



# DIAGNOSTIC AUTOMATION, INC.

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		See external label		2°C-8°C		Σ= tests		cat. #1038-17
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## Δ 4 ANDROSTENEDIONE

Direct immunoenzymatic determination of Δ4 androstenedione in serum or plasma

### INTENDED USE

Competitive immunoenzymatic colorimetric method for quantitative determination of Δ4 androstenedione concentration in serum and plasma

#### 1. 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<sub>2</sub>O<sub>2</sub>) 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 in the sample.

### 2. REAGENT, MATERIAL AND INSTRUMENTATION

#### 2.1 Reagent and material supplied in the kit

\* Androstenedione Standards

STD<sub>1</sub> (1 vial) 1 mL

STD<sub>2</sub> (1 vial) 1 mL

STD<sub>3</sub> (1 vial) 1 mL

STD<sub>4</sub> (1 vial) 1 mL

1. Incubation buffer (1 bottle) 30 mL

Phosphate buffer 50 mM pH 7.4; BSA 1 gr/L

2. Conjugate (1 bottle) 0.4 mL

Androstenedione-HRP conjugate

3. Coated Microplate

Anti-Androstenedione IgG adsorbed on microplate (1 microplate breakable)

4. TMB-substrate (1 bottle) 11 mL

H<sub>2</sub>O<sub>2</sub>-TMB 0.25gr/L (avoid any skin contact)

5. Stop solution (1 bottle) 11 mL

Sulphuric acid 0.15 mol/L (avoid any skin contact)

#### 2.2 Notes

Store all reagents between +2 and + 8°C in the dark.

Open the bag of reagent 3 (Coated Microplate) only when it is at room temperature and close immediately after use.

.Don't remove the adhesive sheet from the unused strips

#### 2.3 Reagents necessary which are not supplied with the kit

Distilled water.

#### 2.4 Auxiliary materials and instrumentation

Automatic dispenser.

Microplates reader

#### 2.5 Preparation of reagents

\*. Standard (S<sub>0</sub>,S<sub>1</sub>,S<sub>2</sub>,S<sub>3</sub>,S<sub>4</sub>,S<sub>5</sub>) (liquid)

Before use, mix for 2 min. with rotating mixer.

The standard has the following concentration of

Androstenedione:

	S <sub>0</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>
ng/ml	0	0.1	0.4	1.2	4.0	10.0

Stable when stored at +4°C until the expire date of kit;

#### 2.6 Diluted Conjugate . (Prepare immediately before use)

Add 10 μl stock solution (reagent 2) to 1.0 mL of Incubation Buffer (reagent 1).

Mix gently for 5 minutes, with rotating mixer

Stable for 3 hours at room temperature

### 3. PREPARATION OF THE SAMPLE

The determination of Androstenedione can be performed in plasma as well as in serum.

Store the sample at -20°C if the determination is not performed on the same day of the sample connection.

#### 3.1 Precaution

-Maximum precision is required for dispensation of reagents.

-This method allows the determination of Androstenedione from 0.1 ng/mL to 10.0 ng/mL.

-The clinical significance of Androstenedione determination can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.

#### 4. PROCEDURE

As it is necessary to perform the determination in duplicate, prepare two wells for each of the six points of the standard curve (S<sub>0</sub>-S<sub>5</sub>), two for each sample, one for Blank.

Pipette:

	Standard	Sample	
Blank			
Sample	---	50 µl	---
Standards S <sub>0</sub> -S <sub>5</sub>	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.

Pipette:

	Standard	Sample
Blank		
TMB-Substrate 100mL		100µL

Incubate at room temperature (20-25°C) for 15 minutes in the dark.

Pipette:

	Standard	Sample	
Blank			
Stop solution (6)	100 µl	100 µl	100 µl

Read the absorbance (E) at 450 nm against Blank.

#### 5. STANDARD CURVE - CALCULATION OF RESULTS

##### 5.1 Mean absorbance and relative percentage

Calculate the mean of the absorbances (Em) for each point of the standard curve and of each sample. Express data as the percentage of the mean absorbance of B<sub>0</sub> (EmS<sub>0</sub>) with the following formula:

$$(B/B_0)\% = \frac{Em}{(Em S_0)} \times 100$$

##### 5.2 Standard curve

Plot the values of the standards expressed as (B/B<sub>0</sub>)% on the enclosed logit-log paper.

Extrapolate the line passing through the points.

##### 5.3 Calculation of results

Interpolate the values of the samples expressed as (B/B<sub>0</sub>)% on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL.

#### 6. REFERENCE VALUES

The serum or plasma Androstenedione reference values are:

WOMAN	Follicular phase	0.75 - 2.16
ng/mL	Luteinic phase	0.94 - 2.33
ng/mL		
MAN		0.60 - 1.85
ng/mL		

#### 7. PERFORMANCE CHARACTERISTICS

##### 7.1 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 %
5a dihydrotestosterone	0.1 %
dehydroepiandrosterone	0.1 %
progesterone	0.001%
estrone	0.001%
cortisol	0.001%

##### 7.2 Sensitivity

The sensitivity of this method, calculated as two times the S.D. from B<sub>0</sub>, is 5 pg when the value of (B/B<sub>0</sub>)% is approx. 90%.

##### 7.3 Precision

The inter and intra-run precision had a coefficient of variation of 3.6% and 5.8% respectively.

##### 7.4 Accuracy

The recovery of 0.1, 0.4, 1.2, 3.0, 10.0 ng/mL of Androstenedione added to "plasma-free" sample gave an average value (±SE) of 102.9% ± 4.2% with reference to the original concentrations.

##### 7.5 Correlation with RIA

Correlation with RIA performed on the same samples:

$$y = 0.19 + 0.968x$$

$$r = 0.991$$

$$n = 32$$

$$p < 0.001$$

#### WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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