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Instructions for use

Androstenedione Saliva ELISA Free

REF SA E-6700R

2°C–8°C

Σ 96

RUO For research use only – Not for use in diagnostic procedures

Androstenedione Saliva ELISA

1. INTENDED USE

Competitive immunoenzymatic colorimetric method for the quantitative determination of Androstenedione concentration in saliva.

2. PRINCIPLE

Androstenedione (antigen) in the sample competes with the antigenic Androstenedione conjugated with horseradish peroxidase (HRP) for binding onto 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 blue color that changes into yellow when the Stop Solution (H_2SO_4) is added.
The colour intensity is inversely proportional to the Androstenedione concentration of in the sample.
Androstenedione concentration in the sample is calculated through a standard curve.

3. REAGENT, MATERIAL AND INSTRUMENTATION

3.1 Reagent and material supplied in the kit

Standards and **Controls** - ready to use

Cat. no.	Component	Standard	Concentration	Volume / Vial
SA E-6701	STANDARD A	Standard A	0 pg/ml	1 ml
SA E-6702	STANDARD B	Standard B	20 pg/ml	1 ml
SA E-6703	STANDARD C	Standard C	100 pg/ml	1 ml
SA E-6704	STANDARD D	Standard D	400 pg/ml	1 ml
SA E-6705	STANDARD E	Standard E	1000 pg/ml	1 ml
SA E-6751	CONTROL 1	Control A		1 ml
SA E-6752	CONTROL 2	Control B		1 ml

SA E-6713 INC-BUFF **Incubation Buffer** - ready to use
Contents: Phosphate buffer pH 7.5 BSA 1 g/l
Volume: 1 x 30 ml

SA E-6740 CONJUGATE-CONC **Enzyme Conjugate** - concentrated
Contents: Androstenedione conjugated with horseradish peroxidase (HRP)
Volume: 1 x 1 ml

SA E-6731 96 **Microtiterwells**
Contents: (1 microplate breakable); Anti-androstenedione antibody adsorbed on microplate

MS E-0055 SUBSTRATE **Substrate Solution** - ready to use
Contents: H_2O_2-TMB 0.26 g/l (avoid any skin contact)
Volume: 1 x 15 ml

MS E-0080 STOP-SOLN **Stop Solution** - ready to use
Contents: Sulphuric acid 0.15 mol/l (avoid any skin contact)
Volume: 1 x 15 ml
Hazards identification:

H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.

SA E-0030 WASH-CONC 50x **Wash Solution** - 50x concentrated
Contents: NaCl 45 g/l; Tween20 55 g/l
Volume: 1 x 20 ml

3.2 Reagents necessary not supplied

Distilled water

3.3 Auxiliary materials and instrumentation

Automatic dispenser
Microplate reader (450 nm, 620-630 nm)
Saliva Collection Device

Note
Store all reagents at 2 °C - 8 °C in the dark.
Open the bag of reagent 5 (Coated Microplate) only when it is at room temperature and close immediately after use; once opened, the microplate is stable until the expiry date of kit. Do not remove the adhesive sheets on the unused strips.

4. WARNINGS

1. This kit is intended for research use only.
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 as preservatives. 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 5 pg/ml to 1000 pg/ml.

5. 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 °C - 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 °C - 28 °C) and mix well prior to use.
4. Do not interchange kit components from different lots. The expiry date printed on box and vials labels 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. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
8. 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
9. 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.
10. Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
11. Maximum precision is required for reconstitution and dispensation of the reagents.
12. Samples microbiologically contaminated, highly lipaemic or haemolysed should not be used in the assay.
13. Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1 Preparation of the Standard

(*Standard A, Standard B, Standard C, Standard D, Standard E)*
Before use, mix for 5 minutes with rotating mixer
The standards are ready to use and have the following concentration of Androstenedione:

	Std A	Std B	Std C	Std D	Std E
pg/ml	0	20	100	400	1000

For samples with Androstenedione concentration greater than 1000 pg/ml dilute the sample (1:2) with Standard A.
Once opened, the standards are stable 6 months at 2 °C – 8 °C.
For SI UNITS: pg/ml x 3.487 = pmol/l

6.2 Preparation of Diluted Conjugate

Prepare immediately before use.
Add 10 µl of Conjugate to 1.0 ml of Incubation Buffer. Mix gently.
Stable for 3 hours at 22 °C - 28 °C

6.3 Preparation of Wash Solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled water to a final volume of 1000 ml prior to use. For smaller volumes respect the 1:50 dilution ratio.
The diluted wash solution is stable for 30 days at 2 °C – 8 °C.

