EIASON® 3α Diol G

IVD

Enzyme immunoassay for the determination of 5α-Androstane-3α, 17β-diol-Glucuronide (3α Diol G) in human serum

Kit instruction

For in-vitro use only

Product of

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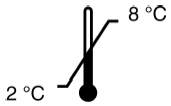
REF E02-001-96

2 °C 8 °C

CE

Used IFU-Symbols

Symbol	English	Symbol	English
IVD	In vitro diagnostic device	MPL	Microplate
REF	Order number	CAL 0-5	Calibrators
	Product of	CO1 CO2	Control low Control high
	Storage	BU	Assay puffer
CE	European Conformity	CONJ	Enzyme Conjugate
	Expiry date	SUB	Substrate
LOT	Batch code	STOP	Stop
		WASH	Wash solution

Intended use

For in-vitro use only.

The EIASON® 3α Diol G ELISA kit is an enzyme immunoassay for the quantitative in vitro diagnostic measurement of 5α-Androstane-3α,17β-diol-Glucuronide in human serum.

Summary

5α-Androstane-3α, 17β-diol glucuronide is a C19 steroid and is either abbreviated as 3α Diol G, 5α Diol G or simply, α Diol G. It is produced mainly as a metabolite of testosterone and dihydrotestosterone (DHT). It is largely produced in target peripheral tissues such as the skin, especially around hair follicles. The stimulation by large amounts of 3α Diol G leads to excessive hair formation, notably where hair is not normally present in women.

In recent years the interest in the measurement of this steroid has increased among clinical investigators studying women suffering from idiopathic hirsutism.

Among the steroids known to be precursors for 3α Diol G are dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS), dihydrotestosterone (DHT), androstenedione and testosterone. Only 3α Diol G has been shown to increase with hirsutism and decrease with treatment. This correlation has also been demonstrated in patients with polycystic ovarian syndrome (PCO). 3α Diol G determinations have therefore proved to be a useful indicator in a variety of ways including monitoring the progress of treatment of idiopathic hirsutism and women with PCO.

Furthermore, diabetic patients (both men and women) under cyclosporine A therapy have shown increased 3α Diol G levels, a side effect resulting in the appearance of hair in previously hairless areas.

Assay principle

The EIASON® 3α Diol G ELISA kit is a solid phase competitive enzyme immunoassay. Competition occurs between an unlabeled antigen (present in standards, controls and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of 3α Diol G in the sample. A set of standards is used to plot a standard curve from which the amount of 3α Diol G in
patient samples and controls can be directly read.

Warnings and precautions

The EIASON® 3α Diol G ELISA Kit is for in vitro diagnostic use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in the Package Insert. IASON will not be held responsible for any loss or damage (except as required by statute) caused, arising out of non-compliance with the instructions provided.

CAUTION: this kit contains material of human and/or animal origin. Handle kit reagents as if capable of transmitting an infectious agent.
Source material from Human origin which is used in this kit was tested and found negative for HbsAG and HIV as well as for HCV antibodies. However, since there is no diagnostic procedure that excludes these agents with 100 percent certainty all components should be handled as potentially hazardous material.
Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents.

Shelf life and storage of reagents

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.

Opened kit-components retain activity until expiration date, if not stated otherwise.

Storage and preparation of serum samples

Serum or plasma (EDTA- or heparin plasma) can be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

Specimen collection

Serum:
Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.
Store at 4 °C for up to 24 hours or at -10 °C or lower if the analyses are to be done at a later date.

Materials provided

Allow all reagents to reach room temperature before use.

1. MPL ELISA strip wells coated with anti-T4 antibody (monoclonal). (96 wells in total, 8 wells per strip). Before opening the packet of strip wells, allow it to stand at room temperature (20-25°C) for at least 30 minutes. After opening, keep any unused wells in the original foil packet (reseal with adhesive tape) and in the self-seal plastic bag with the desiccant provided.
2. CAL 0-5 Calibrator-Set, 6 vials, 2 mL CAL 0 and 0.5 mL CAL 2-6, ready to use;
   Concentrations: 0; 0.25; 1; 3; 10 and 50 ng/mL.
3. CO1 and CO2 Control low & high, 2 vials, 0.6 mL each, ready to use;
   For control values and ranges please refer to vial label or QC-Datasheet.
4. CONJ Enzyme conjugate x50 concentrate, 1 vial, 300 µL;
   3α Diol G-Horseradish Peroxidase (HRP) Conjugate.
   Dilute 1:50 in assay buffer before use (e.g. 40 µL of HRP in 2 mL of BU).
   If the whole plate is to be used dilute 240 µL of HRP in 12 mL of BU.
   Discard any that is left over.
5. BU Assay Buffer; for dilution of CONJ; 15 mL; ready to use.
6. SUBS Substrate Solution, 1 vial, 16 mL, ready to use;
   Tetramethylbenzidine (TMB).
7. STOP Stop solution (1 M H_2SO_4, 1 vial, 6 mL, ready to use).
8. WASH Wash Solution, 1 vial, 50 mL (10X concentrated); Dilute 50 mL of concentrated Wash Solution with 450 mL deionized water to a final volume of 500 mL.

