Androstenedione ELISA Kit

Cat.No: DEIA-XY52
Lot. No. (See product label)

Size

96T

Intended use

The Androstenedione ELISA kit is a competitive immunoenzymatic colorimetric method for the quantitative measurement of Androstenedione concentration in saliva. This Kit is intended for laboratory use only.

Principle Of The Test

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.

Reagents And Materials Provided

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,
3. **Enzyme Conjugate** (1 vial, 1.0 mL)
Androstenedione conjugated with horseradish peroxidase (HRP)
4. **Microtiterwells** (1 microplate breakable)
Anti-androstenedione antibody adsorbed on microplate
5. **Substrate Solution** (1 vial, 15 mL)
H_2O_2-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)
7. 50x Conc. **Wash Solution** (1 vial, 20 mL)
NaCl 45 g/L; Tween20 55 g/L

Materials Required But Not Supplied

1. Distilled water
2. Automatic dispenser
3. Microplate reader (450 nm)
4. Saliva Collection Device

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Storage

2°C to 8°C

Reconstitution And Storage

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.

Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Wash Solution

Add deionized water to the 40x concentrated Wash Solution. Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL. The diluted Wash Solution is stable for 2 weeks at room temperature.

Assay Procedure

Preparation of the Standard(S0, S1, S2, S3, S4)

Before use, mix for 5 minutes with rotating mixer The standards are ready to use and have the following concentration of Androstenedione:
S0 = 0 pg/mL
S1 = 20 pg/mL
S2 = 100 pg/mL
S3 = 400 pg/mL
S4 = 1000 pg/mL pg/mL
For samples with Androstenedione concentration greater than 1000 pg/mL dilute the sample (1:2) with S0 Once opened, the standards are stable 6 months at 2°C - 8°C.
For SI UNITS: pg/mL x 3.487 = pmol/L

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.

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.

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.

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:
a. If saliva collection is carried out in the morning ensure that this is carried out prior to brushing teeth.

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b. During the day allow 1 hour after a meal, oral intake of pharmaceutical drugs or tooth cleaning.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.

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.

Saliva Processing Instructions with Salivette Sardstedt
a. Remove the swab from the suspended insert of the Salivette
b. Gently chewing the swab for 1 minute produces a sufficient quantity of saliva.
c. Replace the swab into the Salivette and firmly close the tube using the stopper.
d. Centrifuge the Salivette for 2 minutes at 1000 g (rcf) for saliva generation.
e. Remove the insert complete with the swab from the centrifuge vessel and discard. The clear saliva is now ready for analysis (at least 1 mL of saliva should be recovered with this method).

Procedure
Allow all reagents to reach room temperature (22°C - 28°C). 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 (S0-S4), 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 3 times with 300 µL of diluted Wash Solution.			
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 Blank within 5 minutes.			

Quality Control

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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-torun 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.

Calculation

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

Typical Standard Curve

Plot the mean value of absorbance of the standards (Em) (S0 - S4) against concentration. Draw the best-fit curve through the plotted points. (e.g.: Four Parameter Logistic).

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. 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
8. 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.
9. Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
10. Maximum precision is required for reconstitution and dispensation of the reagents.
11. Samples microbiologically contaminated , highly lipaemic or haemolysed should not be used in the assay.
12. Plate readers measure vertically. Do not touch the bottom of the wells.

References

1. Judd H. and Yen S. J. Clin. Endoc.& Metab.,36 475 (1973)
2. Abraham G. J. Clin.Endoc. & M.39, 340 (1974)

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3. Hillier S.G. 79th Year book Medical Publishers Inc: Chicago. (1985)
4. Venturoli S. et al Fertility and Sterility, 48(1), 78 (1987)
5. Venturoli S. et al Hormone Res., 24, 269 (1986)
6. D. Riad et al Endocr. Reviews, 3 (4) 304 367 (1982)

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