EAGLE BIOSCIENCES

Androstenedione ELISA Assay Kit

Catalog Number:
ASD31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 2.0 (22 APR 24)

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INTENDED USE

The Eagle Biosciences Androstenedione ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of Androstenedione in human serum by an enzyme immunoassay. The Eagle Biosciences Androstenedione ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.

INTRODUCTION

Androstenedione is produced by the adrenals and gonads. As a result, the determination of the level of androstenedione in serum is important in the evaluation of the functional state of these glands. Androstenedione is a precursor of testosterone and estrone. Besides the adrenals, in females, the ovaries have been shown to be an important source of androstenedione. It has been reported that there is a fluctuation day by day of androstenedione during the ovulatory cycle.
The principle production of testosterone in females is from the conversion of other related androgens, especially androstenedione. An abnormal testosterone level in women should be accompanied by the estimation of serum androstenedione. The use of serum testosterone determination in conjunction with the enzyme immunoassay of androstenedione can be used to determine if the source of the excess androgen production is adrenal or ovarian.

PRINCIPLE OF THE ASSAY

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, controls and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of androstenedione in the sample. A set of standards is used to plot a standard curve from which the amount of androstenedione in patient samples and controls can be directly read.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
    a. Do not pipette by mouth.
    b. Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.

c. Wear protective clothing and disposable gloves. d Wash hands thoroughly after performing the test. e Avoid contact with eyes, use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.

3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Do not use this kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or saliva pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The TMB Substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
15. Do not use blood contaminated saliva samples.
16. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit may be reported as >10 ng/mL. If further dilution and retesting is required, only Calibrator A may be used to dilute samples. The use of any other reagent may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
20. To prevent contamination of reagents, do not pour reagents back into the original containers.
21. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
22. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.

25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
26. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of saker used can influence the optical densities and test results. If a different type of shake and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse the microplate wells, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
29. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the participant is established.
30. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be nonreactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. No test method however, can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.
All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

This assay is a direct system. No specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 25, 50, 100, 150 and 300 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker

5. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)

REAGENTS PROVIDED

1. **Rabbit Anti-Androstenedione Antibody-Coated Break-Apart Well Microplate**— Ready To Use
   - Contents: One 96-well (12x8) polyclonal antibody-coated microplate in a resealable pouch with desiccant.
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

2. **Androstenedione-Horseradish Peroxidase (HRP) Conjugate** — Ready to Use
   - Contents: One bottle containing Androstenedione-HRP conjugate in a protein-based buffer with a nonmercury preservative.
   - Volume: 14 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

3. **Androstenedione Calibrators** — Ready To Use
   - Contents: Six vials containing androstenedione in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum with a precise quantity of androstenedione.

   * Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
A	0 ng/mL	2.0 mL
B	0.1 ng/mL	0.5 mL
C	0.3 ng/mL	0.5 mL
D	1 ng/mL	0.5 mL
E	3 ng/mL	0.5 mL
F	10 ng/mL	0.5 mL

   - Storage: Refrigerate at 2–8°C.
   - Stability: 12 months in unopened vials or as indicated on label.

4. **Controls** — Ready to Use
   - Contents: Two vials containing androstenedione in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum with defined quantities of androstenedione. Refer to vial labels for expected value and acceptable range.
   - Volume: 0.5 mL/vial
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months in unopened vials or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. **Wash Buffer Concentrate** — Requires Preparation x10
   - Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.

Volume: 50 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute the wash buffer concentrate 1:10 in distilled or deionized water to prepare the working wash buffer. If one whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

6. **TMB Substrate** — Ready To Use
   Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
   Volume: 16 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.

7. **Stopping Solution** — Ready To Use
   Contents: One bottle containing 1M sulfuric acid.
   Volume: 6 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.

ASSAY PROCEDURE

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the wash buffer.
2. Remove the required number of well strips from the microplate and assemble into a plate frame. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette **25 µL** of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette **100 µL** of the Androstenedione-HRP conjugate into each well. (We recommend using a multichannel pipette.)
5. **Incubate** on a plate shaker (~200 rpm) for 60 minutes at room temperature.
6. **Wash** the wells 3 times each time with 300 µL/well of diluted wash buffer per well. After washing tap the plate firmly against absorbent paper to remove any residual liquid (the use of a washer is strongly recommended).
7. Pipette **150 µL** of the TMB substrate into each well at timed intervals.
8. **+** on a plate shaker for 10-15 minutes at room temperature (or until calibrator A attains dark blue colour for desired OD).
9. Pipette **50 µL** of stopping solution into each well at the same timed intervals as in step 7.
10. Measure the absorbance at 450 nm in all wells with a microplate reader, within 20 minutes after addition of the stopping solution.

CALCULATIONS

1. Calculate the mean optical density of each calibrator duplicate.

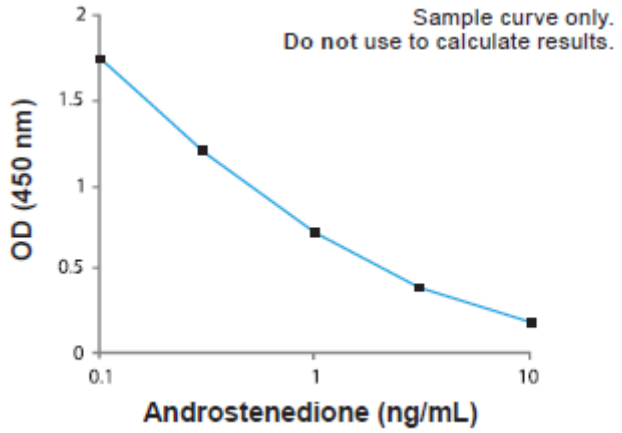
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 10 ng/mL then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	Mean OD	% Binding	Aldosterone (ng/mL)
A	2.443	100	0
B	1.746	71	0.1
C	1.195	49	0.3
D	0.721	30	1
E	0.385	16	3
F	0.184	8	10
Unknown	0.482	-	2.1

TYPICAL CALIBRATOR CURVE



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection (LoD) was determined from the analysis of 64 samples of the blank and a low value sample and it was calculated as follows:
LoD = μB + 1.645σB + 1.645σS, where σB and σS are the standard deviation of the blank and low value sample and μB is the mean value of the blank.
The Limit of Detection (LoD) was determined to be **0.04 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with aldosterone cross-reacting at 100%:

Steroid	% Cross-Reactivity
Androstenedione	100
DHEA	1.8
Testosterone	0.2
Estrone	<0.1
Estradiol	<0.1
Progesterone	<0.1
17-OH-Progesterone	<0.1
5a-DHT	<0.1
Cortisol	<0.01
DHEA-S	<0.01

INTRA-ASSAY PRECISION

Four samples were assayed 24 times each on the same calibrator curve. The results are tabulated below (in ng/mL):

Sample	Mean	SD	CV %
1	0.083	0.006	7.1
2	0.832	0.051	6.2
3	3.28	0.193	5.9
4	9.36	0.927	9.9

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.528	0.046	8.7
2	1.534	0.149	9.7
3	5.905	0.457	7.7

RECOVERY

Spiked samples were prepared by adding defined amounts of androstenedione to three patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1.Unspiked	0.882	-	-
+ 0.75	1.589	1.632	97
+ 1.5	2.521	2.382	106
+ 3.0	4.522	3.882	116
2.Unspiked	1.527	-	-
+ 0.75	2.466	2.277	108
+ 1.5	3.666	3.627	101
+ 3.0	5.756	6.027	96
3.Unspiked	0.585	-	-
+ 0.75	1.268	1.335	95
+ 1.5	1.878	2.085	90
+ 3.0	3.471	3.585	97

LINEARITY

Three patient serum samples were serially diluted with calibrator A.
The results (in ng/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1	2.317	-	-
1:2	1.220	1.158	105
1:4	0.615	0.579	106
1:8	0.329	0.290	113
2	6.594	-	-
1:2	3.212	3.297	97
1:4	1.594	1.648	97
1:8	0.818	0.824	99
3	7.456	-	-
1:2	3.588	3.728	96
1:4	1.835	1.864	98
1:8	0.963	0.932	103

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	N	Mean (ng/mL)	Range (ng/mL)
Males	20	2.0	0.4-3.5
Females	20	1.4	0.3-2.4

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Warranty Information

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