Materials required but not provided in the kit

- microtiter plate calibrated reader (450 ± 10 nm) (e.g. the IASON Instruments Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction.

Assay procedure

General marks

All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
Once the test has been started, all steps should be completed without interruption.
Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
As a general rule the enzymatic reaction is linearly proportional to time and temperature.
Controls and standard curve should always be included in each assay run.

Assay procedure

1. Secure the desired number of MPL Microtiter wells in the holder.
2. Dispense 50 µL of each CAL 0-5, CO and samples with new disposable tips into appropriate wells.
3. Dispense 100 µL CONJ into each well.
4. Incubate 30 min at room temperature (18 – 25 °C) on a plate shaker (approximately 200 rpm).
5. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted WASH (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add 150 µL of SUB to each well.
7. Incubate for 10-15 minutes at room temperature (18 – 25 °C) on a plate shaker (or until CAL 0 attains dark blue colour for desired OD).
8. Stop the enzymatic reaction by adding 50 µL of STOP to each well.
9. Read the OD at 450±10 nm with a microtiter plate reader within 20 minutes after adding the Stop Solution.

or fully automated on:

- **IASON® Quardette**
- **IASON® PersonalLab**
- **IASON® Gladiator**

Calculation of results

- Draw a standard curve by plotting the absorbance of each standard against its concentration. Read off the values of the test samples. Samples with concentrations higher than that of the highest standard have to be further diluted with CAL 0. For the calculation of the concentrations this dilution factor has to be taken into account.
- Alternative data reduction techniques may be employed but users should confirm that the selected curve fit is appropriate and gives acceptable results. 4PL (4 parameter logistics) or point-to-point fits are recommended

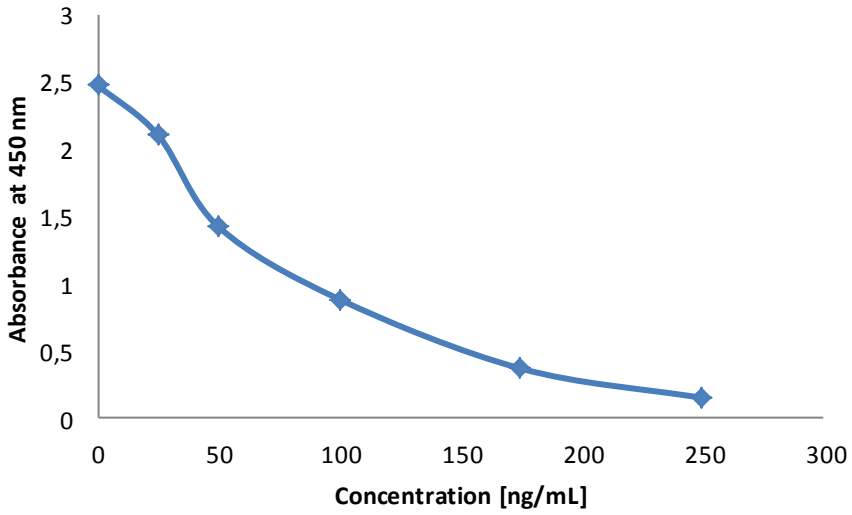
Sample assay data

Example of typical results obtained with EIASON® 3α Diol G calibrators:

Calibrator	Optical density (at 450 nm)
CAL0 (0 ng/mL)	2.477
CAL1 (0.25 ng/mL)	2.104
CAL2 (1 ng/mL)	1.421
CAL3 (3 ng/mL)	0.880
CAL4 (10 ng/mL)	0.364
CAL5 (50 ng/mL)	0.145

This data is for illustration only and must not be used for the calculation of any sample result.

Typical calibration curve



This sample calibration curve is for illustration only.

Expected Values

	ng/mL
Males	1.53 – 14.82
Premenopausal females	0.22 – 4.64
Postmenopausal females	0.61 – 3.71
Puberty (females)	0.51 – 4.03

Each laboratory is recommended to determine ranges for their local population. The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests

Quality control

The regular use of control samples at several analyte levels is advised to ensure day-to-day validity of results. Controls should be tested as unknowns. Quality control charts should be maintained to follow the assay performance.
Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or IASON directly.

Test characteristics

Assay dynamic range

The range of the assay is between 0.25 – 50 [ng/mL].

Specificity (cross-reactivity)

The following compounds were tested for cross-reactivity with the 3α Diol G ELISA kit with 3α Diol G cross-reacting at 100%.

