AccuDiag™ Androstenedione ELISA Kit

REF 1038-17

IVD 2°C 8°C Σ 96 Tests

Test	Androstenedione ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Competitive Enzyme Immunoassay
Detection Range	0.1-10 ng /ml
Sample	25 µL serum
Total Time	~75 min.
Shelf Life	12 Months from the manufacturing date
Sensitivity	0.01 ng/mL

INTENDED USE

Competitive immunoenzymatic colorimetric method for quantitative determination of Androstenedione concentration in human serum or plasma.
Androstenedione ELISA kit is intended for research use only. Do not use for diagnostic purpose.

SUMMARY AND EXPLANATION

Androstenedione (also known as Δ4-androstenedione) is a 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 has relatively weak androgenic activity, estimated at ~ 20% of testosterone. However, serum androstenedione levels often exceed testosterone in both normal and disease states. Secretion and production rates also exceed those of testosterone in women in whom significant extra-adrenal conversion of androstenedione to testosterone occurs.

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.

Measurement of serum androstenedione provides a useful marker of androgen biosynthesis. Elevated androstenedione levels have been demonstrated in virilizing congenital adrenal hyperplasia. Serum androstenedione levels are also increased in polycystic ovary syndrome, and in case of hirsutism in women. Elevated serum androstenedione levels may also occur in adrenal and ovarian virilizing tumors.

TEST PRINCIPLE

The Androstenedione (antigen) in the sample competes with the antigenic Androstenedione conjugated with horseradish peroxidase (HRP) for binding to the limited number of antibodies anti Androstenedione coated on the microplate (solid phase).

After incubation, the bound/free separation is performed by a simple solid-phase washing.

Then, the enzyme HRP in the bound-fraction reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blu color that changes into yellow when the Stop Solution (H_2SO_4) is added.

The color intensity is inversely proportional to the Androstenedione concentration of in the sample.

Androstenedione concentration in the sample is calculated through a calibration curve.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. Calibrators (6 vials, 1 mL each)
   - CAL0 REF 1038-17
   - CAL1 REF 1038-17
   - CAL2 REF 1038-17
   - CAL3 REF 1038-17
   - CAL4 REF 1038-17
   - CAL5 REF 1038-17
2. Control (1 vial, 1 ml) Control concentration is indicated on the Certificate of Analysis
3. Conjugate (1vial, 21 mL) Androstenedione conjugated with Horseradish Peroxidase (HRP)
4. Coated Microplate (1 breakable microplate)Anti Androstenedione antibodies adsorbed on the microplate
5. TMB-Substrate 1 vial, 15 mL) H_2O_2-TMB (0.26 g/L) *(avoid any skin contact)*
6. Stop solution (1vial, 15 mL) Sulphuric acid 0.15 mol/L (avoid any skin contact)
7. 10 X Conc. Wash Solution (1 vial, 50 ml) 0.2M Phosphate Buffer, pH 7.4

Materials required but not provided

1. Distilled water
2. Automatic dispenser
3. Microplates reader (450 nm, 620-630 nm)

Notes

Store all reagents between 2- 8C°in the dark.
Open the bag of reagent 4 (Coated Microplate) only when it is at room temperature and close immediately after use opened, it is stable until the expiry date of the kit.

Reagent Preparation

1. **Preparation of the Calibrators (C_0…C_5)**

Before using, leave the Calibrators on a rotating mixer for at least 5 minutes.
The Standards are ready for use and have the following concentration of Androstenedione:

	C_0	C_1	C_2	C_3	C_4	C_5
ng/ml	0	0.1	0.4	1.2	4.0	10.0

Diagnostic Automation/ Cortez Diagnostics, Inc.
21250 Califa St, Suite 102 and 116, Woodland Hills, CA 91367 USA Phone: 818-591-3030, Fax : 818-591-8383
Email: onestep@rapidtest.com Website: www.rapidtest.com

The Calibrators are stable until the expiry date printed on the label. Once opened, the calibrators are stable six months at 2-8°C.

2. **Preparation of the Sample**
The determination of Androstenedione can be performed in human plasma as well as in serum. Store reagent at -20°C if the determination is not performed on the same day of the sample connection.
Avoid repetitive freezing and thawing of samples.
Dilute the samples higher than 10 ng/mL (1:2) with Standard 0.
The Control is ready for use.

3. **Preparation of the Wash Solution**
Dilute the contents of each vial of the "10X Conc. Wash Solution" with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C. In concentrated wash solution is possible to observe the presence of crystals; in this case mix at room temperature until the complete dissolution of crystals; for greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care to transfer completely the crystals, then mix until crystals are completely dissolved.

