DRG® Androstenedione Saliva ELISA (SLV-4780)

CE

REVISED 12 APR. 2011 RM (VERS. 4.1) **RUO IN THE USA**

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

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 INTENDED USE

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

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 (H_2O_2) 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 x1 vial = 1 mL)
2. **Incubation Buffer** (1 bottle) 30 mL
   Phosphate buffer pH 7.5 BSA 1 g/L, stabilizer
3. **Conjugate** (1 bottle) 1.0 mL
   Androstenedione-HRP conjugate
4. **Coated Microplate** (1 microplate breakable)
   Anti-Androstenedione IgG adsorbed on microplate
5. **TMB-Substrate** (1 bottle) 15 mL
   H_2O_2-TMB 0.26 g/L (avoid any skin contact)
6. **Stop Solution** (1 bottle) 15 mL
   Sulphuric acid 0.15 mol/L (avoid any skin contact)

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.
The microplate, once opened, it stable until the expiry date of kit. Do not remove the adhesive sheets on the unused strips

4 PRECAUTION

- The reagents contain Proclin 300 as preservative.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Do not use different lots of reagents.
- This method allows the determination of Androstenedione from 5 pg/mL to 1000 pg/mL.
- Samples with expected concentrations > 1000 pg/mL should be further diluted (1+1) with S0.
- The clinical significance of Androstenedione determination can be invalidated if the specimen was treated with cortisone or natural or synthetic steroids.

5 PROCEDURE

5.1 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

5.2 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.

5.3 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.

5.3.1 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.

5.3.2 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.

5.4 Procedure

As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the standard curve (S_0-S_4), two for each sample, one for Blank.

Reagent	Standard	Samples	Blank
Standard S0-S4	**50 µL**		
Samples		**50 µL**	
Diluted Conjugate	**150 µL**	**150 µL**	
Incubate at *+37°C* for *1 hour* Remove the contents from each well. Wash the wells with 300 µL of distilled water. Repeat the washing procedure by draining the water completely			
TMB substrate	**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 Blank			

6 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.

7 RESULTS

7.1 Mean Absorbance

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

7.2 Standard Curve

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

7.3 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.

8 WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

9 BIBLIOGRAPHY

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10 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