6.4 Preparation of the Sample

This kit allows the determination of Androstenedione concentration in saliva samples.
It is recommended to collect saliva samples with a centrifuge glass tube and a plastic straw, or Sali Set (catalogue no. SA D-6100, 100 pieces).
Other commercially available sample collectors have not been tested.
The controls are ready to use.

6.4.1 Method and Limitations

Collect saliva samples at the times indicated.
If no specific instructions have been given, saliva samples may be collected at any time, paying attention to the following indications:

1. If saliva collection is carried out in the morning ensure that this is carried out prior to brushing teeth
2. During the day allow 1 hour after a meal, oral intake of pharmaceutical drugs or tooth cleaning before collecting saliva samples
3. It is very important that a good clear sample is received – i.e. no contamination with food, lipstick, blood (bleeding gums) or other extraneous materials.

6.4.2 Saliva Processing Instructions

1. Let the saliva flow down through the straw into the centrifuge glass tube
2. Centrifuge the sample for 15 minutes at 3000 rpm
3. Store at -20 °C for at least 1 hour
4. Centrifuge again for 15 minutes at 3000 rpm
5. The saliva sample is now ready to be tested.
6. Store the sample at 2 °C - 8 °C for one week or at -20 °C for longer time.

6.5 Procedure

Allow all reagents to reach room temperature (22 °C - 28 °C). At the end of the assay, store immediately the reagents at 2 °C - 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 °C - 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 (Standard A-E), two for each Control, two for each sample, one for Blank.

Reagent	Standard	Sample/Control	Blank
Standard A-E	**50 µl**		
Sample/Control		**50 µl**	
Diluted Conjugate	**150 µl**	**150 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.			
Substrate Solution	**100 µl**	**100 µl**	**100 µl**
Incubate at room temperature 22 °C - 28 °C for *15 minutes* in the dark.			
Stop Solution	**100 µl**	**100 µl**	**100 µl**
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

7. 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.

8. RESULTS

8.1 Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the standard curve and of each sample.

8.2 Standard Curve

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

8.3 Calculation of Results

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in pg/ml.

9. REFERENCE VALUE

As the values of salivary Androstenedione have a circadian pattern we suggest collecting the samples at the same hour (8 A.M.):
The following values can be used as preliminary guideline until each laboratory established its own normal range.

		pg/ml
WOMEN	Normal	20 – 160
	P.C.O.-Hirsute	120 – 300
MEN		20 – 150

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.

10. PERFORMANCE AND CHARACTERISTICS

10.1 Precision

10.1.1 Intra Assay Variation

Within run variation was determined by replicate measurements (16x) of two different saliva control in one assay. The within assay variability is ≤ 8.5%.

10.1 2 Inter Assay Variation

Between run variation was determined by replicate measurements (10x) of two different saliva control with different lots of kit. The between assay variability is ≤ 11%.

10.2 Accuracy

The recovery of 50 – 200 – 500 pg/ml of Androstenedione added to sample gave an average value (±SD) of 102.60 % ± 13.23 % with reference to the original concentrations.

10.3 Sensitivity

The lowest detectable concentration of Androstenedione that can be distinguished from the Standard A is 5 pg/ml at the 95 % confidence limit.

10.4 Specificity

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

Androstenedione	100 %
Testosterone	1.2 %
Epitestosterone	0.2 %
5α-dihydrotestosterone	0.1 %
DHEA	0.1 %
Progesterone	$1x10^{-3}$ %
Estrone	$1x10^{-3}$ %
Cortisol	$1x10^{-3}$ %

10.5 Correlation

The Androstenedione saliva ELISA kit was compared to another commercially available Androstenedione saliva assay. 38 saliva samples were analysed according in both test systems. The linear regression curve was calculated:

$y = 0.46x + 5.51$

$r^2 = 0.983$

y = Androstenedione saliva Elisa kit

x = Salimetrics Salivary Androstenedione Elisa kit

11 WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

12 BIBLIOGRAPHY

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13 TROUBLESHOOTING

POSSIBLE ERROR CAUSES / SUGGESTIONS

No colorimetric reaction
- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)
- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)
- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers
- contamination of pipettes, tips or containers
- insufficient washing (conjugates not properly removed)

Too high within run (CV%)
- reagents and/or strips not pre-warmed to room temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

Too high between-run (CV%)
- incubation conditions not constant (time, temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!