ASSAY PROCEDURE

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay, store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the Standard curve (C_0-C_5), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample / Control	Blank
Sample / Control		**25 µL**	
Calibrator C_0-C_5	**25 µL**		
Conjugate	**200 µL**	**200 µL**	
Incubate at *+37°C* for *1 hour* Remove the contents from each well. Wash the wells 3 times with 300 µL of diluted wash solution. **Important note**: 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. **Automatic washer**: if you use automated equipment, wash the wells at least 5 times.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate at room temperature 22-28°C for *15 minutes* in the dark.			
Stop solution	**100 µL**	**100 µL**	**100 µL**
Shake gently the microplate. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

RESULTS

1. **Mean Absorbance**
Calculate the mean of the absorbance (Em) for each point of the standard curve (C_0-C_5) and of each sample.

2. **Standard Curve**
Plot the mean value of absorbance (Em) of the standards (C_0-C_5) against concentration. Draw the best-fit curve through the plotted points. (e.g.: Four Parameter Logistic).

3. **Calculation of results**
Interpolate the values of the samples on the calibration curve to obtain the corresponding values of the concentrations expressed in ng/mL.

REFERENCE VALUE

The serum or plasma Androstenedione reference values are:

WOMEN	Follicular phase	0.75 - 3.1 ng/mL
	Luteinic phase	0.94 - 3.2 ng/mL
MEN		0.60 - 2.7 ng/mL

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.

QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of Androstenedione for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

PERFORMANCE CHARACTERISTICS

1. **Precision**
Intra Assay Variation
Within run variation was determined by replicate (20x) the measurements of three different control sera in one assay. The within assay variability is ≤ 10.0%.

Inter Assay Variation
Between run variation was determined by replicate (10x) the measurements of three different control sera in different lots. The between assay variability is ≤ 9.5%.

2. **Accuracy**
The recovery of 0.4 - 0.8 - 1.6 - 3.2 ng/mL of Androstenedione added to sample gave an average value (±SD) of 100.91% ± 5.61% with reference to the original concentrations.

The dilution test performed on three sera diluted 2 - 4 - 8 - 16 times gave an average value (±SD) of 107.18% ± 3.03%.

3. **Specificity**
The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

Androstenedione	100 %
5 α-dihydrotestosterone	0.05 %
DHEA	0.05 %
Epitestosterone	0.04 %
DHEA-S	0.027 %
Cortisol	0.008 %
Progesterone	0.007 %
Estrone	0.007 %
Testosterone	<0.001%
17B-Estradiol	<0.001%
Estriol	<0.001 %
Aldosterone	<0.001 %

4. **Sensitivity**
The lowest detectable concentration of Androstenedione that can be distinguished from the zero standard is 0.01 ng/mL at the 95 % confidence limit.

5. **Correlation with RIA**
The DAI Androstenedione ELISA was compared to another commercially available Androstenedione assay. 37 serum samples were analysed according in both test systems.

The linear regression curve was calculated:
(Androstenedione DAI) = 0.89* (Androstenedione RIA) + 0.16
$r^2 = 0.859$

DAI Androstenedione kit (Y) was compared to the previous DAI Androstenedione assay (X).
78 serum samples were analyzed.
The linear regression curve was calculated:
(Y) = 079*(X) + 0.53
$r^2 = 0.825$

PRECAUTIONS

1. Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
2. All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
3. Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
4. Do not interchange kit components from different lots. The expiry dates printed on the labels of the box and of the vials must be observed. Do not use any kit component beyond their expiry date.
5. If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
6. The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
7. 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.
8. 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.
9. Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
10. Maximum precision is required for reconstitution and dispensation of the reagents.
11. Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
12. Plate readers measure vertically. Do not touch the bottom of the wells.

WARNINGS

1. This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
2. Use appropriate personal protective equipment while working with the reagents provided.
3. Follow Good Laboratory Practice (GLP) for handling blood products.
4. Some reagents contain small amounts of Proclin 300^R as preservative. Avoid the contact with skin or mucosa.
5. The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
6. The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
7. Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.
8. This method allows the determination of Androstenedione from 0.1 ng/mL to 10 ng/mL.
9. The clinical significance of Androstenedione determination can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.

REFERENCES

1. Judd. H. Yen S. J.Clin. Endocr. & Metab., 36, 475 (1973)
2. Abraham G, J. Clin. Endoc. & M. 39,340 (1974)
3. Hillier S. G. and De Zwart F.
4. 79th Year book Medical Publishers Inc. Chicago (1985)
5. Venturoli S. et al Aspects in Adolescence with Menstrual Irregularities Fertility and Sterility, 48 (1), 78 (1987)
6. Venturoli S. et al Hormone Res., 24,269 (1986)

ISO 13485
ISO 9001

Diagnostic Automation/Cortez Diagnostics, Inc.
21250 Califa Street, Suite 102 and 116,
Woodland Hills, California 91367 USA

Date Adopted	**2017-Jan-06**
REF **1038-17**	**AccuDiag™- Androstenedione ELISA**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands.** www.cepartner4u.eu
Revision Date: 2015-Oct	