Instructions for Use

Androstane-diol Glucuronide ELISA

Enzyme immunoassay for the direct quantitative determination of Androstane-diol Glucuronide in human serum.

REF **DB52171**

Σ **96**

2-8°C

EU: IVD CE

I B L I N T E R N A T I O N A L G M B H

Flughafenstrasse 52a
D-22335 Hamburg, Germany

Phone: +49 (0)40-53 28 91-0
Fax: +49 (0)40-53 28 91-11

IBL@IBL-International.com
www.IBL-International.com

1. INTENDED USE

For the direct quantitative determination of 5α-Androstane-3α,17β-diol Glucuronide (3αDiol G) by enzyme immunoassay in human serum. For in vitro diagnostic use only.

2. SUMMARY AND EXPLANATION

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.

3. TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the competition principle. An unknown amount of antigen present in the sample and a fixed amount of enzyme labelled antigen compete for the binding sites of the antibodies coated onto the wells. After incubation the wells are washed to stop the competition reaction. After the substrate reaction the intensity of the developed colour is inversely proportional to the amount of the antigen in the sample. Results of samples can be determined directly using the standard curve.

4. WARNINGS AND PRECAUTIONS

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. A calibrator curve must be established for every run.
6. The control should be included in every run and fall within established confidence limits.
7. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
8. When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
9. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
10. The assay buffer is sensitive to light and should be stored in the original dark bottle away from direct sunlight.
11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
15. Avoid contact with Stop solution. It may cause skin irritations and burns.
16. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.

Once opened, Standards and Controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

The Microtiter Plate and other reagents are stable up to 12 months or indicated on label, when stored at 2-8 °C.

6. SPECIMEN COLLECTION AND STORAGE

Serum

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Approximately 0.2 mL of serum is required per duplicate determination. Collect 4-5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer.

Storage	4°C	≤ -10°C (Aliquots)	Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles.
Stability	24 h	≥ 24 h	

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Contents: One 96 well (12x8) polyclonal antibody-coated microwell plate in a resealable pouch with desiccant.
1 x 0.3 mL	ENZCONJ CONC	**Enzyme Conjugate**, Concentrate (50 x) Contains: 3α Diol G-HRP conjugate in a protein-based buffer with a non-mercury preservative.
1 x 2.0 mL	CAL A	**Standard A** 0 pg/mL Ready to use. Contains: 3α Diol G, in protein-containing buffer, non-mercury preservatives. Exact concentrations see vial labels or QC certficate.
1 x 5 x 1.0 mL	CAL B-F	**Standard B-F** 0.25; 1.0; 3.0; 10.0; 50.0 ng/mL Ready to use. Contains: 3α Diol G, in protein-containing buffer, non-mercury preservatives. Exact concentrations see vial labels or QC certficate.
1 x 1.0 mL	CONTROL LOW	**Control Low** Ready to use. Contains: 3α Diol G in protein-containing buffer, non-mercury preservatives. Acceptable ranges see vial labels or QC Certificate.
1 x 1.0 mL	CONTROL HIGH	**Control High** Ready to use. Contains: 3α Diol G in protein-containing buffer, non-mercury preservatives. Acceptable ranges see vial labels or QC Certificate.
1 x 18 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains: TMB and hydrogen peroxide in a non-DMF or DMSO containing buffer.
1 x 8 mL	TMB STOP	**TMB Stop Solution** Ready to use. 1 M H_2SO_4.
2 x 50 mL	WASHBUF CONC	**Wash Buffer,** Concentrate (10 x) Contains: Buffer with non-ionic detergent and non-mercury preservatives.
1 x 17 mL	ASSAYBUF	**Assay Buffer** Ready to use. Contains: protein-containing buffer, non-mercury preservatives.

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 50, 100, 150, 300 µL
2. Vortex mixer
3. Orbital shaker (600 rpm) (e.g. EAS 2/4, SLT) or Linear shaker (200rpm)
4. 8-Channel Micropipettor with reagent reservoirs
5. Wash bottle, automated or semi-automated microtiter plate washing system
6. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
7. Bidistilled or deionised water
8. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of lyophilized or concentrated components

Dilute / dissolve	Component	with	Diluent	Relation	Storage	Stability
50 mL	**WASHBUF CONC**	450 mL	bidist. water	1:10	2-8°C	12 months
40 µL	**ENZCONJ CONC**	1960 µL	**ASSAYBUF**	1:50	Discard any that is left over.	

