2 - 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose reagent to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For Laboratory use.
3. Not for Internal or External Use in Humans or Animals.
4. There should be no eating or drinking within work area.
5. Always wear gloves and a protective lab coat.
6. No pipetting should be done by mouth. Handle all specimens and reagents as potentially infectious and biohazardous.
7. Do not add sodium azide to samples as preservative.
8. Do not use external controls containing sodium azide.
9. Use disposable pipette tips to avoid contaminating chromogenic substrate reagent. Discard reagent if it turns blue.
10. Do not pour chromogenic substrate back into container after use.
11. Do not freeze reagents.
12. Do not mix reagents from different kit lot numbers.
13. Keep reagents out of direct sunlight.
14. Handle stop reagent with care, since it is corrosive.
15. Bring all reagents to room temperature.
16. Viscous forensic samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting.
17. Ensure the bag containing the micro-plate strips and desiccant is sealed well, if only a few strips are used.

SPECIMEN COLLECTION HANDLING

1. Collect blood specimens and separate the serum immediately.
2. Typically, specimens may be stored refrigerated at (2-8° C) for 1 week. If storage time exceeds 1 week, store frozen at (-20° C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

PREPARATION OF REAGENTS

20XWash 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 (20-25° C).

ASSAY PROCEDURE

All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming. Once the test has been started, all steps should be completed without interruption.

1. Secure the desired number of microwells strips in the holder.
2. Dispense 25µl Androstenedione Standards, controls and samples into appropriate wells.
3. Dispense 50µl Enzyme Conjugate into each well.
4. Dispense 50µl 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

LIMITATION OF THE TEST

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