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Instructions for use

Androstanediol-Glucuronide ELISA

REF **AA E-1500R**

2°C – 8°C

Σ 96

RUO For research use only – Not for use in diagnostic procedures

Androstanediol-Glucuronide ELISA

INTENDED USE
For the direct quantitative determination of 3α Diol G by enzyme immunoassay in human serum.

PRINCIPLE OF THE TEST
The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, controls and samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate. 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 samples and controls can be directly read.

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 standard 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 Standard A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.

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 nonreactive 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. No test method however, 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

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

REAGENTS PROVIDED

1. AA E-0030 WASH-CONC 10x **Wash Buffer Concentrate** – requires preparation **X10**

Content:	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.

2. AA E-0055 SUBSTRATE **TMB Substrate** – Ready To Use

Content:	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.

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

Content:	One vial containing 1 M sulfuric acid.
Volume:	6 ml/vial
Storage:	Refrigerate at 2 – 8 °C
Stability:	12 months or as indicated on label.
Hazards identification:	

H315 Causes skin irritation.
H319 Causes serious eye irritation.

4. Standards and **Controls** – Ready To Use

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

Cat. no.	Symbol	Standard	Concentration	Volume/Vial
AA E-1501	STANDARD A	Standard A	0 ng/ml	2.0 ml
AA E-1502	STANDARD B	Standard B	0.25 ng/ml	0.6 ml
AA E-1503	STANDARD C	Standard C	1 ng/ml	0.6 ml
AA E-1504	STANDARD D	Standard D	3 ng/ml	0.6 ml
AA E-1505	STANDARD E	Standard E	10 ng/ml	0.6 ml
AA E-1506	STANDARD F	Standard 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

Content: 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.

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

Content: 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.

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

Content: 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.

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

Content: 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 (e.g. 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. Standards, 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 **standard, 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 Standard 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 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 specimen/control samples.*

CALCULATIONS

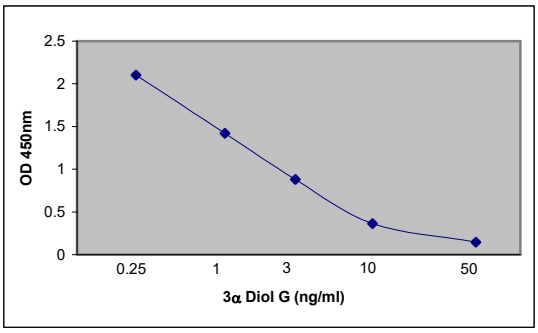
1. Calculate the mean optical density of each standard duplicate.
2. Draw a standard curve on semi-log paper with the mean optical densities on the Y-axis and the standard concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate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

TYPICAL TABULATED DATA:

Standard	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 STANDARD 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 Standard 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 standard 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 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 serum samples were diluted with Standard 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
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

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CHANGE HISTORY

Previous Version:	7.0-r	**New Version:**	7.0a-r
Changes:	***REAGENTS PROVIDED*** Hazard labelling for component AA E-0080 updated		

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date		Batch code		
	Consult instructions for use		Content		
	Caution		Catalogue number		Distributor
	Date of manufacture				For research use only!