



User's Manual

Androstenedione ELISA

REF

IB79119



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IVD

For *in-vitro* diagnostic use.

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1 INTRODUCTION

1.1 Intended Use

The **IBL-AMERICA Androstenedione ELISA** is an enzyme immunoassay for the quantitative *in vitro* diagnostic measurement of Androstenedione in serum and EDTA plasma. This assay is for *in-vitro* diagnostic use.

1.2 Summary and Explanation

The steroid hormone Androstenedione is one of the main androgens, besides Testosterone and Dehydroepiandrosterone. Testosterone, the most important biological active androgen, is derived from peripheral enzymatic conversion of Androstenedione.

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 may be 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.

2 PRINCIPLE OF THE TEST

The IBL-AMERICA Androstenedione ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.

The microtiter wells are coated with a polyclonal [rabbit] antibody directed towards an antigenic site of the Androstenedione molecule.

During the first incubation, the androstenedione in the added sample competes with the added enzyme conjugate, which is an androstenedione molecule conjugated to horseradish peroxidase, for binding to the coated antibody.

After a washing step, to remove all unbound substances, the solid phase is incubated with the substrate solution. The reaction is abruptly stopped by addition of the stop solution and optical density (OD) of the resulting yellow product is measured. The intensity of the color is inversely proportional to the concentration of the analyte in the sample.

A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

3 WARNINGS AND PRECAUTIONS

1. This kit is for *in-vitro* diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21 °C - 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where samples or kit reagents are handled.
12. Wear disposable latex gloves when handling samples and reagents. Microbial contamination of reagents or samples may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 1 N acidic solution. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from IBL-America.

4 REAGENTS

4.1 Reagents provided

1. **SORB** **MT** **Microtiterwells**, 12 x 8 (break apart) strips, 96 wells; Wells coated with anti-Androstenedione antibody (polyclonal).
2. **CAL** **0** – **5** **Standard (Standard 0-5)**, 6 vials, 1 mL, ready to use Concentrations: 0 – 0.1 – 0.3 – 1.0 – 3.0 – 10 ng/mL. Conversion: ng/mL x 3.492 = nmol/L, Standards are calibrated against Reference Material of the National Measurement Institute Australia (NMI-M955) Contain non-mercury preservative.
3. **CONTROL** **low** – **high** **Control Low & High**, 2 vials, 1.0 mL each, ready to use; For control values and ranges please refer to vial label or QC-Datasheet. Contain non-mercury preservative.
4. **ENZ** **CONJ** **Enzyme Conjugate**, 1 vial, 25 mL, ready to use, Androstenedione conjugated to horseradish peroxidase; Contains non-mercury preservative.
5. **SUB** **TMB** **Substrate Solution**, 1 vial, 25 mL, ready to use, Tetramethylbenzidine (TMB).
6. **STOP** **SOLN** **Stop Solution**, 1 vial, 14 mL, ready to use, contains 1 N acidic solution, Avoid contact with the stop solution. It may cause skin irritations and burns.
7. **WASH** **SOLN** **40x** **Wash Solution**, 1 vial, 30 mL (40X concentrated), see „Preparation of Reagents“.

Note: Additional *Standard 0* for sample dilution is available upon request.

4.2 Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm)
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

4.3 Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.

Opened kits retain activity for 8 weeks if stored as described above.

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Wash Solution

Add deionized water to the 40X concentrated Wash Solution.

Dilute 30 mL of concentrated *Wash Solution* with 1170 mL deionized water to a final volume of 1200 mL.

The diluted Wash Solution is stable for 2 weeks at room temperature.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheet.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, IBL-AMERICA has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SAMPLE COLLECTION AND PREPARATION

Serum or EDTA plasma can be used in this assay.

Do not use Heparin or Citrate plasma. Heparin plasma leads to slightly reduced values. For citrate plasma the results are significantly increased.

Note: Samples containing sodium azide should not be used in the assay. In general, it should be avoided to use haemolytic, icteric or lipaemic specimens. For further information refer to chapter "Interfering Substances".

5.1 Sample Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Samples receiving anticoagulants may require increased clotting time.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.

(E.g. Sarstedt Monovette for EDTA plasma)

5.2 Sample Storage and Preparation

Samples should be capped and may be stored for up to 5 days at 2 °C to 8 °C prior to assaying. Samples held for a longer time (up to 12 months) should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Sample Dilution

If in an initial assay, a sample is found to contain more than the highest standard, the sample can be diluted with *Standard 0* and reassayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

- a) dilution 1:10: 10 µL sample + 90 µL *Standard 0* (mix thoroughly)
b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly).

