AccuDiag™ Androstenedione ELISA Kit

Cat# 1038-17

IVD See external Label 2°C 8°C Σ 96 Tests

Test	Androstenedione ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Competitive Enzyme Immunoassay
Detection Range	0.01-10 ng /ml
Sample	25 µL serum
Total Time	~75 min.
Shelf Life	12 -14 Months from the manufacturing date
Specificity	100 %
Sensitivity	0.01 ng/mL

INTENDED USE

Competitive immunoenzymatic colorimetric method for quantitative determination of Androstenedione concentration in serum and plasma**. Androstenedione kit is intended for laboratory use only.**

SUMMARY AND EXPLANATION

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 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

Androstenedione (antigen) in the sample competes with horseradish peroxidase Androstenedione (enzyme-labelled antigen) for binding onto the limited number of anti-Androstenedione coated on the microplates (solid phase).

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

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

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

The enzyme substrate (H_2O_2) and the TMB-substrate (TMB) are added. After an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbance are determined.

Androstenedione concentration in the sample is calculated based on a series by a set of standard.

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

Reagent Preparation

1. Preparation of the Standard (S_0,S_1,S_2,S_3,S_4,S_5) and Control
The standard has the following concentration of Androstenedione:

	S_0	S_1	S_2	S_3	S_4	S_5
ng/ml	0	0.1	0.4	1.2	4.0	10.0

Stability: until the expiration date printed on the kit at 2-8 °C.
Once open, the standards are stable six months at 2-8 °C.

2. **Preparation of the Sample**
The determination of Androstenedione can be performed in 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.
Dilute the samples higher than 10 ng/mL (1/2) with the **"Sample diluent"**.

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.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. Androstenedione Standards 6x (1 vial = 1 mL)

STD0	REF DAS0/1038-17
STD1	REF DAS1/1038-17
STD2	REF DAS2/1038-17
STD3	REF DAS3/1038-17
STD4	REF DAS4/1038-17

STD5 REF **DAS5/1038-17**

2. Control (1 vial = 1ml) REF **DA-Con/1038-17**

3. Conjugate (1 bottle) 21 mL
Androstenedione-HRP conjugate REF **DA-C/1038-17**

4. Coated Microplate :Anti-Androstenedione IgG absorbed on microplate
(1 microplate breakable) REF **DA-P/1038-17**

5. TMB-substrate (1 bottle) 15 mL. H_2O_2-TMB 0.25gr/L
(avoid any skin contact) REF **DA-T/1038-17**

6. Stop solution (1 bottle) 15 mL Sulphuric acid 0.15 mol/L
(avoid any skin contact) REF **DA-S/1038-17**

7. **10 X Conc. Wash Solution (1 vial, 50 ml)**
NaCl 160g/L ; tween-2010 g/L 0.2M Phosphate Buffer, pH 7.4
REF **DA WC/1038-17**

Materials required but not provided

1. Distilled water.
2. Automatic dispenser.
3. Microplates reader (450 nm).

Notes

Store all reagents between 2÷8C°in the dark.
Open the bag of reagent 3 (Antibody) only when it is at room temperature and close immediately after use. Do not remove the adhesive sheets on the unused strips.

ASSAY PROCEDURE

- **Allow all reagents to reach room temperature (22-28°C).**
- 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 calibration curve (C_0-C_5), two for each Control, two for each sample, one for Blank.

Reagent	**Standard**	**Sample**	**Blank**
Sample		**25 µL**	
Standard S0-S5	**25 µL**		
Control	**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. Repeat the washing procedure by draining the water completely			
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 Blank

RESULTS

1. **Mean Absorbance**
Calculate the mean of the absorbance (Em) for each point of the standard curve (S0-S5) and of each sample.

2. **Standard Curve**
Plot the mean value of absorbance (Em) of the standards (S0-S5) against concentration. Draw the best-fit curve through the plotted points. (es: Four Parameter Logistic).

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

4. Range of Control:
Please refer to the range information from the COA for the given Lot number. The obtained value for the control should fall within the specified range.

REFERENCE VALUE

The serum or plasma Androstenedione reference values are:

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

1.1.1. 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%.

1.1.2. 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. Serum samples of 16 females and 21 males were analysed according in both test systems.

The linear regression curve was calculated
y = 0.928 x + 0.02
r = 0.946 (r^2 = 0.895)
Androstenedione kit (Y) was compared to the previous Androstenedione DAI assay (X).
78 serum samples were analyzed.
The linear regression curve was calculated:
(Y) = 079*(X) + 0.53
r2 = 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.
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

- This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Some reagents contain small amounts of Proclin 300^R as preservative. Avoid the contact with skin or mucosa.
- 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.
- 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.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.
- This method allows the determination of Androstenedione from 0.1 ng/mL to 10 ng/mL.
- The clinical significance of Androstenedione determination can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.

WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

REFERENCES

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ISO 13485
ISO 9001

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2012-03-01	AccuDiag™- Androstenedione ELISA -2013
EC REP	CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu
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