Samples containing concentrations higher than the highest standard have to be diluted further up to 1:8 with Standard A and reassayed. The result obtained should be multiplied by the dilution factor.

11. TEST PROCEDURE

1.	Pipette **50 µL** of each **Calibrator, Control and specimen sample** into the respective wells of the Microtiter Plate.
2.	Pipette **100 µL** of freshly prepared **Enzyme Conjugate (1:50)** into each well.
3.	**Incubate 30 minutes** at **RT (18-25°C)** on an orbital shaker (approx. 600 rpm) or linear shaker (200 rpm).
4.	Wash plate **3 x** with **300 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
5.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
6.	Pipette **150 µL** of **TMB Substrate Solution** into each well.
7.	**Incubate 10-15 min at RT (18-25°C)** on an orbital shaker (approx. 600 rpm) or linear shaker (200 rpm) or until calibrator A attains dark blue colour for desired OD.
8.	Stop the substrate reaction by adding **50 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
9.	**Measure** optical density with a photometer at **450 nm*** within **20 min.**

*) If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the vial labels. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logisitcs or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

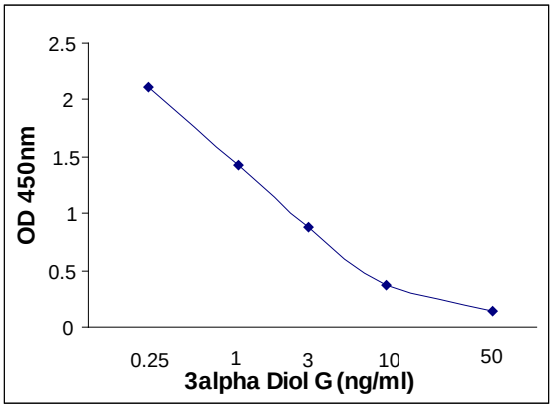
The concentration of the samples can be read directly from the standard curve.

Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

Typical Calibration Curve

(Example. Do not use for calculation!)

Calibrator	OD 1	OD 2	Mean OD	Value (ng/mL)
A	2.480	2.474	2.477	0
B	2.102	2.106	2.104	0.25
C	1.428	1.413	1.421	1
D	0.877	0.883	0.880	3
E	0.360	0.368	0.364	10
F	0.147	0.143	0.145	50
Unknown	0.598	0.596	0.597	5.4



14. EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (ng/mL)
Males	1.53-14.82
Premenopausal	0.22-4.64
Postmenopausal	0.61-3.71
Puberty (Female)	0.51-4.03

15. PERFORMANCE

SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the Androstane-diol Glucuronide ELISA is 0.1 ng/mL.

SPECIFICITY (CROSS REACTIVITY)

The following compounds were tested for cross-reactivity with the Androstane-diol Glucuronide ELISA 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

The following steroids were tested but cross-reacted at less than 0.01%: Corticosterone, Dehydroepiandrosterone, Dihydrotestosterone, Epiandrosterone, 17β-Estradiol and Estrone.

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV%
1	0.87	0.07	7.8
2	6.86	0.49	7.2
3	21.26	1.29	6.0

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV%
1	0.98	0.10	10.4
2	7.05	0.46	6.5
3	20.92	2.26	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	Obs.Result	Exp.Result	Recovery%
1 Unspiked	0.67	-	-
+0.5	1.07	1.17	91.4
+5.0	4.99	5.67	88.0
+15.0	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
+15.0	17.64	16.83	104.8
3 Unspiked	12.76	-	-
+0.5	15.32	13.26	115.5
+5.0	19.22	17.76	108.2
+15.0	22.68	27.76	81.7

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Obs.Result	Exp.Result	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

16. REFERENCES

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Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.


IBL International GmbH
Flughafenstr. 52A, 22335 Hamburg, Germany

Tel.: + 49 (0) 40 532891 -0 Fax: -11
E-MAIL: IBL@IBL-International.com
WEB: http://www.IBL-International.com