LDN Labor Diagnostika Nord GmbH & Co. KG
Am Eichenhain 1, 48531 Nordhorn
Telefon: +49-5921-8197 0
Telefax: +49-5921-8197 222
e-mail: info@ldn.de
Internet: http://www.ldn.de

LDN®

Instructions for use

Androstanediol-Glucoronide ELISA

REF **AA E-1500**

Σ 96

+2 +8 °C

IVD

CE

5α-ANDROSTANE-3α, 17β-DIOL GLUCURONIDE (3α DIOL G) ELISA

INTENDED USE

For the direct quantitative determination of 3α Diol G by enzyme immunoassay in human serum.
For *in vitro* diagnostic use only.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. 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.

CLINICAL APPLICATIONS

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.

PROCEDURAL CAUTIONS AND WARNINGS

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. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
9. 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.
10. 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.
11. The assay buffer is sensitive to light and should be stored in the original dark bottle away from direct sunlight.
12. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
13. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
14. 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.
15. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of 3α Diol G in human serum. The kit is not calibrated for the determination of 3α Diol G in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. 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.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

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. 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. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Precision pipettes to dispense 50, 100, 150 and 300 µl
- Disposable pipette tips
- Distilled or deionized water
- Plate shaker
- Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10).

REAGENTS PROVIDED

AA E-0030	WASH-CONC 10x	**Wash Buffer Concentrate – X10**
Contents:	One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.	
Volume:	50 ml/bottle	
Storage:	Refrigerate at 2-8°C	
Stability:	12 months or as indicated on label.	
Preparation:	Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.	

AA E-0055	SUBSTRATE	**TMB Substrate** - Ready To Use.
Contents:	One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.	
Volume:	16 ml/bottle	
Storage:	Refrigerate at 2-8°C	
Stability:	12 months or as indicated on label.	

AA E-0080 STOP-SOLN **Stopping Solution** - Ready To Use.

Contents: One vial containing 1M sulfuric acid.

Volume: 6 ml/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Calibrators and **Controls**- Ready To Use.

Listed below are approximate concentrations, please refer to vial labels for exact concentrations:

Cat. no.	Symbol	Calibrator	Concentration	Volume/Vial
AA E-1501	STANDARD A	**Calibrator A**	0 ng/ml	2.0 ml
AA E-1502	STANDARD B	**Calibrator B**	0.25 ng/ml	0.6 ml
AA E-1503	STANDARD C	**Calibrator C**	1 ng/ml	0.6 ml
AA E-1504	STANDARD D	**Calibrator D**	3 ng/ml	0.6 ml
AA E-1505	STANDARD E	**Calibrator E**	10 ng/ml	0.6 ml
AA E-1506	STANDARD F	**Calibrator F**	50 ng/ml	0.6 ml
AA E-1551	CONTROL 1	**Control 1**	Refer to vial labels for expected value and acceptable range!	0.6 ml
AA E-1552	CONTROL 2	**Control 2**		0.6 ml

Contents: 3α Diol G in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of 3α Diol G.

Storage: Refrigerate at 2-8°C

Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

AA E-1513 ASSAY-BUFF **Assay Buffer** - Ready To Use. ⚠*Warm to completely dissolve before use.*

Contents: One vial containing a protein-based buffer with a non-mercury preservative.

Volume: 15 ml/bottle

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

AA E-1531 96 **Rabbit Anti-3α Diol G Antibody Coated Microwell Plate-Break Apart Wells** - Ready To Use.

Contents: One 96 well (12x8) polyclonal antibody-coated microwell plate in a resealable pouch with desiccant.

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

AA E-1540 CONJUGATE-CONC 50x **3α Diol G-Horseradish Peroxidase (HRP) Conjugate Concentrate – X50**

Contents: 3α Diol G -HRP conjugate in a protein-based buffer with a non-mercury preservative.

Volume: 300 µl/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:50 in assay buffer before use (eg. 40 µL of HRP in 2 ml of assay buffer). If the whole plate is to be used dilute 240 µL of HRP in 12 ml of assay buffer. Discard any that is left over.

ASSAY PROCEDURE

Specimen Pretreatment: ***None.***

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1.	Prepare **working solutions** of the **3α Diol G-HRP conjugate** and **wash buffer**.
2.	Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
3.	**Pipette 50 µl** of each **calibrator, control and specimen sample** into correspondingly labelled wells in duplicate.
4.	Pipette **100 µL** of the **conjugate working solution** into each well. *(We recommend using a multichannel pipette).*
5.	**Incubate** on a plate shaker (approximately 200 rpm) for **30 minutes** at **room temperature**.
6.	Wash the wells **3 times** with **300 µl of diluted wash buffer** per well and tap the plate firmly against absorbent paper to ensure that it is dry *(The use of a washer is recommended).*
7.	Pipette **150 µl** of **TMB substrate** into each well at timed intervals.
8.	Incubate the plate on a plate shaker for **10-15** minutes at **room temperature**. *(or until Calibrator A attains dark blue colour for desired OD).*
9.	Pipette **50 µl** of **stopping solution** into each well at the same timed intervals as in step 7.
10.	Read the plate on a microwell plate reader at **450 nm** within 20 minutes after addition of the stopping solution.
⚠	*If the OD exceeds the upper limit of detection or if a 450nm filter is unavailable, a 405 or 415nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.*

CALCULATIONS

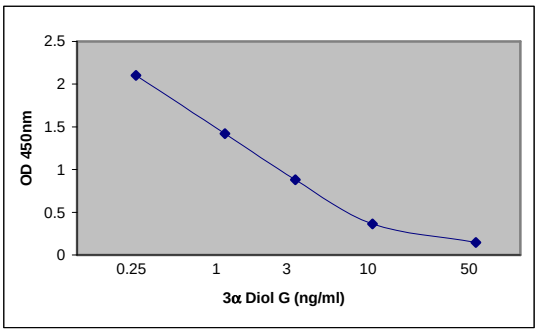
1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 50 ng/ml then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA:

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

TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results:



PERFORMANCE CHARACTERISTICS

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 Direct 3α Diol G ELISA kit is **0.1 ng/ml.**

SPECIFICITY (CROSS REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct 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

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

EXPECTED NORMAL 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

REFERENCES

- 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.
- 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.
- Moghissi, E., et al., Origin of plasma androstanediol glucuronide in men. J. Clin. Endocrinol. Metab. 59/3:417- 421, 1984.
- 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.
- 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.
- 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.
- 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.
- Riddick, L., et al., 3α-Androstanediol glucuronide in premature and normal pubarche. J. Clin. Endocrinol. Metab. 72:46-50, 1991.
- 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.

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!