



REVISED 6 SEPT. 2011 RM (VERS. 5.1)

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This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

Competitive immunoenzymatic colorimetric method for determination of Androstenedione concentration in saliva. Androstenedione Saliva kit is intended for laboratory use only

2 PRINCIPLE

Androstenedione (antigen) in the sample competes with horseradish peroxidase androstenedione (enzyme-labelled antigen) for binding onto the limited number of anti- androstenedione (antibody) sites on the microplates (solid phase). After incubation, the bound/free separation is performed by a simple solid-phase washing.

The enzyme substrate (H₂O₂) and the TMB-Substrate (TMB) are added. After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the absorbances are determined. Androstenedione concentration in the sample is calculated based on a series of standard.

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

3 REAGENT, MATERIAL AND INSTRUMENTATION

3.1 Reagent and material supplied in the kit

1. **Androstenedione Standards** S0 – S4 (5 vials, 1 mL each)
2. **Incubation Buffer** (1 vial, 30 mL)
Phosphate buffer pH 7.5 BSA 1 g/L, stabilizer
3. **Conjugate** (1 vial, 1.0 mL)
Androstenedione conjugated with horseradish peroxidase (HRP)
4. **Coated Microplate** (1 microplate breakable)
Anti-Androstenedione antibody adsorbed on microplate
5. **TMB-Substrate** (1 vial, 15 mL)
H₂O₂-TMB 0.26 g/L (avoid any skin contact)
6. **Stop Solution** (1 vial, 15 mL)
Sulphuric acid 0.15 mol/L (avoid any skin contact)

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7. **50X Conc. Wash Solution** (1 vial, 20 mL)
NaCl 45 g/L; Tween-20 55 g/L

3.2 Reagents necessary not supplied

Distilled water

3.3 Auxiliary materials and instrumentation

Automatic dispenser

Microplate reader (450 nm)

Saliva Collection Device

Note

Store all reagents at 2 °C - 8 °C in the dark.

Open the bag of reagent 4 (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

- Use appropriate personal protective equipment while working with the reagents provided.
- Some reagents contain small amounts of Proclin 300[®] as preservatives. 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/H₂O₂ to directed sunlight, metals or oxidants.
- This method allows the determination of Androstenedione from 5 pg/mL to 1000 pg/mL.
- For samples with Androstenedione concentration greater than 1000 pg/mL dilute the sample (1:1) with S0.
- The clinical significance of Androstenedione determination can be invalidated if the specimen sample donor was treated with cortisone or natural or synthetic steroids.

5 PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- 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.



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- If you use automated equipment is your responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- 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
- 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.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated should not be used in the assay. Highly lipemic or haemolysed specimens should similarly not be used
- Plate readers measure vertically. Do not touch the bottom of the wells.

6 PROCEDURE

6.1 Preparation of the Standard

(S0, S1, S2, S3, S4)

Before use, mix for 5 min. with rotating mixer

The standard has the following concentration of Androstenedione:

	S0	S1	S2	S3	S4
pg/mL	0	20	100	400	1000

Once opened, the standards are stable 6 months at 2-8°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 (reagent 3) to 1.0 mL of Incubation Buffer (reagent 2). 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.



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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 DRG SALI TUBES 100 (REF SLV-4158)

Do not use sample collector commercially available as “SALIVETTE”. Other sample collectors commercially available have not been tested.

6.4.1 Method and Limitations

Collect saliva samples at the times indicated.

If no specific instructions have been given oral fluid (saliva) samples may be collected at any time for saliva collection, the following should be noted:

- a. If saliva collection is carried out in the morning ensure that this is carried out prior to brushing teeth
- b. During the day allow 1 hour after any food or drink before collecting saliva samples
- c. 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

Let the saliva flow down through the straw into the centrifuge glass tube

1. Centrifuge the sample for 15 minutes at 3000 rpm
2. Store at – 20 °C for at least 1 hour
3. Defrost samples
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.



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6.5 Procedure

As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the standard curve (S₀-S₄), two for each sample, one for Blank.

Reagent	Standard	Samples	Blank
Standard S ₀ -S ₄	50 µL		
Samples		50 µL	
Diluted Conjugate	150 µL	150 µL	
Incubate at +37°C for <i>1 hour</i> Remove the contents from each well; wash the wells 3 times with 300 µL of diluted Wash Solution.			
TMB substrate	100 µL	100 µL	100 µL
Incubate at room temperature 22 °C - 28 °C for <i>15 minutes</i> in the dark.			
Stop solution	100 µL	100 µL	100 µL
Shake the microplate gently. Read the absorbance (E) at 450 nm against Blank			

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 (E_m) for each point of the standard curve and of each sample.



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Plot the mean value of absorbance of the standards (E_m) 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 WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

10 BIBLIOGRAPHY

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11 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

**REVISED 6 SEPT. 2011 RM (VERS. 5.1)****RUO** IN THE USA**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

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