Steroid	Cross-reactivity [%]
3α Diol G	100
Testosterone	0.2
Progesterone	0.16
Androstenedione	0.14
Cortisol	0.05

Analytical sensitivity

The analytical sensitivity of the EIASON® 3α Diol G ELISA was calculated by subtracting 2 standard deviations from the mean of 10 replicate analyses of the CAL0 and was found to be 0.1 ng/mL.

Reproducibility

Intra-assay-precision (n=24)			Inter-assay-precision (n=10)		
Sample	**Mean [ng/mL]**	**CV [%]**	**Sample**	**Mean [ng/mL]**	**CV [%]**
1	0.87	7,8	1	0.98	10.4
2	6.86	7,2	2	7.05	6.5
3	21.26	6,0	3	20.92	10.8

Recovery

Spiked samples were prepared by adding defined amounts of 3α Diol G to three patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Measured conc. [ng/mL]	Expected conc. [ng/mL]	Recovery [%]
1 (unspiked)	0.67	-	-
+0.5	1.07	1.17	91.4
+5.0	4.99	5.67	88.0
+15	12.66	15.67	80.8
2 (unspiked)	1.83	-	-
+0.5	2.07	2.33	88.8
+5.0	6.18	6.83	90.5
+15	17.64	16.83	104.8
3 (unspiked)	12.76	-	-
+0.5	15.32	13.26	115.5
+5.0	19.22	17.76	1108.2
+15	22.68	27.76	81.7

Linearity

Sample	Measured conc. [ng/mL]	Expected conc. [ng/mL]	Recovery [%]
1	6,24	-	-
1:2	2,83	3.12	90.7
1:4	1,55	1.56	99.4
1:8	0,74	0.78	94.9
2	13.55	-	-
1:2	6.00	6.77	88.6
1:4	2.71	3.39	80.0
1:8	1.70	1.64	103.6
3	17.05	-	-
1:2	6.93	8.53	81.2
1:4	4.09	4.26	96.0
1:8	2.34	2.13	109.8

Limitation of use

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.
The wash and mix-steps are critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

Legal aspects

Reliability of results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact IASON.

Therapeutic consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under Reliability of Results. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point Therapeutic Consequences are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

References

1. Gunther, H.J. and Wilson, J.D., Formation of 5α- Androstane-3α,17β-diol by normal and hypertrophic human prostate. J. Clin. Endocrinol. Metab. 44/1:107-115, 1977.
2. Deslypere, J.P., et al., Plasma 5α-Androstane-3α,17β-diol and urinary 5α-Androstane-3α,17β-diol glucuronide, parameters of peripheral androgen action: A comparative study. J. Clin. Endocrinol. Metab. 54/2:386-391, 1982.
3. Moghissi, E., et al., Origin of plasma androstanediol glucuronide in men. J. Clin. Endocrinol. Metab. 59/3:417- 421, 1984.
4. Scanlon, M.J. et al., Serum androstanediol glucuronide concentrations in normal and hirsute women and patients with thyroid dysfunction. Clin. Endocinol. 29:529-538, 1988.
5. Reiner, B.J., et al., Serum 3α-Androstanediol glucuronide measurements in sexually mature women with congenital adrenal hyperplasia during therapy. J. Clin. Endocrinol. Metab. 69-105-109, 1989.
6. Vexiau, P., et al., Increase in plasma 5α-Androstane-3α,17β-diol glucuronide as a marker of peripheral androgen action in hirsutism: A side-effect induced by cyclosporine A. J. Steroid Biochem. 35/1:133-137, 1990.
7. Pang, S., et al., 3α-Androstanediol glucuronide in virilizing congenital adrenal hyperplasia: A useful serum metabolic marker of integrated adrenal androgen secretion. J. Clin. Endocrinol. Metab. 73/1:166-174, 1991.
8. Riddick, L., et al., 3α-Androstanediol glucuronide in premature and normal pubarche. J. Clin. Endocrinol. Metab. 72:46-50, 1991.
9. Check, J.H., et al, Falsely elevated steroidal assay levels related to heterophile antibodies against various animal species. Gynecol Obstet Invest 40:139-140, 1995.

Pipetting scheme

Allow all reagents to reach room temperature before use

1. Pipetting	CAL0 – CAL6 **50µL**	CO **50µL**	Samples **50µL**
2. Pipetting	CONJ		**50µL**
3. Incubation	30 min at room temperature (18-25°C) on a shaker (200 rpm)		
4. Washing	3x washing: Wash 3 times with **300µL** WASH aspirate or decant and dry on an absorbent material		
5. Pipetting	SUB		**150µL**
6. Incubation	15 min at room temperature (18-25°C) on a shaker		
7. Pipetting	STOP		**50µL**
8. Reading	450nm (RF 620nm) Overrange Filter: 405nm, Factor: 3 (dependent on photometer), reading within 20 min. Calculation: 4-parameter or point to point		

Expected Values

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Males	1.53 – 14.82
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