6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and samples 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.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **20 µL** of each **Standard, Control** and **samples** with new disposable tips into appropriate wells.
3. Dispense **200 µL Enzyme Conjugate** into each well.
Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **60 minutes** at room temperature.
5. Briskly shake out the contents of the wells.
Rinse the wells **4 times** with diluted *Wash Solution* (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.

Important note:

The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

6. Add **200 µL** of **Substrate Solution** to each well.
7. Incubate for **30 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **100 µL** of **Stop Solution** to each well.
9. Determine the absorbance (OD) of each well at **450 nm (reading) and at 620 nm to 630 nm (background subtraction, recommended)** with a microtiter plate reader.
It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Results

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using semi-logarithmic 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. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. 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.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 10.0 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only can **cannot** be used in place of data generations at the time of assay.

Standard	Optical Units (450nm)
Standard 0 (0 ng/mL)	1.93
Standard 1 (0.1 ng/mL)	1.76
Standard 2 (0.3 ng/mL)	1.39
Standard 3 (1.0 ng/mL)	0.87
Standard 4 (3.0 ng/mL)	0.47
Standard 5 (10.0 ng/mL)	0.23

7 EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with serum of apparently normal healthy donors, using the IBL-AMERICA Androstenedione ELISA the following values are observed:

Females

Age (years)	N	Mean (ng/mL)	Median (ng/mL)	2.5 th Percentile (ng/mL)	97.5 th Percentile (ng/mL)	Range (min. – max.) (ng/mL)
0-10	29	0.39	0.30	0.02	0.86	0.00 – 0.99
11-17	17	1.36	1.18	0.25	2.78	0.10 – 2.83
18-53	66	2.22	2.12	0.75	3.89	0.30 – 4.39
54-82	26	1.32	1.20	0.35	2.49	0.25 – 2.72

Males

Age (years)	N	Mean (ng/mL)	Median (ng/mL)	2.5 th Percentile (ng/mL)	97.5 th Percentile (ng/mL)	Range (min. – max.) (ng/mL)
0-10	34	0.40	0.26	0.01	1.31	0.00 – 1.54
11-17	16	1.75	1.75	0.33	3.30	0.11 – 3.50
18-53	36	2.15	2.15	0.45	4.20	0.44 – 4.56
54-82	44	1.95	1.95	0.30	3.93	0.26 – 4.26

8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or IBL-AMERICA directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 0.021 – 10 ng/mL.

9.2 Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross reactivity of the assay:

Compound	Cross reactivity %
Androstenedione	100
Androsterone	< 0.01
Aldosterone	0.0
Cortisol	0.2
Dihydrotestosterone	< 0.01
Dihydroepiandrosterone	< 0.01
Estriol	1.8
16-Epiestriol	< 0.01
Estradiol	< 0.01
Estriol-3-glucuronide	< 0.01
Estriol-16-glucuronide	< 0.01
Estriol-16-sulfate	< 0.01
Estrone	< 0.01
17a-Pregnenolone	< 0.01
17OH-Progesterone	0.3
Progesterone	< 0.01
Testosterone	0.6

9.3 Sensitivity

The analytical sensitivity of the IBL-AMERICA ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the Standard 0 (S0) and was found to be 0.021 ng/mL.

9.4 Reproducibility

9.4.1 Intra Assay

The within assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	20	0.7	9.2
2	20	1.2	5.3
3	20	8.0	6.9

9.4.2 Inter Assay

The between assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	40	1.0	8.7
2	40	3.0	8.1
3	40	4.3	8.3

9.4.3 Inter-Lot

The inter-lot variation was determined by measuring each sample 6 times with 3 different kit lots.

Sample	n	Mean (ng/ml)	Inter Lot CV (%)
1	18	3.4	5.2
2	18	5.3	8.7
3	18	7.1	5.6

9.5 Recovery

Samples have been spiked by adding Androstenedione solutions with known concentrations in a 1:1 ratio. The expected values were calculated by addition of half of the values determined for the undiluted samples and half of the values of the known solutions. The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

		Sample 1	Sample 2	Sample 3
Concentration [ng/mL]		1.5	1.4	2.0
Average Recovery		106.6	102.6	104.0
Range of Recovery [%]	from	96.4	99.1	93.3
	to	112.9	108.6	114.0

9.6 Linearity

		Sample 1	Sample 2	Sample 3
Concentration [ng/mL]		6.2	4.7	7.2
Average Recovery		97.0	96.3	93.4
Range of Recovery [%]	from	92.3	91.6	86.7
	to	102.9	101.3	105.6

10 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of Androstenedione in a sample.

10.3 High-Dose-Hook Effect

No hook effect was observed in this test.

11 LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact IBL-AMERICA.

11.2 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

12 REFERENCES

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SYMBOLS USED WITH IBL-AMERICA ELISAS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità