ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	**#1200**	Human Albumin (Urinary)
#1750	Human IgG (total)	**#1760**	Human IgM
#1800	Human IgE	**#1810**	Human Ferritin
#1210	Human Transferrin (Tf)	**#0020**	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		
#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	**#1830**	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	**#1510**	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		
#0030	Human Insulin	**#0040**	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	**#0410**	HCG-free beta
#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	**#1110**	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	**#1700**	Human T3 (total)
#1850	Human Cortisol	**#1860**	Human Progesterone
#1865	Human Pregnolone	**#1875**	Human Aldosterone
#1880	Human Testosterone	**#1885**	Human free Testosterone
#1910	Human Androstenedione	**#1920**	Human Estradiol
#1925	Human Estrone	**#1940**	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		
#3000	Human Rheumatoid Factors IgM (RF)		
#3100	Human anti-dsDNA		
#3200	Anti-Nuclear Antibodies (ANA)		

Instruction Manual No. M-1915

ANDROSTENEDIONE
Saliva

ELISA KIT Cat. No. 1915

For Quantitative Determination of Androstenedione In Human Saliva

For In Vitro Research Use Only

India Contact:

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Email: customerservice@atzlabs.com
Web: www.atzlabs.com

DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

ANDROSTENEDIONE SALIVA ELISA KIT Cat. No. 1915

Kit Contents: (reagents for 96 tests)

Components	Cat. #
Anti-Androstenedione coated strip **plate** (96 wells)	1916
Androstenedione Saliva **Std. A** (0 pg/mL), 10 ml	1917A
Androstenedione Saliva **Std. B** (5 pg/mL), 1 ml	1917B
Androstenedione Saliva **Std. C** (20 pg/mL), 1 ml	1917C
Androstenedione Saliva **Std. D** (100 pg/mL), 1 ml	1917D
Androstenedione Saliva **Std. E** (300 pg/mL), 1 ml	1917E
Androstenedione Saliva **Std. F** (1000 pg/mL), 1 ml	1917F
Androstenedione **reference**/c**ontrol**, 1 ml (see lot sp concn on the vial	1915C
Standards and controls are prepared in protein-based buffer with non-mercury preservative	
Androstenedione-**HRP Conjugate (20X);** 0.8 ml, Dilute 1:20 with assay buffer.	1918S
Assay Buffer, 15 ml	AB1915
Wash Buffer Conc. (10X), 50 ml	WB-10
HRP substrate (TMB) Solution; 16 ml	TMB-10
Stop solution, 6 ml	ST-10
Complete Instruction Manual	M1915

Introduction

Androstenedione, a major androgen found in peripheral blood, is secreted by the ovary, testis and the adrenal gland. Androstenedione is the immediate precursor to testosterone in the biosynthetic pathway. A significant percentage of secreted androstenedione is converted peripherally, principally to testosterone. Androstenedione is increased in a high percentage of women with polycystic ovarian syndrome (PCO), hirsutism, various signs of hyperandrogenicity, tumors of the adrenal gland, and congenital adrenal hyperplasia (CAH). Information on the concentration of androstenedione might also be of interest in some of the reproductive system disorders such as sexual precocity, female phenotype at birth, or sexual ambiguity. The correlation has been found between salivary androstenedione and plasma total androstenedione. Salivary levels of androstenedione were also found to be approximately equal to and highly correlated with the free plasma level. As a result, the determination of salivary androstenedione combines a highly sensitive technique and non-invasive sample collection that is of value in clinical and research studies.

ADI's Androstenedione ELISA kit provides for the measurement of Androstenedione in human saliva.

2. PRECISION

Intra-assay precision:

	Sample A	Sample B	Sample C
N	10	10	10
Mean (pg/mL)	47.57	59.48	134.37
S.D.(pg/mL)	2.18	1.61	5.24
C.V. (%)	3.4	5.24	3.9

Inter-assay precision:

	Sample A	Sample B	Sample C
N	10	10	10
Mean (pg/mL)	37.81	116.00	153.46
S.D.(pg/mL)	2.18	6.32	8.4
C.V. (%)	5.18	5.4	5.5

3. ACCURACY/RECOVERY

Three saliva samples were spiked with known concn. of androstenedione. The Androstenedione values were measured and % of recovery determined.

Initial Values (pg/mL)	Observed values (ng /ml)	Expected values (ng /ml)	Recovery (%)
Sample A Unspiked	34.3		
+ 100	130.2	134.3	96.9
Sample B Unspiked	51.7		
+ 20	69.9	71.7	97.5
+ 60	93.5	111.7	97.5
+ 200	238.5	251.7	94.8
Sample C Unspiked	99.0		
+ 200	337.8	299	91.8

3. LINEARITY

Three saliva samples were serially diluted (1:2, 1:4, 1:8 with Std. A. The Androstenedione values were measured and % of recovery was determined.

Initial Values (pg/mL)	Observed values (pg/mL)	Expected values (ng /ml)	Recovery (%)
Sample A Undiluted	130.2		
Dilution 1:2	74.98	65.1	115.2
Dilution 1:4	34.31	32.6	105.2
Dilution 1:8	19.78	16.3	121.3
Sample B Undiluted	337.8		
Dilution 1:2	155.17	168.9	91.9
Dilution 1:4	104.8	84.5	124.0
Dilution 1:8	51.6	42.2	122.3
Sample C Undiluted	500.2		
Dilution 1:2	247.2	250.1	98.5
Dilution 1:4	137.6	125.3	109.8
Dilution 1:8	76.2	62.6	120.6

EXTRACTION VS. NON-EXTRACTION COMPARITIVE STUDY

Androstenedione Saliva ELISA method was validated by the following comparative study between:

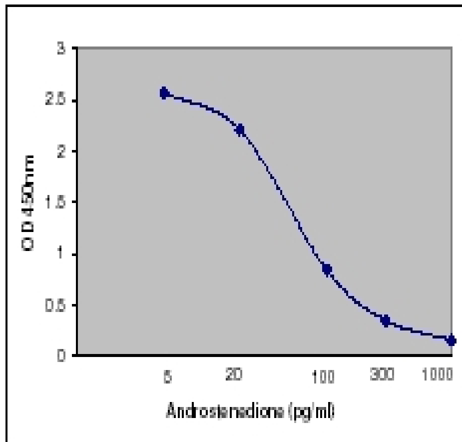
1. Direct assay of saliva samples
2. Prior extraction of saliva samples with diethyl ether
3. Prior heating of saliva samples for 1 hour at 60-70oC

Ten random saliva samples (range 17-115 pg/ml) were tested in Direct, Extraction, and prior heating methods. There was 95-105% correlation among the three methods.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (pg/mL)	Net Mean $A_{450\ nm}$
A1, A2	**Std. A** (0 pg/mL)	2.917
B1, B2	**Std. B** (5 pg /ml)	2.568
C1, C2	**Std. C** (20 pg /ml)	2.206
D1, D2	**Std. D** (100 pg /ml)	0.852
E1, E2	**Std. E** (300 pg /ml)	0.345
E1, E2	**Std. F** (1000 pg /ml)	0.150

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

Androstenedione Saliva ELISA kit is based upon competitive solid phase ELISA. The patient sample competes with enzyme-linked Androstenedione for a fixed and limited number of antibody binding sites on the coated plates. In the assay, the Androstenedione standard or samples sera are incubated with Androstenedione-HRP conjugate in the anti-Androstenedione coated wells. In this solid-phase system, the antibody bound Androstenedione will remain on the well while unbound Androstenedione will be removed by washing. A color (blue) is developed when the substrate, TMB is mixed with the antibody bound Androstenedione-HRP conjugate. After a short incubation, the enzyme reaction is stopped (blue color turns yellow) and the intensity of the color (yellow) is measured using an ELISA plate reader. The color is inversely proportional to the concentration of Androstenedione in the sample.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International Androstenedione Saliva ELISA test is intended for *in vitro research* use only. The reagents contain procilin as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses; therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).
All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SPECIMEN COLLECTION AND STORAGE

Approximately 1 ml of saliva is required per duplicate determination. Collect 4-5 ml of saliva into a clean glass tube without force or inducement and before eating, drinking or brushing the teeth. Simply rinse the mouth with water before collection. Do not use blood-contaminated specimens. Store samples at 4oC for up to 24 hours or at -10oC 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

Specimen tubes are to be placed into a freezer and allowed to freeze. When ready to use, the specimens are to be thawed and centrifuged. The supernatants are to be collected and poured into freshly labelled tubes.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. HRP substrate should be colorless at the time of use. If solutions have turned light blue in color, these should be replaced. Do not expose these solutions to strong light during storage or use. The unused portions of the standards should be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C. Addition of the HRP substrate solution starts a kinetic reaction. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dispense 200-300 ul of wash buffer to all wells. Mix for 5 seconds and discard or aspirate the solution. The step should be done just before adding the samples, do not allow the wells to dry at any time during the assay.

1. Pipet **100 µl** of standards, control, and samples into appropriate wells in *duplicate*.

2. Add **100 ul** of Androstenedione-HRP conjugate into each well**.** Mix gently. Cover the plate and incubate for **60 minutes** at room temperature on **plate shaker**. If plate shaker is not available, plates can be mixed manually every 15-20 min.

3. Remove reaction mixture and **wash 3X** with wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

Pipette **100 ul** of HRP-substrate solution (TMB). Mix gently. Cover the plate and incubate for **10-15 minutes** (at room temperature on plate shaker (or until calibrator A attains dark blue color or A450=2.0-2.5). If plate shaker is not available, plates can be mixed manually.

4. Stop the reaction by adding **50 µl** of stopping solution to all wells**.** Mix gently. Measure the absorbance at **450 nm** using an ELISA reader within 30 min.

Note: If the OD exceeds the upper limit of detection or if a 450nm filter is unavailable, a 405 or 415nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

CALCULATION OF RESULTS

1. Calculate the net mean OD from the duplicates of standards, controls, and patients samples.
2. Plot the concentration (X) of each reference standard against its Absorbance (Y) using a semi-log paper. Draw a point-to-point line through the mean of the duplicate point.
3. Obtain the value of sample Androstenedione by standard curve. The data given in the example is for demonstration purpose only and must not be used in place of data for each assay.
4. If a sample reads more than 1000 pg/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.

DILUTION OF SAMPLES and LIMITATIONS

It is recommended that each laboratory must determine its own normal and abnormal ranges. Extrapolation of Androstenedione values beyond the standard curve may yield variable results. Samples containing >1000 pg/mL Androstenedione can be diluted with 0 standard (no more than 1:16 dilution) and retested. The results must be multiplied by dilution factor. Controls from other manufacturers may contain serum preservatives incompatible with ADI's ELISA reagents should not be used. Whenever laboratory data conflict with clinical findings or impressions, clinical judgment should be exercised and additional evaluation undertaken.

EXPECTED VALUE

1. It is recommended that each laboratory should determine its own normal and abnormal range. The following values can be used as preliminary guidelines until the laboratory establishes its own normal values.

Sample	N	Range (pg/ml)
Male	28	48-309
Female	23	32-330

PERFORMANCE CHARACTERISTICS

Specificity

The following compounds were tested for crossreactivity of the assay: Androstenedione (100%), Androstandione (2.79%), testosterone (0.2%), Dihydrotestosterone (0.1%), and epiandrosterone (0.1%).   , testosterone, dehydroandrosterone, 5-a-DHT, Androstenedione sulphate, androsterone, estrone, progesterone, dihydroandrosterone, estradiol, and Cortisol (0.2-0.001%). The following steroids were tested but cross-reacted at less than 0.1%: Androstenedione Sulfate, Androsterone, Cortisol, Dehydroisoandrosterone, Dehydroepiandrosterone Sulfate, Dihydroandrosterone, 5 -Dihydrotestosterone, 17 -Estradiol, Estrone, Etiocholanolone Glucuronide and Progesterone.

Sensitivity

The minimal detectable conc. of Androstenedione is estimated to be 1 pg/mL. The minimal detectable conc. is defines as the concn. of Androstenedione, which corresponds to the absorbance, that is 2 S.D. smaller than the mean abs. Value of the zero std.